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Impaired water barrier function in acne vulgaris

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Abstract **In acne vulgaris, abnormal follicular keratinization is important for comedo formation, yet the precise mechanisms of comedogenesis are not known. The present study examined the interrelationship between sebum secretion rate (SSR), lipid content and water barrier function (WBF) of the stratum corneum (SC) in 36 acne patients and 29 control subjects. All major SC lipid classes were separated and qantified by thin-layer chromatography/photodensitometry. WBF was evaluated by measuring transepidermal water loss (TEWL), and the hygroscopic properties and water-holding capacity of the SC. The SSR over a period of 3 h was significantly higher in patients with moderate acne than in control subjects, but no significant difference was noticed between patients with mild acne and control subjects. Significant differences between patients with both moderate and mild acne and control subjects were noted in the amount of sphingolipids (ceramides and free sphingosine), but not for any other lipid classes. Furthermore in acne patients, lower amounts of sphingolipids were observed corresponding with a diminished WBF. These results suggest that an impaired WBF caused by decreased amounts of ceramides may be responsible for comedo formation, since barrier dysfunction is accompanied by hyperkeratosis of the follicular epithelium.**

Key words Water barrier function · Acne vulgaris
Ceramides · Sphingosine · Sebum secretion rate

Introduction

Acne vulgaris is a disease well known throughout the world. Although one of the primary events in the pathogenesis of acne is abnormal follicular keratinization [8, 12], leading to comedo formation, no precise mechanism of comedogenesis has yet been clarified. There are two hypotheses concerning the role played by lipids in comedo formation. First, Downing and co-workers have postulated a linoleic acid deficiency of the follicular epithelium [5, 20]. They have demonstrated that increasing sebum production during puberty reduces the proportion of linoleate in ceramide 1. Second, Melnik et al. have suggested that an imbalance of free sterol and cholesterol sulphate causes follicular retention hyperkeratosis [12]. Both of these groups formulated a concept of a dilutional effect of sebum on epidermal lipids in acne [16]. Recent studies have indicated that epidermal lipid and DNA synthesis might be regulated by the water barrier function (WBF) of the stratum corneum [17]. If this is correct, a diminished WBF may cause hyperproliferation of the epithelium. Because sebaceous follicles have long canals through which sebum flows, hyperkeratinization of the follicular epithelium may easily cause plugged follicles.

In this study, the sebum secretion rate (SSR) and the lipid content and WBF of the stratum corneum were examined in acne. We report here, for the first time, a possible comedogenic mechanism involving an impaired WBF in acne.

Materials and methods

Subjects

A group of 36 male patients with facial acne, aged 14–26 years (mean age 21.1 ± 3.4 years), were examined. The clinical severity of the acne was graded according to the criteria of Plewig and Kligman [15]: grade I (mild type), less than 10 comedones without papules on one cheek; and grade II (moderate type), 10–20 comedones with less than 10 papules on one cheek. Of the 36 patients, 25 were categorized as grade I (mild group, mean age 21.4 ± 3.4 years) and 11 as grade II (moderate group, mean age 20.5 ± 3.2years). None of the patients had received topical or systemic treatment prior to the study. A group of 29 healthy male subjects aged 16–24 years (mean age 21.8 ± 2.9 years) without skin disease were similarly examined as controls. All subjects were asked to refrain from washing their face for 1 day prior to examination.

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Measurement of water barrier function

WBF was evaluated by measuring transepidermal water loss (TEWL), and the hygroscopic properties and water-holding capacity (WHC) of the stratum corneum of the cheeks of the subjects. TEWL was measured using an evaporimeter (EP1; ServoMed, Stockholm, Sweden) [14]. Based on a study by Tagami et al. [21], a water sorption–desorption test was performed using a skin surface hydrometer (SKICON-200; IBS, Hamamatsu, Japan). First, the conductance of the skin surface was measured and then a drop of distilled water was applied to the same area for 10 s to evaluate the hygroscopic properties [21]. After wiping the test area with a pad of gauze, conductance values, were measured serially at intervals of 30 s for 2 min to estimate WHC [21]. To avoid non-physiological conditions, these serial measurements were carried out under the fixed conditions of 20° C and 50% relative humidity.

Estimation of sebum secretion rates

Sustained SSR was measured on the forehead of each subject as previously described [24]. Briefly, the forehead was first swabbed with lipid-free cotton wool moistened with ether, and a lipid-free absorbent paper was attached to a defined area of the forehead for 3 h. Lipids were then extracted from the recovered paper and the amount was measured gravimetrically [24].

Collection and measurement of stratum corneum lipids

Stratum corneum lipids were collected from the cheeks using a cup method [3]. A glass cup (open end, 30 mm ID) containing 10 ml hexane/methanol (2:3 v/v) was tightly pressed onto the skin surface for 60 s and the extracted lipids recovered by evaporation of the solvent in a rotary evaporator [18].

Separation and measurement of lipid classes

Determination of lipid classes was accomplished using high-performance thin-layer chromatography (HPTLC) separation [13]. Approximately 50 μg of each extract was applied to the HPTLC plates (Kieselgel 60; Merck, Darmstadt, Germany). The chromatogram was first developed with hexane and then with benzene to the top of the plate. Relatively polar lipids on the plate were developed to a level of 6 cm with chloroform/methanol/water (95:20:1) and then to 14.5 cm with hexane/ether/acetic acid (80:20:10) [18]. After development, the chromatogram was sprayed with 10% (w/v) cupric sulphate hydrate in 8% (w/v) phosphoric acid [12] and charred by heating at 180° C for 60 min in a convection oven (STAC 5200; Shimadzurika Instruments, Tokyo, Japan) [18]. After cooling, the charred chromatogram was quantitated using an absorbance reflection method at a wavelength of 500 nm and linear scanning on a photodensitometer (CS-9000; Shimadzu, Kyoto, Japan). The amount of each lipid class was calculated by the measurement of the densitometric area for each spot; the areas were proportional to the weight of the respective constituents in the lipid mixture. Lipid standards were obtained from Sigma (St. Louis, Mo., USA).

Separation and measurement of sphingolipids

Sphingolipids were separated using a silica gel column. The lipids collected by the cup method were suspended in ether and passed through a column of silica gel. Continued elution with ether removed non-polar lipids, and subsequent elution with chloroform/methanol/water (C/M/W, 200:100:3) recovered the polar lipids. Ceramides were separated from the other polar lipid fractions by chromatography on a 0.25 mm thick layer of silica gel (Alltech, Deerfield, Ill., USA) with a mobile phase of chloroform/methanol/acetic acid (190:9:1) [20]. After drying, the chromatogram was sprayed with 50% sulphuric acid and charred by heating at 220° C on a hot-plate. The charred chromatogram was quantitated using a photodensitometer as described above. The amount of ceramides was determined by comparison with the densitometric area for cochromatographed pig epidermal ceramides.

Approximately 800 μg of C/M/W extract was applied to the TLC plates (Alltech) and was first developed to a level of 10 cm with chloroform/methanol/ammonia (40:10:1) and then with chloroform/methanol/acetic acid (190:9:1) to the top of the plate [22]. After development, the plate was sprayed with 0.5% ninhydrin in *n*-butanol (w/v) and placed silica-side down over a steaming hot water-bath for 5 min, after which it was turned over [22]. After cooling, it was scanned with a photodensitometer at a wavelength of 520 nm. The amount of free sphingosine was estimated by comparison with a sphingosine standard (Sigma).

Statistical analysis

Student's *t*-test was employed for statistical analysis.

Results

Water barrier function

The TEWL level was higher and the conductance value before the water sorption-desorption test was lower in both mild and moderate groups than in the control group (Table 1). A negative correlation was found between the TEWL and the conductance value of all subjects (data not shown).

Figure 1 shows the results of the water sorption–desorption test for both the mild and moderate groups and the control group. The conductance value increased abruptly after the application of water and then began to reduce gradually over 2 min. The values were consistently lower in acne patients than in the control subjects and lower in the moderate group than in the mild group.

Sebum secretion rate

The results of the SSR measurements on the forehead are shown in Table 2. The values were significantly higher in

Table 1 Comparison of water barrier function between acne patients and control subjects

	Acne patients		Control subjects ($n = 29$)
	Moderate ($n = 11$)	Mild ($n = 25$)	
TEWL (g/m²/h)	16.8 ± 3.8**	14.4 ± 2.5**	10.3 ± 2.4**
Conductance ($\mu\Omega^{-1}$)	52.1 ± 6.7**	92.8 ± 8.0**	188.9 ± 12.7**

Values are mean ± SD
** All values significantly different ($P < 0.01$) from one another

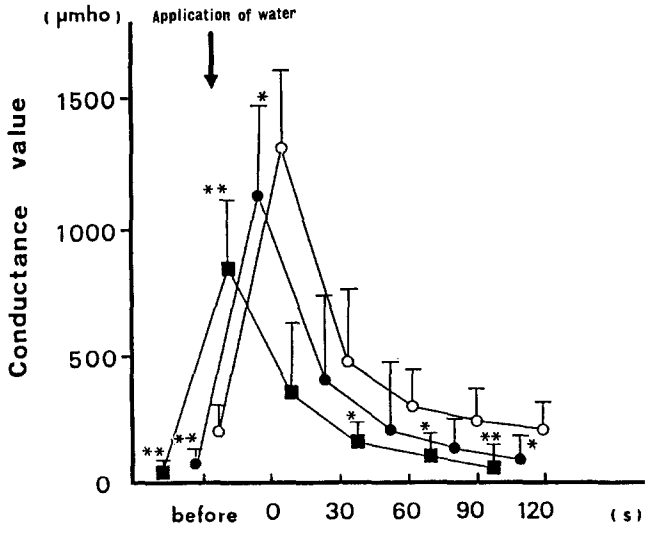

Fig. 1 Results of the water sorption–desorption test for patients with mild (●) and moderate acne (■) and control subjects (○). *Bars* represent SD. * $P < 0.05$, ** $P < 0.01$ versus control subjects

the moderate group than in the control group, but no significant difference was found between the mild group and the control group.

Composition of stratum corneum lipids

The mean weights of the stratum corneum lipid fraction of the acne patients and control subjects are presented in Table 2. Stratum corneum lipids extracted by the cup method are of both epidermal and sebaceous origin [18]. Squalene, wax esters and triglycerides originate purely from sebaceous glands. Free fatty acids, cholesterol and cholesterol esters derive from both epidermis and sebaceous glands, while cholesterol sulphate, ceramides and phospholipids are epidermal in origin. No significant differences between the acne patients and control subjects were noted for any lipid fraction except ceramides (Table 2). The total amount of lipids tended to be larger in the moderate group than in control group (Table 2).

Table 3 shows the mean percentages of sphingolipids from the polar lipid fractions of stratum corneum lipids from acne patients and control subjects. The proportions of free sphingosine and of total ceramides were significantly lower in both mild and moderate acne patients than in control subjects (Table 3). The composition of the ceramides in the acne patients and control subjects were found to be identical (data not shown).

Discussion

This study revealed that the acne patients showed a reduced WBF (Table 1 and Fig. 1) and decreased sphingolipids (Tables 2 and 3). Furthermore, lower levels of sphingolipids were observed corresponding with the diminished WBF in the patients (compare Table 1 with Table 3). Despite a significant difference in WBF between the mild group and the control group, both groups showed a similar SSR that was significantly lower than that of the moderate group (compare Table 1 with Table 2). This suggests that without a high SSR, follicular impaction may

Table 2 Sebum secretion rates (mg/40 cm^2/3 h) and mean weights (μg/cm^2) of the stratum corneum lipid fraction of acne patients and control subjects

	Acne patients		Control subjects ($n = 29$)
	Moderate ($n = 11$)	Mild ($n = 25$)	
Sebum secretion rates	13.23 ± 2.85*	9.83 ± 2.55	10.58 ± 4.30
Squalene	27.42 ± 4.46	16.37 ± 3.80	16.66 ± 2.58
Wax esters	51.03 ± 7.10	36.60 ± 5.50	39.21 ± 3.39
Triglycerides	40.88 ± 6.00	35.33 ± 3.50	35.81 ± 3.18
Free fatty acids	27.81 ± 1.32	31.24 ± 3.57	31.54 ± 3.16
Cholesterol	5.79 ± 0.71	6.53 ± 0.80	5.72 ± 0.65
Cholesterol esters	7.65 ± 0.30	6.85 ± 0.94	5.77 ± 0.87
Cholesterol sulphate	0.54 ± 0.18	0.74 ± 0.20	0.87 ± 0.18
Total ceramides	3.40 ± 0.45*	4.07 ± 0.87*	6.49 ± 0.98
Phospholipids	0.77 ± 0.15	0.75 ± 0.21	0.79 ± 0.09
Total lipids	165.29 ± 20.1	138.48 ± 10.22	142.86 ± 15.75

Values are mean ± SD
* $P < 0.05$ versus control subjects

Table 3 Mean percentages of free sphingosine and total ceramides in the polar lipid fractions from the stratum corneum of acne patients and control subjects

Sphingolipid	Acne patients		Control subjects ($n = 29$)
	Moderate ($n = 11$)	Mild ($n = 25$)	
Free sphingosine	0.18 ± 0.05**	0.30 ± 0.07**	0.74 ± 0.09**
Total ceramides	29.08 ± 2.07**	35.46 ± 3.94**	44.96 ± 5.83**

Values are mean ± SD
** All values significantly different ($P < 0.1$) from one another

occur during adolescence together with enlarged pilosebaceous canals affected by androgens [9]. Knutson [9] observed morphological differences between the infundibulum of normal and comedogenic follicles and suggested that decreases in membrane-coating granules (MCGs) are related to the abnormal follicular keratinization in early comedones. Currently, ceramides derived from the glycolipids of MCGs are considered to be responsible for WBF of keratinized epithelium [6, 19]. Presumably, decreased ceramides may cause a reduced WBF and hyperkeratosis of the follicular epithelium in acne patients. Proksch et al. [17] speculated that barrier disruption may cause lipid synthesis and hyperproliferative skin diseases such as ichthyosis, psoriasis, atopic dermatitis and irritant contact dermatitis. Downing et al. [5] and Stewart et al. [20] postulated that the decreased proportion of linoleate in ceramide 1 caused by the dilutional effect of sebum induces the characteristic responses of hyperkeratosis and decreased barrier function. We did not examine the linoleate content of the stratum corneum in this study, but in a previous study [25] we found no evidence that sebaceous fatty acids affect the composition of the esterified fatty acid of ceramide 1 in pubertal children and young adults aged 15 to 25 years. The ratio of free sterols to cholesterol sulphate in the stratum corneum, which has been indicated as an important factor in comedogenesis [12], was examined in this study, but no significant difference was observed between acne patients and control subjects (data not shown, but estimated in Table 2). Acne is common during puberty in Japan, but is not as severe (generally confined to grades I and II) as in Caucasians [15] and regresses naturally in the early-20s age group. There may be ethnic differences in lipid metabolism between Caucasians and Japanese. Some investigators have reported that ceramide synthesis may also be affected by aging as well as SSR [4, 11].

In addition to the essential role of ceramides in comedogenesis, the mechanism of inflammation in acne is also important. Our patients with moderate acne who had some inflamed lesions showed a lower WBF and a higher SSR than those with mild acne. Many studies have suggested a potential role for *Propionibacterium acnes* in comedogenesis [8, 9, 15, 16]. However, an ultrastructural study by Lavker et al. [10] demonstrated that abnormal keratinization in comedones occurs without *P. acnes*. However, more recent work has indicated that *P. acnes* may release mediators of inflammation or induce cytokine release by keratinocytes [7]. Presumably, *P. acnes* can readily colonize a hyperkeratotic follicular epithelium with an impaired WBF utilizing the abundant sebum as a nutrient [5, 10, 20]. As also reported by Wertz and Downing [23], free sphingosine was present in the stratum corneum lipids of all subjects (Table 3). It has been suggested that free sphingosine is produced by ceramide hydrolysis [22]. Therefore, low levels of free sphingosine in the acne patients were caused by decreased synthesis of ceramides (Table 3). Free sphingosine is known to have broad biological activity [22, 23]. Bibel et al. [1, 2], demonstrated that sphingosine can inhibit the proliferation of gram-positive bacteria including *P. acnes*. Therefore, there may be a possibility that acne patients have a disorder not only of WBF but also of the antimicrobial barrier. More studies are needed to clarify the relationship between free sphingosine and *P. acnes*.

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