

ROLE OF TOPICAL GLYCOLIC ACID AND PHOSPHATIDYLCHOLINE LINOLEIC ACID-RICH IN THE PATHOGENESIS OF ACNE. *LINOLEIC ACID VERSUS SQUALENE*

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Synopsis

Acne is a disease that commonly occurs during adolescence with the production of testosterone and the activation of the sebaceous glands. Factors such as stress, the environment, drugs, greasy cosmetics and mechanical irritants may contribute to or aggravate this disease.

Many facts indicate that free fatty acids released in sebaceous follicles through the action of bacterial lipases on sebaceous triglycerides play an important role in the overall pathogenesis of acne.

Moreover, it has been shown that there is a significant decrease in the levels of linoleic acid in sebum of patients with acne and a contemporary increase of squalene and oleic acid.

Supported by our recent data that demonstrate an anti-acne activity carried out by an emulsion based on phosphatidylcholine and glycolic acid buffered through a special mixture of aminoacids we controlled TEWL, corinebacterium acnes colonies and the presence, at cutaneous level, of linoleic acid and squalene on 20 patients with an average age of 18 ± 2 with a mild to moderate acne.

The data obtained proved a remarkable decrease of squalene and a contemporary increase of linoleic acid in the stratum corneum lipids, together with a considerable improvement of the skin's look of the patients.

Therefore it seems possible to assert that phospholipidic creams particularly rich in linoleic acid can be used as new cosmeceutical means adjuvant in the acne juvenilis therapy.

Riassunto

L'acne è una malattia che si verifica di solito durante il periodo dell'adolescenza con la produzione di testosterone e l'attivazione delle ghiandole sebacee. Fattori come lo stress, l'ambiente, i farmaci, i cosmetici grassi e gli irritanti meccanici possono contribuire o aggravare la malattia.

Molti fattori indicano che gli acidi grassi liberi rilasciati nei follicoli sebacei attraverso l'azione di lipasi batteriche sui trigliceridi sebacei giocano un ruolo importante nella patologia complessiva

dell'acne. Inoltre è stato provato che si verifica una diminuzione significativa nei livelli di acido linoleico nel sebo dei pazienti affetti da acne ed un contemporaneo aumento di squalene ed acido oleico.

Sostenuti dai nostri recenti risultati che dimostrano un'attività anti-acne svolta da un'emulsione basata su fosfatidilcolina e acido glicolico tamponato da una speciale miscela di aminoacidi, abbiamo controllato la TEWL, le colonie di *corinebacterium acnes* e la presenza, a livello cutaneo, di linoleico e squalene su 20 pazienti con una età media di 18 ± 2 affetti da acne debole o media.

I risultati ottenuti hanno dimostrato una considerevole diminuzione di squalene ed un contemporaneo aumento di acido linoleico nei lipidi dello strato corneo, insieme ad un miglioramento considerevole dell'aspetto cutaneo dei pazienti.

Per questi motivi sembra possibile affermare che le creme fosfolipidiche particolarmente ricche di acido linoleico possono essere utilizzate come nuovi mezzi cosmetici adiuvanti nella terapia dell'acne juvenilis.

INTRODUCTION

Acne is a disease that commonly occurs during adolescence with the production of testosterone and the activation of the sebaceous glands. Factors such as stress, the environment, drugs, greasy cosmetics and mechanical irritants may contribute to or aggravate this disease (1-3).

A connection between acne and high rates of sebum secretion is well recognized (4-5)

Many facts indicate that free fatty acids released in sebaceous follicles through the action of bacterial lipases on sebaceous triglycerides play an important role in the overall pathogenesis of the acne (5,6).

Since hyperkeratosis is part of the essential fatty acid deficiency syndrome, it has been also seen that a decrease in the amount of essential fatty acids might be involved in irritating hyperkeratinization within the follicle (7,8).

It is known that in the condition of essential fatty acid deficiency, essential fatty acids of the n-6 family are replaced by non-essential fatty acids of the n-9 family.

Moreover, it has been shown that there is a significant decrease in the levels of linoleic acid in sebum of patients with acne and a contemporary increase of squalene and oleic acid (9).

Supported by the results obtained by the work of Ghyyczy et al. (10) and by our recent data (11) we decided to study in detail the cutaneous activity carried out by an emulsion based on phosphatidylcholine and glycolic acid buffered through a special mixture of aminoacids on acne affected skin, through the evaluation of two definite parameters: corinebacterium acnes colonies and the presence at cutaneous level of linoleic acid and squalene.

We decided, moreover, to check also the possible variations that may be found in the Transepidermal Water Loss (TEWL), since the reduced presence of essential fatty acids makes the membrane structure more permeable to water.

MATERIALS AND METHODS

Materials

Vehicle: soybean liposome containing 10% lecithin fraction with 80% phosphatidylcholine linoleic acid-rich (cream B)

Active ingredients: vehicle + glycolic acid buffered to pH 4.5 by a special blend of aminoacids, chlorexidine digluconate and salicylic acid (cream A).

Patients

Twenty patients (10 female, 10 male) with an average age of 18 ± 2 with a mild to moderate acne, participated in the study. All were volunteers and the nature of the study was explained to them in full. As described elsewhere (11) exclusion criteria included patients with more than 5 nodules and cysts and patients who had used topical antibiotics, retinoids or benzoyl peroxide in the past 14 days, systemic antibiotics in the past 30 days, systemic retinoids in the past 2 years, any other topical acne treatments including medicated soaps, creams or make up in the past 7 days, topical corticosteroids in the past 14 days, or systemic corticosteroids in the past 12 weeks.

Test procedure

The study was conducted as a 12-weeks double-blind paired comparison, with treatment assignments randomized, as described elsewhere (11). Each patient was supplied with two tubes containing the test creams (A and B) to apply on the left or on the right area of the face on a randomized basis for three months. They were also instructed to apply always the same cream to the designed sites after washing first thing in the morning and just before retiring in the evening. A mild non-irritant washing cream (Mavigen® Idroschiuma) was supplied by us to be used throughout the study. Other instructions included that the patients use no other acne treatment during the study and not to apply the creams the day of evaluations or wash their face 4 hours before evaluations.

Biophysical non-invasive measurements

Measurements were performed on the 1st day (baseline) and after 2, 4, 6, 8, 10 and 12 weeks (end of treatment). The medium value, as described elsewhere (11), was always carried out on two different sites of right or left areas of forehead and automatically evaluated by 3C System® (Dermotech, Rome, Italy) (12).

Transepidermal Water Loss (TEWL)

All evaluations were performed after a 30-minute acclimatization period in a room at 22±2°C and 50% humidity.

Water evaporating from the skin surface was measured quantitatively with the 3C System®

methodology (12).

The 3C System® probe consists of a cylindrical open chamber measuring system, diameter 14 mm, height 10 mm and skin area 0.95 cm², two sensor units, containing thin capacitance film transducer, are placed at 3 and 7 mm distance from the skin surface. TEWL is calculated digitally in g/m² h. The obtained results are shown in Tab. I and Fig. 1.

Squalene and Linoleic acid

All lipids extracted by 3C System® methodology from both the half-forehead from all the twenty patients were separately dampened and collected samples from each half-forehead were

Table I

TEWL MEASUREMENTS OF ACNE AFFECTED PATIENTS TREATED BY PHOSPHATIDYLCHOLINE-CREAM GLYCOLIC ACID ENRICHED

n=20 T=22°C RH=50%

Weeks	Vehicle (cream B)	Active (cream A)
0	22.3±7.2	24.5±8.1
2	20.4±6.8	18.6±7.6
4	15.2±7.0	12.0±3.5
6	14.8±6.5	11.2±2.7
8	12.1±4.8	11.5±3.2
10	11.8±3.1	11.6±2.9
12	11.7±3.5	11.8±2.5

All p values are not significant as to groups and highly significant (p<0.005) as to baseline values.

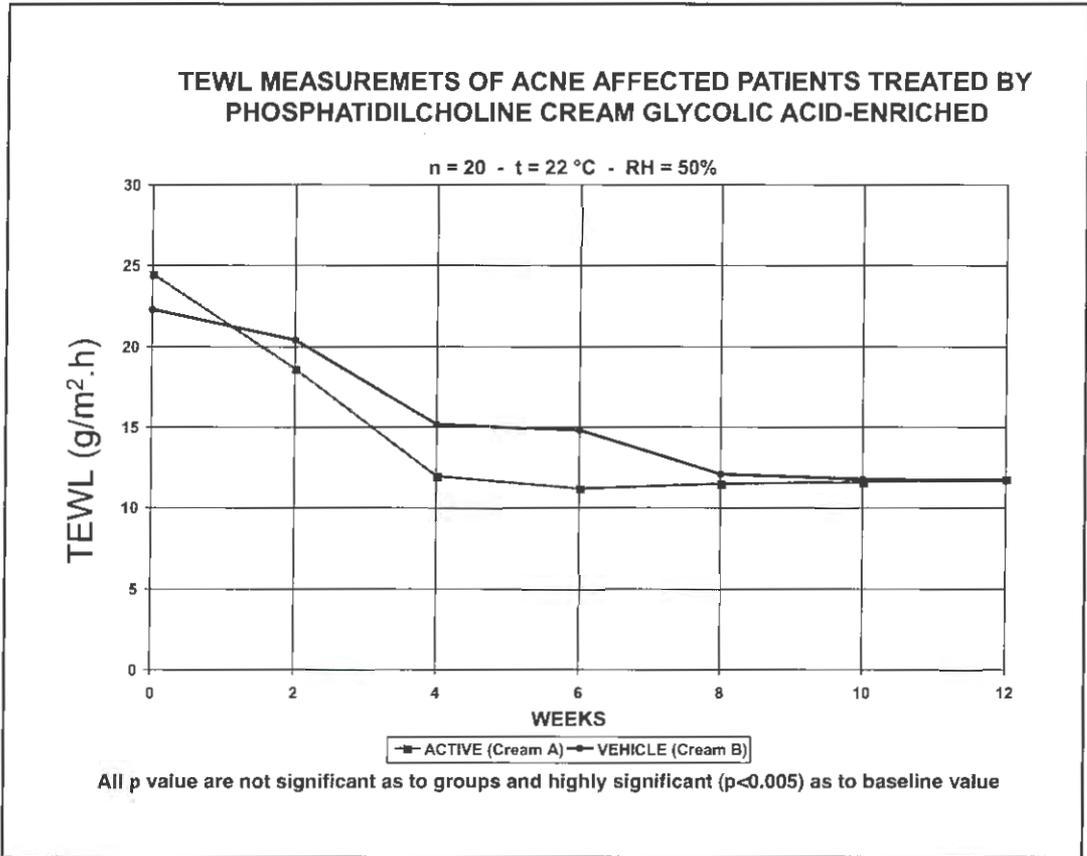


Fig 1

pooled. Collections from the right and the left areas were analyzed separately. All solvents used were chromatography grade. Total lipids were extracted from the 3C plastic foil (1 cm²) using ethyl ether methanol (2:1) for 3 h. at room temperature according to Folch et al. (13). The solvent was dried under nitrogen and the lipids were redissolved in chloroform (2:1) according to Cavina et al. (14). Lipid fractions were chromatographically separated into their individual lipid classes.

Fatty acids were then converted to their methyl esters according to Stoffel et al. (15). Oleic and linoleic acid methyl esters and squalene were quantitatively identified by using internal standards (Sigma Chemical).

The obtained results are shown in Fig. 2 and Tab. II.

Colonies of propionibacterium acnes

The evaluation of *P. acnes* was carried out according to the method of Williamson and Kligman (16). Samples were taken at 1st day and after 2, 4, 6, 8, 10 and 12 weeks of treatment.

Before taking the sample, both cheeks of the subjects were cleaned by using a sterile gauze saturated with a 0.1% sterile solution of Triton x-100, followed by a further cleaning with sterile distilled water and finally rubbed with a gauze saturated with hexane for 30 seconds. The cleansed skin was protected with a porous sterile pla-

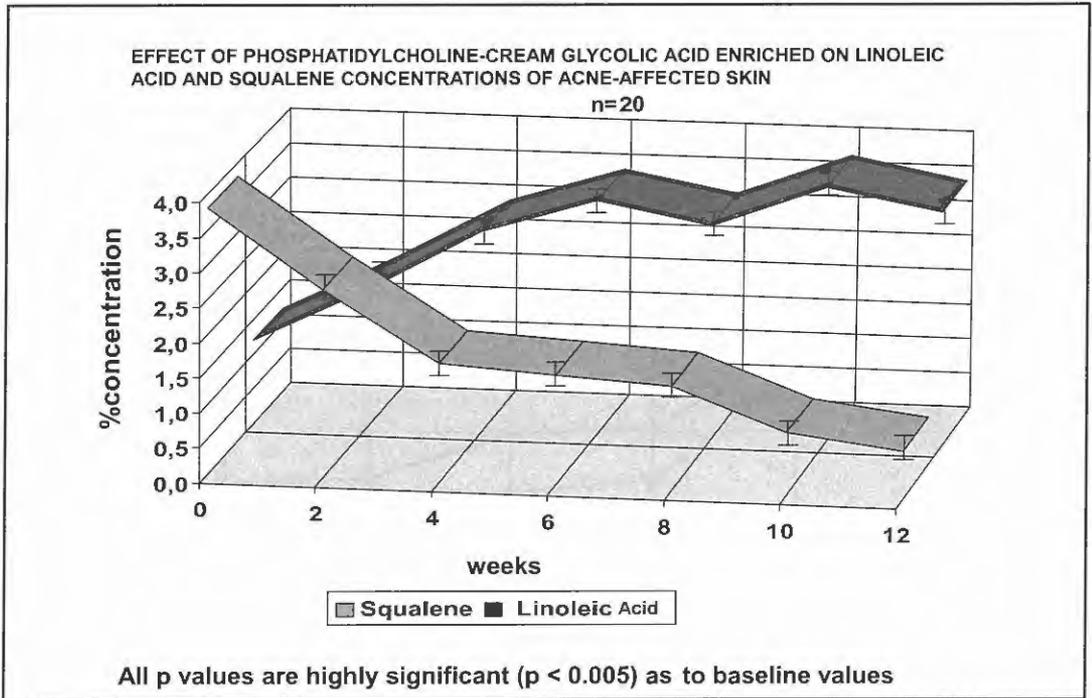


Fig 2

Table II

**TOPICAL APPLICATION EFFECT OF PHOSPHATIDYLCHOLINE-CREAM
GLYCOLIC ACID ENRICHED ON LINOLEIC ACID AND SQUALENE
CONCENTRATIONS OF ACNE-AFFECTED SKIN.**

n=20 T=22°C RH=50%

Weeks	Squalene %concentration	Linoleic acid % concentration
0	3.8±0.2	1.2±0.1
2	3.2±0.1	2±0.1
4	2.7±0.3	2.9±0.2
6	2±0.1	3.2±0.1
8	1.2±0.1	3.4±0.2
10	1±0.1	3.7±0.1
12	0.9±0.1	3.9±0.2

All p values are significant ($p < 0.05$) both as to weeks and to baseline values.

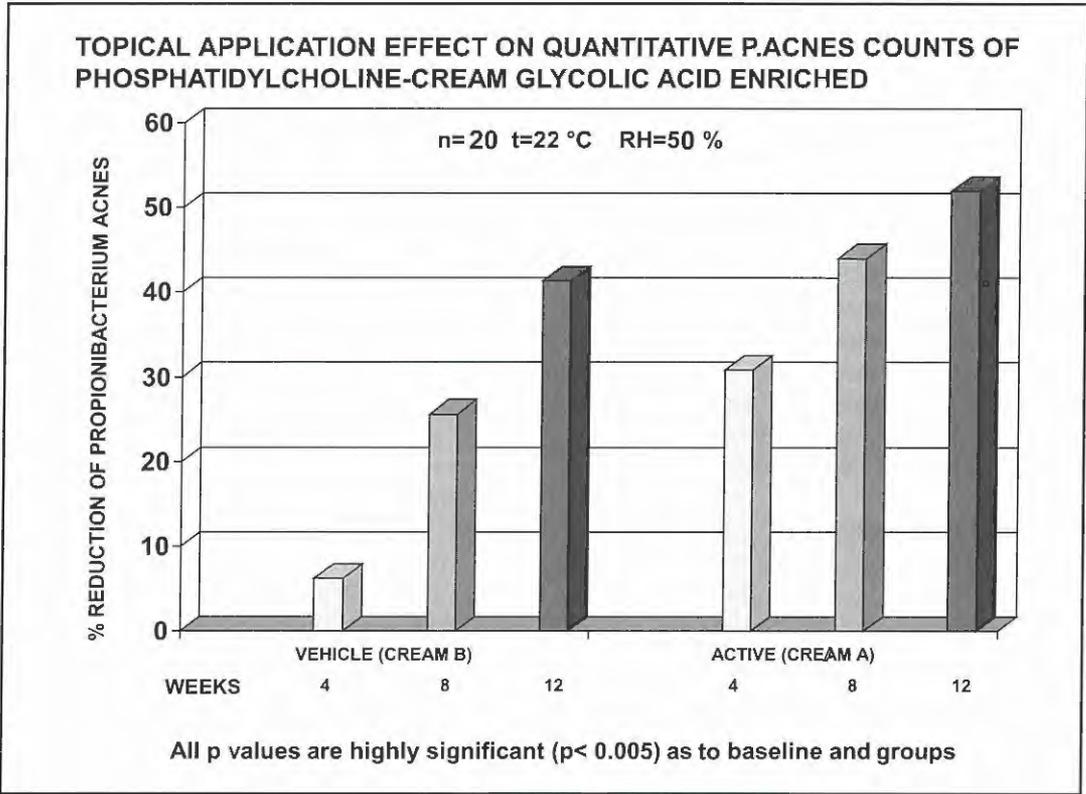


Fig 3

Table III

TOPICAL APPLICATION EFFECT OF A PHOSPHATIDYLCHOLINE-CREAM GLYCOLIC ACID ENRICHED ON QUANTITATIVE P. ACNES COUNTS

n=20

Product	Number of subjects	Week propionibacterium acnes (Log/cm ²)			
		0	4	8	12
Vehicle (cream B)	10	6.0842	5.7087	4.5219	3.5610
Active (cream A)	10	6.1533	4.2538	3.4636	2.9776

All p values are significant ($p < 0.01$) as to groups and highly significant ($p < 0.005$) as to baseline values.

stic gauze, so as to maintain normal evaporative activity. After one hour a cylinder of sterile glass (internal area 3.8 cm²) with hollow base was applied to the area. Into said cylinder 1 ml of sterile solution of 0.1% Triton x-100 in a pH 7.9 phosphate buffer was introduced. Having cleaned the area with a teflon spatula for one minute, two successive samples of liquid were taken. The samples thus obtained were diluted a number of times with a solution of 0.05% Triton x-100, immediately set in culture with a solution of 0.1% Tween 80 and incubated anaerobically for 7 days. The colonies of *P. acnes* were determined according to Mc Finley et al (17). The obtained mean results are shown in Table III and Fig. 3.

Statistical analysis

Differences between means were calculated using the Student's test. Statistical correlations between percent values of squalene and linoleic acid and absolute total amount recovery of lipids were calculated using the Pearson correlation coefficient (Statistical Analysis System, SAS, North Caroline, USA).

RESULTS AND DISCUSSION

According to data obtained from Ghyczy et al (10), and as it can be seen in the fig. 2 and Tab. II, the squalene concentration decreases drastically since the second week of treatment, while at the same time it can be noted a regular increase of the linoleic acid present in the stratum corneum lipids. It seems that this activity can be ascribed exclusively to the phospholipidic vehicle rich in linoleic acid.

The data obtained from the cutaneous areas treated with the cream B (vehicle) compared with the corresponding areas of the same subject treated with the cream A (active), resulted almost similar and are therefore reported as the average of a unique treatment (Fig. 2 and Tab. II).

With regard to the presence of corinebacterium acnes (Fig. 3 and Tab. III), the active cream

(cream A) resulted much more effective than the vehicle (cream B). The vehicle also (cream B) reduces the presence of corinebacterium acnes of about 50% after 12 weeks of treatment. This unexpected result is to be ascribed probably to the antioxidant activity typical of the soya phospholipids, that is likely to interfere with the survival and the development of corineabacterium acnes.

According to what we verified (18) it has also to be pointed out that by adding to the vehicle only the glycolic acid buffered to pH 4.5 it was obtained a further decrease of the whole bacterial charge, apart from the well-known bactericide activity carried out by both the salicylic acid and clorexidine. It has been verified experimentally that the glycolic acid alone buffered with special mixtures of aminoacids increases of a further about 30% the bacteriostatic activity typical of the used phosphatidylcholine.

Finally, as it was expected, it was possible to verify a considerable decrease of TEWL in all patients treated by both the vehicle and the active cream (Fig. 1 and Tab. I).

Considering the results obtained by our previous study, the results obtained by Ghyczy et al. (10,11) and the data obtained with this work it seems possible to assert that phospholipidic creams particularly rich in linoleic acid can be used as new cosmeceutical means adjuvant in the acne juvenilis therapy.

These emulsions have, moreover, proved to be even more active when the right proportion of glycolic acid, properly buffered with aminoacids, is added to them.

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