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THE TREATMENT OF ACNE VULGARIS BY PHOSPHATIDYLCHOLINE FROM SOYBEANS, WITH A HIGH CONTENT OF LINOLEIC ACID

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Synopsis

The efficacy of photosphatidylcholine from soya was evaluated in the treatment of acne vulgaris in 7 studies on 77 subjects.
Results of clinical trials:
- Reduction of the number of comedones and efflorescences;
- Decrease squalene concentration and
- Increase linoleic acid concentration in skin surface lipids;
- The efficacy of photosphatidylcholine is equal if used as liposome or in nonliposomal form.

Riassunto

L'efficacia della fosfatidilcolina di soya è stata valutata nel trattamento dell'acne vulgaris in 7 studi su 77 pazienti.
Risultati dei test clinici:
- Riduzione del numero di comedoni ed eruzioni cutanee;
- Diminuzione della concentrazione di squalene;
- Aumento della concentrazione di acido linoleico nei lipidi di superficie della pelle;
- L'efficacia della fosfatidilcolina è uguale se usata in forma liposomica o non liposomica.

Introduction

Acne vulgaris, with an incidence of nearly 100% in the second decade of life, is the most common skin disorder. The manifestations can differ considerably. According to Gollnick 30% of the affected subjects requires medical consultation and treatment [1]. Nevertheless, the less severe forms, also known as physiological acne, can be a relevant psychological problem for a large proportion of the remaining 70% of the teenage population. OTC drugs and cosmetic products are available to satisfy the demand this creates.

The pathogenesis of acne has not been elucidated in detail. However, four factors are regarded as being of clinical relevance and are, hence, the target of therapeutic intervention. They are differentiated as primary and secondary factors [1].

The primary factors are:

1. Elevated sebum production.
2. Disturbance of follicular keratinization.

The secondary factors are:

1. Follicular inflammation and immune response,
2. Hyperproliferation of Propionibacterium acnes.

The ideal treatment for acne is causal therapy for the primary factors. The only drug substance to afford this is isotretionin, a substance which has to be taken orally and which is associated with severe side effects.

On the other hand, there are several substances whose effects make them suitable for treatment of the secondary factors. Recent studies have suggested the importance of linoleic acid in the process of follicular hyperkeratosis, suggesting a new therapeutic possibility through normalizing the reduced linoleic content in the sebaceous follicle by external application.

Phosphatidylcholine, also misleadingly called lecithin, from soybeans contains chemically bound fatty acids, of which nearly 70% is linoleic acid. Pure phosphatidylcholine and fractions from soylecitine with a high content of phosphatidylcholine, the Phospholipons®, have been used in drugs and cosmetic products for decades. They are regarded as being safe; this is manifested in their GRAS-status [2]. They form liposomes with a typical particle size of 0.1 to 0.3 µm when formulated in water. Since the diameter of the sebaceous duct is 5 to 15 µm, liposomes should enter the sebaceous gland readily, transporting the linoleic acid.

Phosphatidycholine-bound linoleic acid has several advantages compared to the free form. The linoleic acid bound to phosphatidylcholine is more resistant to oxidation, is nearly colourless, has little odor and can formulate itself without further additives. It is beneficial if superfluous chemicals are omitted in the formulation since any chemical substance, used as formulation additive, can have an adverse effect on skin which is already irritated and inflamed as it is in acne.

Triglyceride-bound linoleic acid would add more lipid to the acne-affected skin, which is already suffering from an overproduction of sebum.

Linoleic Acid and Acne

The wax ester content of the sebum and of the epidermal acylceramides containing linoleic acid has been found to be inversely proportional to the activity of the sebaceous glands [3], with sebum fatty acids also being incorporated in epidermal lipids.

An analysis of the lipids in comedones and on the surface of the skin of acne patients and on the surface of the skin of persons not suffering from acne revealed that the acylceramides of the comedones and the skin surface of acne patients contained much less linoleic acid than the acylceramides from the skin surface of control subjects [4,5].

This finding gave rise to the hypothesis that the concentration of linoleic acid in sebum from human sebaceous glands depends both on the amount of linoleic acid that is present in every sebum-producing cell at the start of its differentiation and on the extent to which this amount is reduced by the endogenous lipid synthesis that follows [4,6].

The treatment of female acne patients with a combination of the antiandrogen cyproterone acetate and the estrogen ethinylestradiol led to a reduction of sebum secretion and to a simultaneous increase in the concentration of linoleic acid in all classes of lipids

[3]; a similar effect was also achieved with 13-cis-retinoic acid [7].

All these findings emphasize that sebum production, the development of acne and the concentration of linoleic acid in the sebum and in the epidermal lipids are all closely related, but do not provide any evidence of the pathogenic importance of linoleic acid for the disorder itself.

As early as 1929 Burr and Burr undertook fundamental diet studies in animals, which led to the recognition of the importance of polyunsaturated fatty acids - of linoleic acid in particular [8]. Rats that were fed a fat-free diet for several weeks developed the characteristic symptoms of an essential fatty acid deficiency; these were primarily characterized by an increase in the transepidermal water loss (TEWL) [9] and epidermal hyperproliferation [10].

The topical application of linoleic acid to the skins of rats suffering from essential fatty acid deficiency normalized the transepidermal water loss [11]. This curative effect is not prevented even by large doses of indomethacin, a potent inhibitor of cyclooxygenase, but at least in part by eicosatetraenoic acid, a potent inhibitor of both cyclo- and lipoxygenase [12]. This finding suggests that a lipoxygenase product formed from linoleic acid is of essential importance in addition to linoleic acid, being an essential component of the epidermal lipid barrier in the form of acylceramides.

This substance was later identified as 13-hydroxyoctadecadienoic acid (13-HODE). The application of 0.1% 13-HODE to the skin of guinea-pigs suffering from an epidermal hyperproliferation as a result of the administration of eicosapentaenoic (20:5n-3) and docosahexaenoic (22:6n-3) acids, led to the re-establishment of a histologically normal epidermis and to a normalization of the incorporation of radioactively labelled thymidine [13].

In addition, 13-HODE exhibits anti-inflammatory properties since it inhibits the formation of LTB_4 by human neutrophiles [14]. Linoleic acid itself, or a not yet definitely identified reaction product, possibly 13-HODE, inhibits phagocytosis and the formation of reactive oxygen species in the case of neutrophiles [15].

Thus, linoleic acid or linoleic acid bound to phosphatidylcholine is able to exhibit the following properties:

1. Linoleic acid is essential for the formation of the epidermal lipid barrier, whose inadequate function is the decisive stimulus for the proliferation, metabolic re-adjustment and keratinization of epidermal cells.
2. Linoleic acid is the precursor of 13-HODE, a product with antiproliferative properties, that also inhibits the production of the inflammation mediator LTB_4.
3. Linoleic acid itself , or a not yet characterized reaction product, inhibits phagocytosis and the production of aggressive oxygen metabolites by neutrophile granulocytes and, hence, inhibits the inflammatory process.

These findings suggest the potential of the anti-acne efficacy of linoleic acid bound to phosphatidylcholine from soybeans [16].

Biological Evaluations

The concentration of linoleic acid and squalene was determined in Studies 1 and 2 in order to evaluate the alteration of skin surface lipids as the result of phosphatidylcholine treatment. In Study 3 the anti-acne efficacy was proven, by pooling the results of 7 studies.

PROTOCOL OF STUDY 1

Aim of the study: to evaluate the content of linoleic acid in skin surface lipids

Test product: liposome containing 10% lecithin fraction with 80% phosphatidylcholine (tradename: Natipide® 08010A)

Dosage: 1 mg/cm² of lecithin fraction with 80% phosphatidylcholine; the molecule containing chemicly bound linoleic acid (tradename: Phospholipon® 80)

Duration of the treatment: 8 weeks

Number of subjects: 14, 4 female and 10 male, aged 14 to 17 years, affected by acne grade 1 and 2 [18].

Method of evaluation: the skin surface lipids were sampled by direct skin contact with a mixture of n-hexane/2-propanol (3:2, v/v). This was done by

placing 2 ml of the solvent mixture in a test tube and applying this to the skin so that the solvent mixture made contact with the skin for 2 min. The solvent mixture was then evaporated off in a stream of nitrogen, the residue was dissolved in 2 ml methanol/0.5 ml n-hexane and converted to the corresponding methyl esters with the aid of 0.3 ml boron triflouride methanol complex. Linoleic acid and squalene were determined quantitatively by gas chromatography [17].

PROTOCOL OF STUDY 2

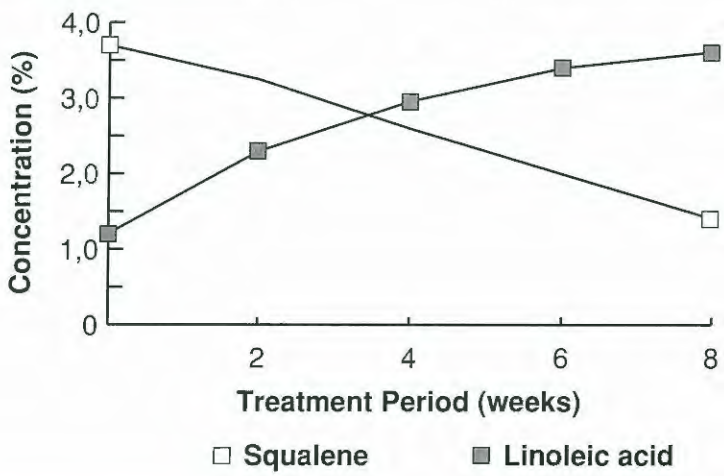
Aim of the study: to evaluate the content of squalene in skin surface lipids
Test product: liposome containing 10% lecithin fraction with 80% phosphatidylcholine (tradename: Natipide® 08010A)
Dosage: 1 mg/cm² of lecithin fraction with 80% phosphatidylcholine; the molecule containing chemicly bound linoleic acid (tradename: Phospholipon® 80)
Duration of the treatment: 8 weeks
Number of subjects: 7, 3 female and 4 male, aged 14 to 17 years, affected by acne grade 1 and 2 [18].
Method of evaluation: see Study 1

RESULT OF STUDIES 1 AND 2

The results of Studies 1 and 2 are summarized in Fig. 1.

PROTOCOL OF STUDY 3

Number of studies: 7
Test products: liposome containing 10% lecithin fraction with 80% phosphatidylcholine (tradename: Natipide® 08010A) and the adequate liposome formulations containing 10 and 20% Phospholipon® 90 [16].
Dosage:1 mg/cm² of lecithin fraction containing 80% phosphatidylcholine and pure phosphatidylcholine (tradename: Phospholipon® 80/90) skin, once per day
Control: no treatment on the contralateral side of the face
Study design: open, single-center
Duration of the treatment: 20 days to 8 weeks
Number of subjects: between 5 and 15 per study (total number of subjects n=77, age of subjects between 13 and 18 years)
Indication: acne vulgaris
Efficacy variables: number of comedones and efflo-



The values were determined for linoleic acid at 0, 2, 4, 6 and 8 weeks, squalene at 0 and 8 weeks.

Figure 1: *Effect of treatment with Natipide 08010A on the linoleic acid and squalene content of the skin surface lipids of acne-affected subjects.*

rescences
Evaluation: a projection foil was laid on the test area and a black marker was used to mark the comedones and a red marker to mark the inflamed lesions on the foil on the same 3 x 3 cm area throughout the course of the study. The marks were counted. In all studies an intrasubject control was provided by the corresponding figures for the untreated contralateral side of the face [18].
Exclusion criteria: -acne vulgaris requiring medication, -treatment of the acne with medicaments or cosmetics during the 2 months prior to the study, -consumption of ovulation inhibitors.

Side effects: the subjects merely noticed a somewhat drier and less oily skin and moderate peeling. These effects were enhanced by the use of liposome containing 20% lecithin fraction with 80% phosphatidylcholine (tradename: Natipide® II), which is a gel and can easily be overdosed. However, these observations did not lead to a negative assessment on the part of the subject or to abandonment of the study.

RESULT OF STUDY 3

The results of the studies were compiled and evaluated in a meta-analysis. The average effects of all the studies (Fig.2, 3) are outlined in the following graphs:

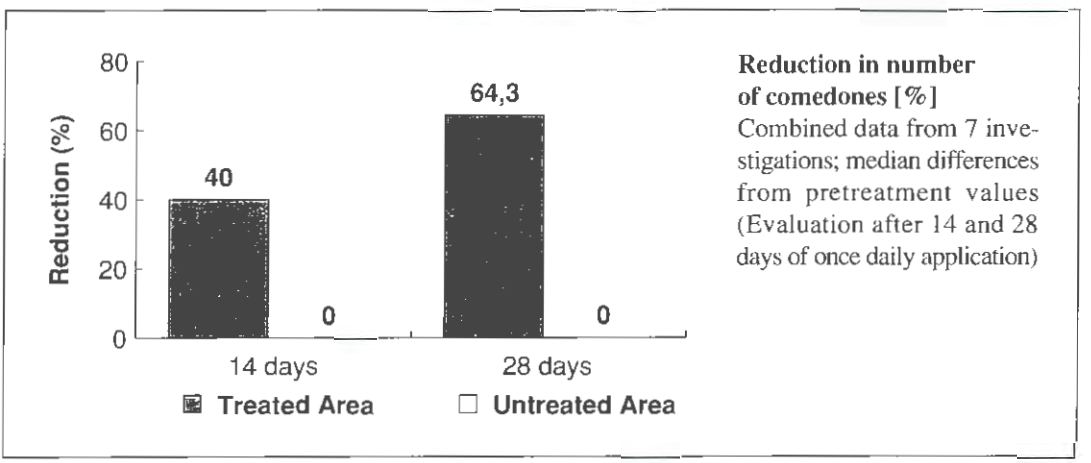


Figure 2: Reduction in number of comedones.

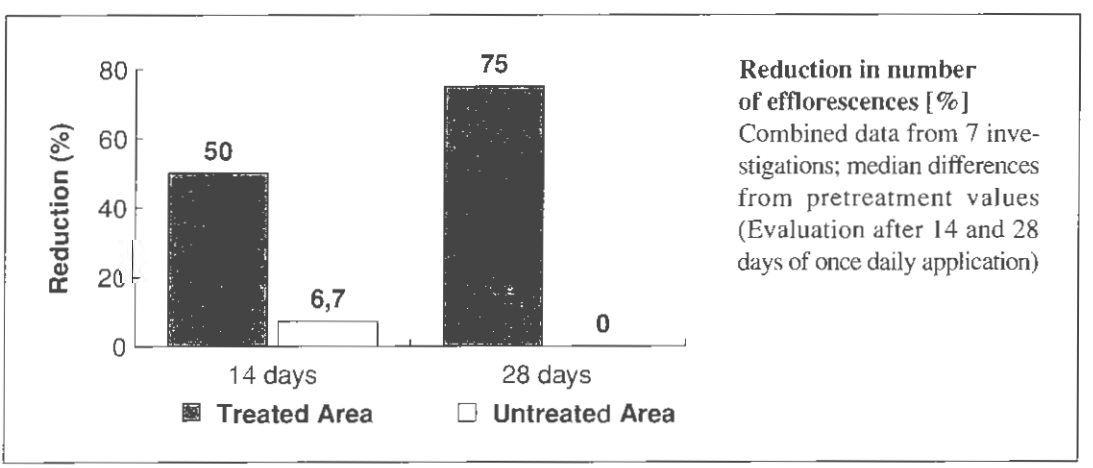


Figure 3: Reduction in number of efflorescences.

The following bar charts (Fig. 4, 5) document the studies separately and give the relative number of subjects where the improvement was more than 60%.

Discussion

Recent investigations suggest that overproduction of sebum leads to a deficiency of linoleic acid and, hence, to disturbance of lipid synthesis and differentiation in the epithelium of the mouth of the sebaceous follicle. This is supported by the analysis of the lipids of the blackheads and of the skin surface of acne patients and of healthy control persons, whereby therapeutic intervention with antiandrogens and 13-cis-retinoic acid lead to the normalization of the linoleic acid content of the lipids in acne patients.
Furthermore, linoleic acid is the precursor of 13-hy-

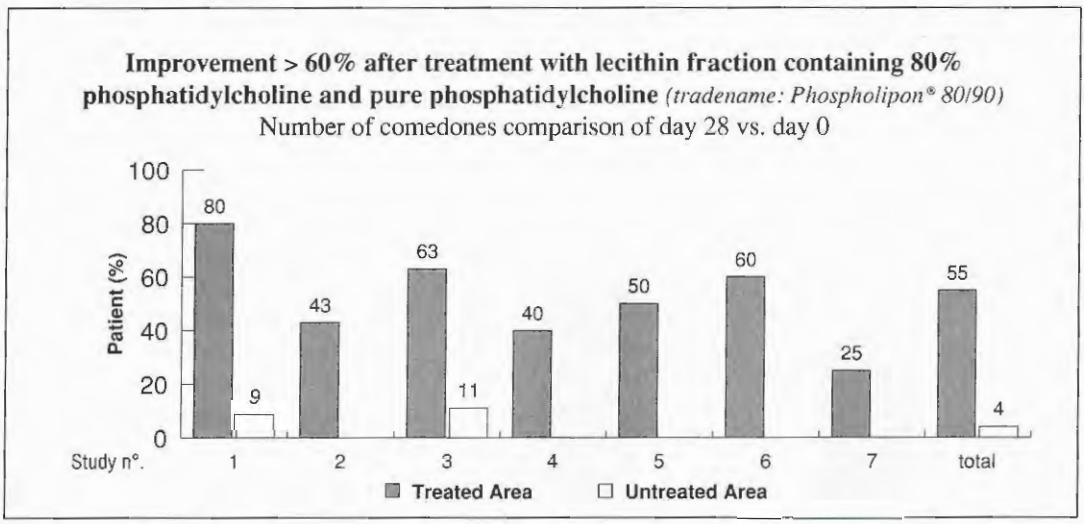


Figure 4: Improvement > 60% with regard to number of comedones.

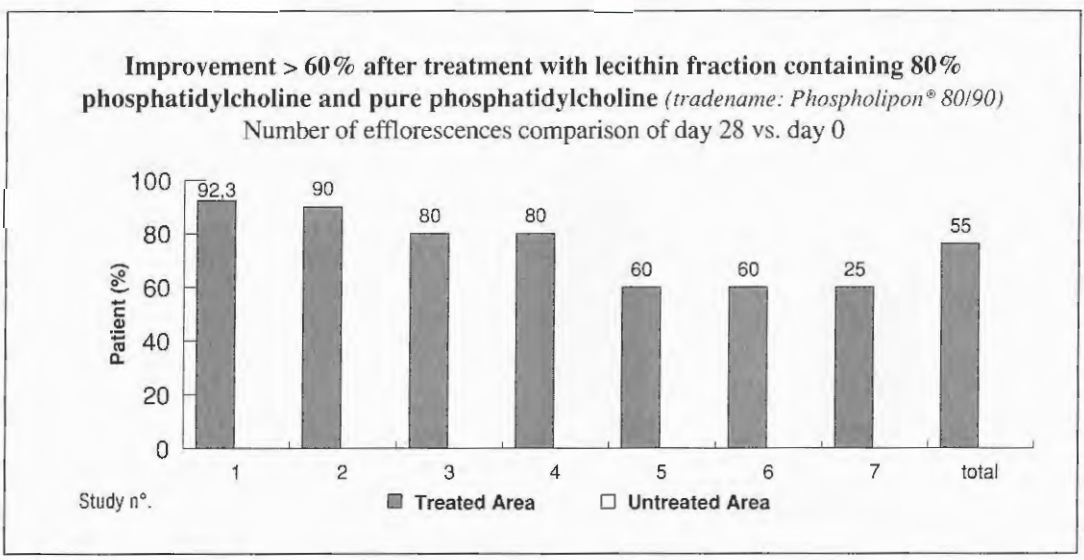


Figure 5: Improvement > 60% with regard to number of efflorescences.

droxyoctadecadienoic acid (13-HODE), a substance with antiproliferative properties, that also inhibits the production of the inflammation mediator LTB_4.
The literature contains no descriptions of the successful treatment of acne by linoleic acid or by derivatives.
Phosphatidylcholine from vegetable sources, especially from soybeans, contains bound fatty acids, of which 70% is linoleic acid. Phosphatidylcholine can be formulated in water to liposomes with a particle size of 0.15 to 0.25 µm without the need for additional substances. These particles may readily pass the sebaceous duct and transport the linoleic acid into the sebaceous gland. The omission of superfluous chemicals in the formulation is beneficial since any chemical substance used as a formulation additive can have adverse effects on skin which is irritated or inflamed as it is in acne.
Seven studies, on 77 subjects, affected by physiological acne, were carried out on the basis of this rationale. Pure phosphatidylcholine and a fraction with 80% phosphatidylcholine, were tested in two different formulations, as liposome with 10% and 20% phospholipids respectively. Formulation containing the same phosphatidylcholine but no water (these are non-liposomal solution) were also investigated.
An average reduction of the comedones by 65% and a reduction of the efflorescences by 75% was recorded for all formulations tested.
In an independent study, the alteration of the skin lipids as the result of treatment by liposome containing 10% lecithin fraction with 80% phosphatidylcholine (tradename: Natipide® 08010A) was studied. A concomitant increase of the linoleic acid content and decrease of squalene content was observed in the course of the treatment. Since squalene is one of the most important indicators for greasy and acne-affected skin, and since the increased sebum production is a primary factor in the pathogenesis of acne, these findings are relevant for the use of phosphatidylcholine; with high content of esterified linoleic acid, thus, suitable for use as a drug substance and as an active ingredient for cosmetics.
These results prove that phosphatidylcholine, purified from soybeans, constitutes a new therapeutic principle for the treatment of acne [19].
The mode of action suggests the use of soybean-derived phosphatidylcholine
> in physiological acne, which is 70% of the affected persons, as a drug or as an active ingredient in cosmetics,
> or in acne needing medical attention, with the combination of phosphatidylcholine and a drug substance with a complementary mode of action.
The results also suggest its application for normalizing greasy skin, using cosmetics to apply adequate concentrations of phosphatidylcholine in topical formulations.

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