EVALUATION OF *P. ACNES* LIPASE ACTIVITY BY HPTLC: EVIDENCE OF AN ANTIBACTERIAL CREAM EFFICIENCY IN A CLINICAL STUDY

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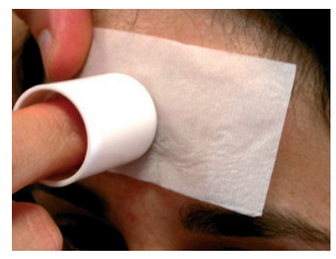
Introduction

The Skin surface is normally protected by sebum, a hydro-lipidic film secreted at the surface. Propionibacterium acnes *(P. acnes*) is involved in the acne pathology[1]. This anaerobic bacteria produces bacterial lipases converting deep triglycerids (TG) into free fatty acids (FFA)[2]. The produced sebum moves into the follicle up to the surface.

We have established a new method to quantify free fatty acids and triglycerids in the sebum which allows the evaluation of the *P. acnes* lipase activity. Lipids from the sebum are collected by a non-invasive method and separated by HPTLC just before densitometric quantification. The AG/TG ratio can give us information on sebum composition and skin colonization by *P. acnes*[3]. A variation of this AG/TG ratio was used successfully to evaluate in vivo antibacterial properties of a new dermatological formulation on acneic patients.

Materials and Methods

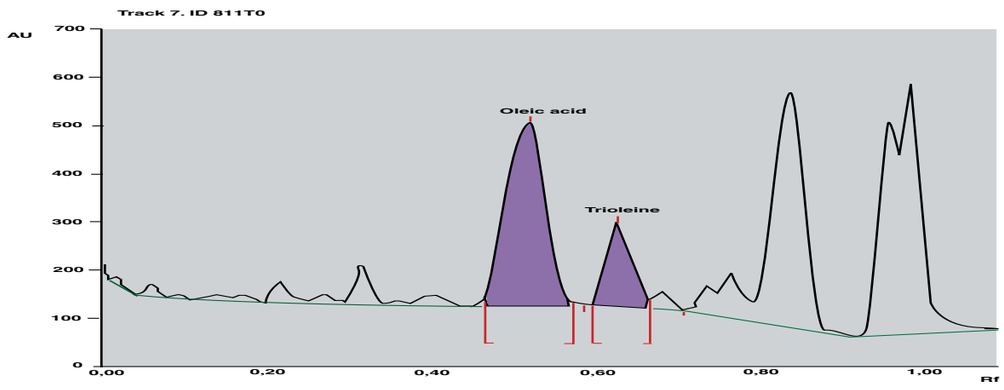
Lipolysis proof of FFA from TG : was made by adding a recombinant lipase of *P. acnes* in a solution of TG in a weak acidic buffer for 2 hours.
The standards, the blank without lipase and the incubation medium were spotted on a thin TLC plate, separated and sprayed to reveal the different lipids.

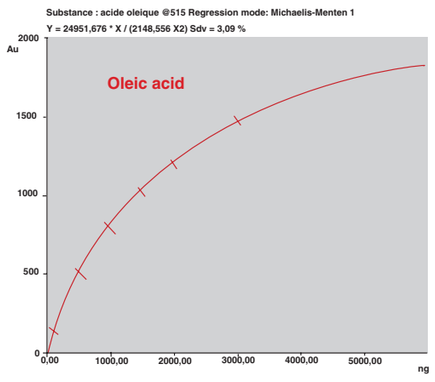


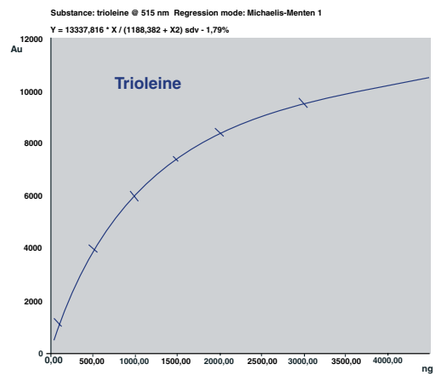
For the clinical study : the sebum was collected on the foreheads of 31 young volunteers, before and after 28 days of topical application of a new cream formulation, by soaking the cigarette paper with sebum. The different lipids were extracted from each paper with ethyl acetate and evaporated on N_2 before being dissolved in the same small volume of ethyl acetate just before analysis on HPTLC.
A six point standard mixture of trioleine and oleic acid was prepared and applied with the samples on HPTLC Plates silica gel 60 F_{254} with Automatic Sampler 3. The separation was achieved in 2 hours on AMD-2 with an 11 step gradient based on ethyl acetate and hexane with acetic acid pre-conditioning[4]. Derivatization was performed by spraying a 10% aqueous copper sulfate solution and heating at 180°C until brown coloration of the lipids. Densitometric evaluation was done with the TLC Scanner 3 by measurement at 515 nm and evaluation of lipid quantity with the calibration curves obtained by a Michaëlis-Menten regression (or kinetic).
Lipolysis of FFA from TG was evaluated by the yield of TG and FFA for each patient and compared between Day 0 and Day 28 results.

Results

Triglycerids lipolysis by *P. Acnes* lipase: After 2 hours incubation of TG with the recombinant lipase of *P. acnes* in an acidic buffer near the skin pH, we observed the hydrolysis of triglycerids to free fatty acids and Diglycerids (DiG). No spontaneous hydrolysis took place with the buffer only in the same conditions (**Fig. 1**).
Track profile and Calibration curves : The separation of FFA and TG on HPTLC plates allowed integration of them separately with an Rf. of 0.5 and 0.65 respectively. The six calibrated levels of FFA and TG give a good correlation with Michaëlis-Menten equation.







HPTLC analysis of skin surface lipids: After TG and FFA quantification, we calculated the TG/FFA ratio for each time of treatment and for each voluntareer, the total analysis is reported in the Fig. 3. Even if the ratios are very different for all the volunteers (from 0.03 to 4.9), the treatment effect was positive for the majority of them (27 from 31). We could consider that only the intra-individual differences are significant of the anti-bacterial effect. The average values give a highly significant (*p= 0.0001, Wilcoxon test*) 2-fold decrease between the starting date (R=1.05) and the end of treatment (R=0.50) due to the anti-bacterial effect of the cream.

Fig. 1 – Lypolysis by recombinant lipase of *P. Acnes*

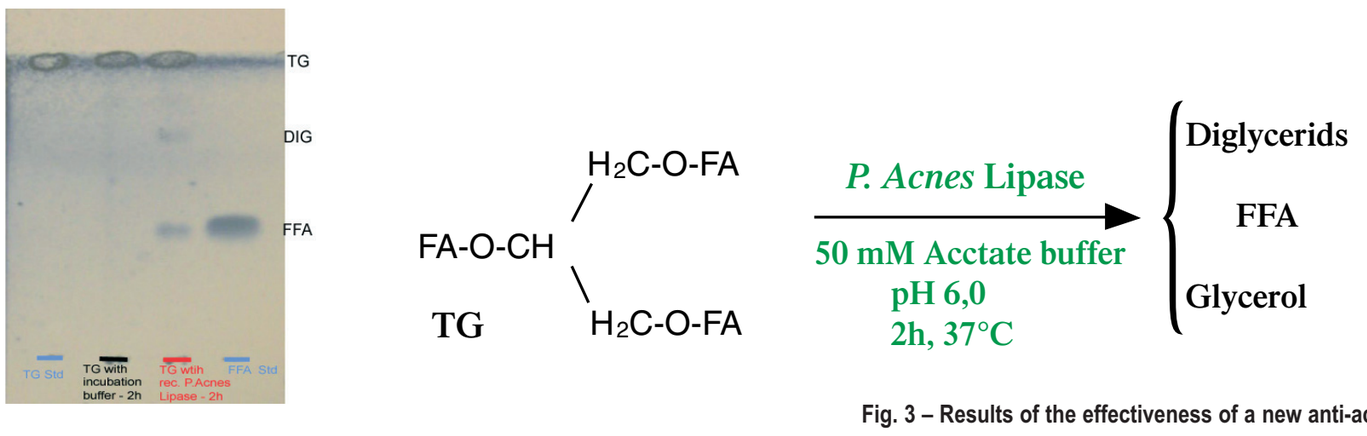


Fig. 2 – HPTLC plate of the skin surface lipids extracts

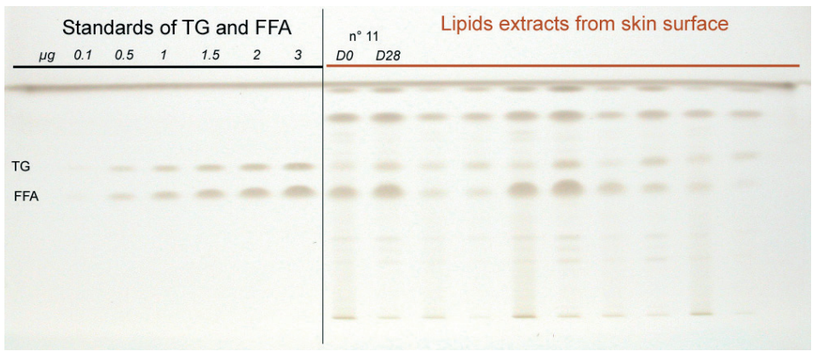
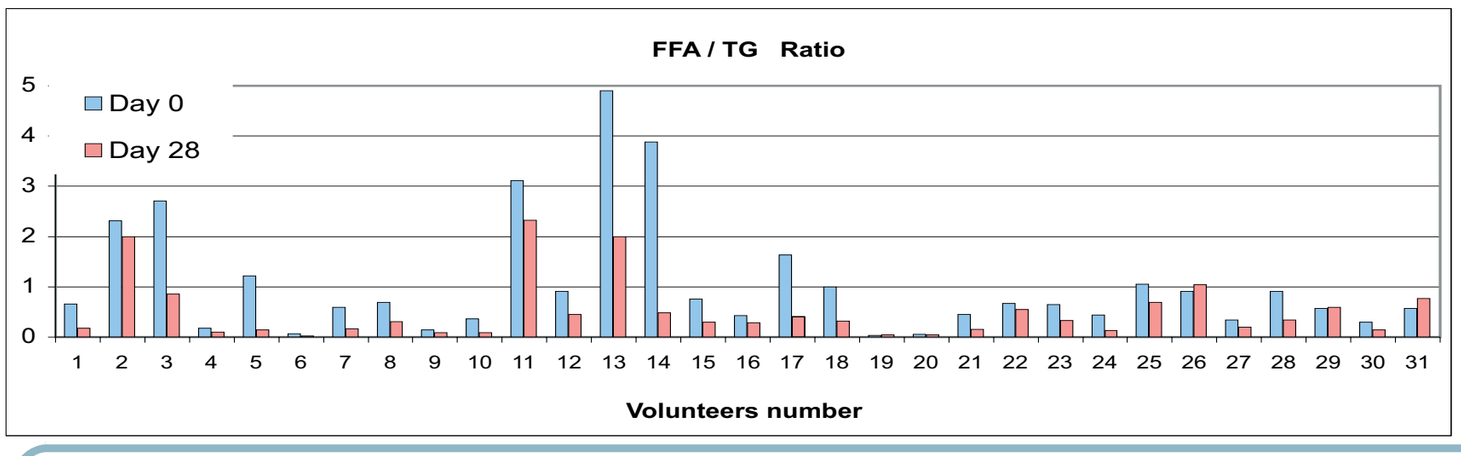
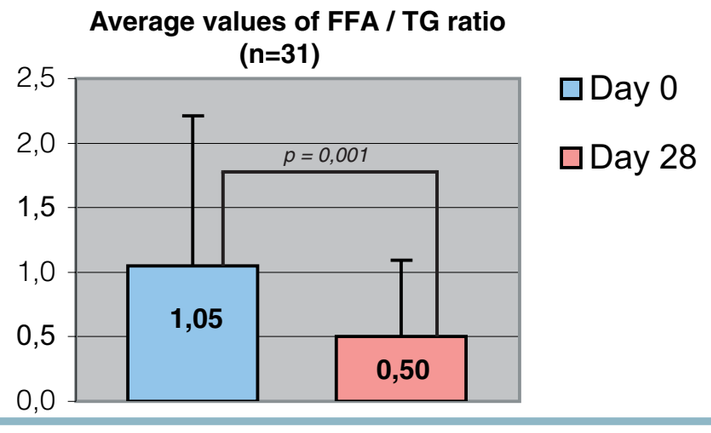


Fig. 3 – Results of the effectiveness of a new anti-acneic cream formulation





Conclusion

This study, conducted on 31 young volunteers suffering from acne, shows the benefits of a new cream formulation. After one month of topical application, we observed a significant decrease in the free fatty acid proportions combined with an increase in triglycerids, supporting the hypothesis of reduction of lipolysis due to anti-microbial activity. The use of cigarette paper collection and HPTLC analysis enabled a simple, rapid and non-invasive triglycerid and free fatty acid analysis of forehead surface lipids and a good monitoring of drug effects on microbial colonization.

References

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