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In vivo monitoring of topical therapy for acne with reflectance confocal microscopy

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Background: Acne vulgaris is a common disease of the pilosebaceous unit. The aim of the study was to evaluate compartment-specific treatment action through the microscopic non-invasive imaging of skin changes.
Methods: Mild-moderate acne patients, that were prescribed a topical anti-acne product, were followed by clinical and reflectance confocal microscopy (RCM) imaging every 14 days to 6 weeks. Mean and standard deviation of the scores were analyzed for each time point.
Results: After 2 weeks, the RCM count of papules/pustules and the RCM scores of exocytosis and dermal inflammation, decreased substantially. After 4 weeks, the RCM number of comedos was reduced. After 6 weeks, the number of regular follicles increased, while the infundibula with thickened bright border decreased significantly.
Conclusion: The progressive reduction in the clinical scores was correlated with the improvement of the RCM parameters. RCM study of acne skin showed a different timing for inflammatory and hyperkeratotic components to achieve a significant reduction during topical therapy with the association of retinoid and antibacterial molecules. The microscopic changes observed showed the regularization of the skin and the improvement of acne related features. RCM may represent a useful tool for the objective assessment of treatment efficacy and individual response evaluation.

Key words: acne – reflectance confocal microscopy – topical therapy – noninvasive

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ACNE VULGARIS is a common disease of the pilosebaceous unit. It primarily affects adolescents and presents a pleomorphic clinical presentation. Alteration of the keratinization process in the infundibulum, increased and altered sebum production, follicular colonization by Propionibacterium Acnes and inflammatory mediators released into the skin are the main drivers of the pathologic process, and represent the key targets for the treatment (1). The choice of topical and systemic therapy is guided by the clinical symptoms and the severity of the disease. Different therapeutic approaches are available today for the treatment of acne (2–4). Mild to moderate forms (comedonal acne, papulopustular acne) are usually treated with topical products, including comedolytic agents, retinoids, anti-inflammatory medications, and antibiotics (2). The use of fixed-dose combination where a topical retinoid is associated to an antimicrobial molecule is part of the first-line treatment for mild-moderate acne, as it is recommended by the current European evidence-based guidelines (2).

Treatment effectiveness and mechanism are usually studied through clinical assessment and laboratory evaluations (5–8). The microscopic changes occurring during the treatment to the lesions and the surrounding skin are difficult to be evaluated due to the need of repeated biopsies on a cosmetic sensitive area. Today, the introduction of reflectance confocal microscopy (RCM) for the study of skin diseases offers the possibility to obtain optical biopsies of the skin at cellular level resolution with no tissue damage or alteration (9–12). Furthermore, RCM allows repeating the imaging over time on the same area due to its non-invasiveness (13,14). This technology showed the possibility to identify *in vivo* the main histopathologic aspects of

the different acne lesions and the subclinical alterations evident in apparently healthy skin of acne patients, consisting of microcomedos and infundibular hyperkeratinization and accumulation of keratotic and sebaceous material (15).

The purpose of this study was to evaluate compartment-specific treatment mechanism and action through the sequential documentation of the microscopic changes induced on the skin by a topical bench-market combination of retinoid and antibacterial molecule on mild-moderate acne vulgaris of the face.

Materials and Methods

The protocol represents an observational study for the dynamic *in vivo* evaluation of microscopic changes induced by the topical treatment. The study was conducted in accordance with the ethical principles originating from the Declaration of Helsinki and Good Clinical Practices and in compliance with local regulatory requirements. The study was reviewed and approved by an institutional review board.

Study population

Patients with mild-to-moderate acne of the face, defined by the presence of less than 30 inflammatory lesions on the face or body, without any nodules or cysts, were considered eligible in case of prescription of a topical product containing a combination of retinoid and antimicrobial agent for a treatment duration of 6 weeks. Patients who had received any topical or oral medication for acne vulgaris in the last 1 month, subjects who had been previously diagnosed with an endocrinologic disorder, subjects who used any systemic medications or that had any facial procedure were excluded from the study. Patients were asked to return for a clinical control and documentation every 2 weeks up to the end of the treatment.

Instrument and image acquisition

According with clinical practice at our Department, clinical imaging and RCM imaging is acquired at baseline and at each next monitoring visit to evaluate the effectiveness of the treatment. Clinical and instrumental imaging was carried at baseline, after 2 weeks (±2 days), after 4 weeks (±2 days), and after 6 weeks (±2 days).

A target area of 4 × 4 mm on the cheek or forehead was selected at baseline in proximity of the largest acne lesion present. The target area of 4 × 4 mm was reported on a transparent plastic film that was modeled on the face of the patient. The plastic film was employed during the following visits for the precise follow-up of the same area.

A clinical digital photography of the full face and one of the target area were acquired by means of a digital camera (Canfield Nikon D90 Digital SLR® and Canfield Close-up Scale®; Canfield Imaging Systems, Fairfield, NJ, USA).

Measurements

All the evaluations were performed in blind from the clinical and RCM images retrieved from the database after blinding the patient's data and visit, and randomization of the sequence. Clinical evaluations included physician global assessment evaluations (PhGA), the total number of acne lesions (TLC) and the number of inflammatory acne lesions (i-TLC).

Reflectance confocal microscopy mosaics were evaluated by two expert confocalists. RCM evaluations were conducted in accordance to the findings of the previous RCM study on acne, focusing in particular on the count of hair follicles presenting normal infundibula, the number of infundibula presenting a thickened bright border, the number of dilated infundibula/comedos (>150 μm), corresponding to roundish structures presenting bright thick hyper-reflecting border (usually in an onion-like fashion) and amorphous material content, and the number of inflammatory lesions (papules and pustules), corresponding to large roundish areas with ill-defined borders, containing bright particles, inflammatory cells and/or bright compact organized amorphous material, and surrounded by abundant inflammatory infiltrate at the periphery of the lesion (15).

Statistics

Mean (M) and standard deviation (SD) of the clinical scores and RCM lesions count were calculated at each time point. Student's *t*-test was calculated (SPSS Inc., Chicago, IL, USA) to compare the changes during time. A *P*-value less than 0.05 was considered significant.

Results

Of 26 eligible patients, a total of 20 patients (four males and 16 females median age 23.4 years, range 14–26 years old) were enrolled, because of prescription of the same topical product containing as active substances Hydroxypinacolone Retinoate/BIOPEP 15 (Biretix Ultra®, Difa Cooper S.P.A., Caronno P.lla, Varese, Italy). Three other different topical products containing a combination of retinoids and antibiotics were prescribed to the other six patients. For statistical reasons, only the homogeneous sample of patients receiving Biretix Ultra® has been included in order to avoid a three-way interaction and preserve data consistency.

The study was completed and analysis performed on 19 patients, being one dropped out as she did not come to the scheduled control visits.

No drug-related side effects have been reported, except a mild irritation of the skin in three patients not requiring any treatment, suspension or discontinuation of the medication.

Mean and SD of the measured parameters are listed in Table 1. At baseline, the average PhGA, TLC and i-TLC were indicative of a mild to moderate acne. RCM evaluations showed an average of three acne lesions and 48 hair follicles detectable per 4 × 4 mm target areas, approximately one-third of which presenting RCM alterations (i.e. Infundibula with thickened bright border). After 2 weeks of treatment, the average number of papules and pustules decreased from 0.75 to 0.55 ($P = 0.04$), while the number of comedos, regular follicles and bright infundibula remained constant. At the same time, exocytosis and dermal inflammation was reduced from a mean score of 0.8–0.5 ($P = 0.05$). After 4 weeks of treatment, the number of comedos was reduced from 2.2 to 0.9 ($P = 0.001$), and then remained constant up to the end of the study. After 6 weeks of treatment RCM scores also significantly improved for the number of regular follicles, increased from an average of 29.3–46.9 ($P = 0.001$), and of the infundibula with thickened bright border, decreased from 19.9 to 6.8 ($P = 0.001$).

Discussion

In this study, RCM was employed for the evaluation of the effect of topical product containing as active substances retinoid and antimicrobial agent. The product was applied once daily on acne lesions and apparently healthy skin. RCM gave us the possibility to repeat the imaging over time on the same area due to its non-invasiveness, thus permitting not only to monitor the evolution of the clinically visible lesions but also to identify the changes occurring on the surrounding skin. The study of acne affected skin area of 4 × 4 mm by means of RCM Vivascope 1500® allowed the identification and quantitation of pilosebaceous infundibular alterations occurring in acneic skin. The presence of the infundibula with hyper-reflecting border, probably related to the altered keratinization, has been already identified as a characteristic pattern of the apparently healthy skin in acne patients (12). The precise counting of the number of the different type of lesions in accordance to their microscopic characteristics and of the number of

TABLE 1. Mean and SD of the measured parameters during time

	Baseline, mean (SD)	After 2 weeks ± 2 days, mean (SD)	After 4 weeks ± 2 days, mean (SD)	After 6 weeks ± 2 days, mean (SD)
Clinical assessment				
PhGA	26.8 (16.4)	25.7 (10.5)	16.8 (10.7)*	4.6 (4.9)*
TLC	23.3 (8.57)	19.2 (9.6)	19.1 (8.9)	12.1 (10.6)*
i-TLC	14.9 (10.1)	12.3 (8.3)	9.3 (7.2)*	4.9 (4.8)*
RCM count of follicular structures				
Regular follicles and infundibula	29.3 (5.4)	30.3 (5.1)	32.8 (5.6)	46.9 (8.1)*
Infundibula with thickened bright border	19.9 (9.1)	19.1 (8.6)	14.6 (5.1)	6.8 (3.4)*
RCM count of acne lesions				
Dilated infundibula/comedos	2.2 (1.3)	1.9 (1.1)	0.9 (0.8)*	0.9 (0.7)*
Inflammatory papules and pustoles	0.75 (0.9)	0.55 (0.8)*	0.2 (0.4)*	0.1 (0.3)*
RCM inflammation				
Exocytosis and dermal inflammation (0–2)	0.8 (0.87)	0.5 (0.60)*	0.35 (0.49)*	0.29 (0.46)*

*Significant compared to baseline ($P < 0.05$).

PhGA, physician global assessment evaluations; TLC, total number of acne lesions; i-TLC, number of inflammatory acne lesions; RCM, reflectance confocal microscopy.

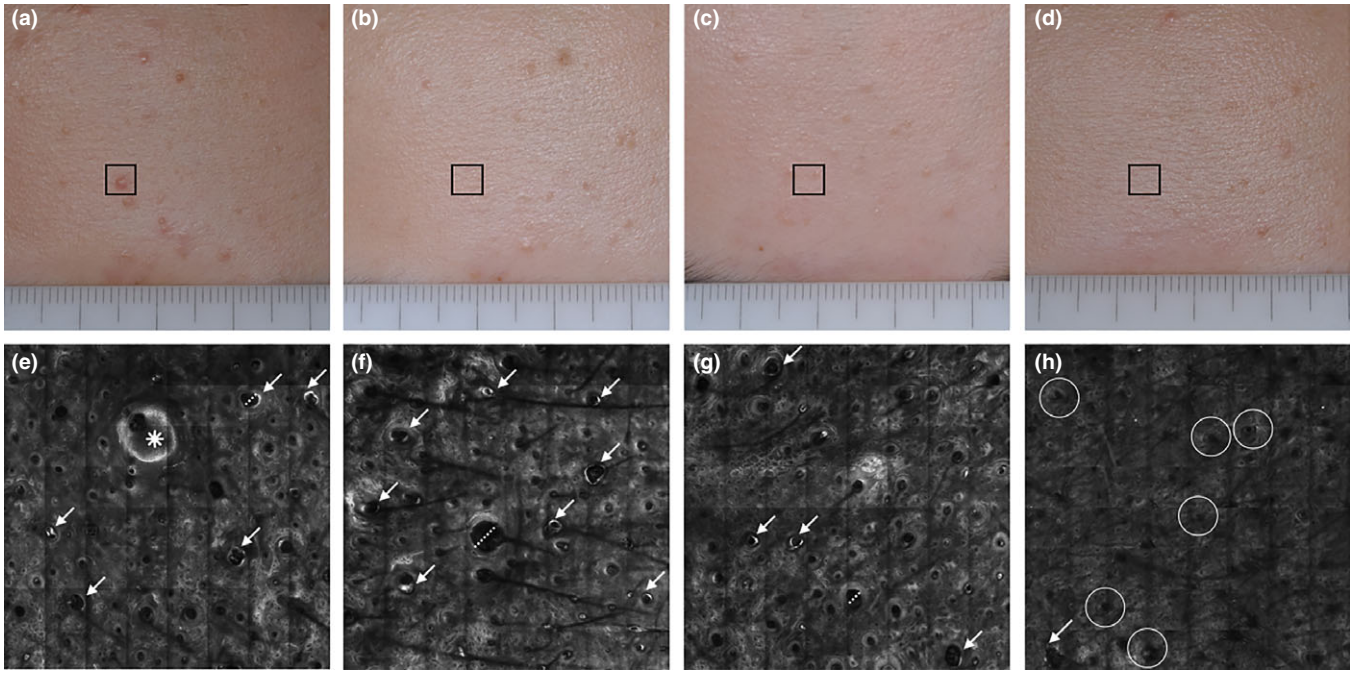

Fig. 1. Clinical and RCM image of the treatment area (4 × 4 mm) time-course, acquired at an average depth of 65 μm from the skin surface. Baseline clinical image (a) showing presence of comedos and papular-pustolar lesions and also visible upon RCM (asterisk) (e). After 2 weeks a slight reduction in lesion number is visible (b). RCM is showing persistence of dilated infundibula (dotted lines) and infundibula with thickened bright border (arrows) (f). After 4 weeks, the clinical aspect is markedly improved (c). Corresponding RCM images displays the reduction in the overall number of acne lesions as well as the reduction in the infundibula with thickened bright border (g). After 6 weeks of treatment, a further clinical improvement is achieved (d). RCM showed the almost complete disappearance of acne lesions and of the hyperkeratotic follicular borders, counterbalanced by the increase in the number of regular infundibula (white circles) with normal reflecting border (h).

infundibula with hyper-reflecting border, on the total including the ones with normal reflecting contour, permitted to quantitatively define the microscopic changes induced by the topical treatment. Overall, the progressive reduction in the clinical scores of acne severity (significant after 4 weeks of treatment) was correlated with the improvement of all the RCM parameters associated with acne (Fig. 1). RCM study of skin pathophysiology showed a different timing for inflammatory and hyperkeratotic components of acne skin to achieve a significant reduction with respect of pretreatment values. In fact, just after 2 weeks of therapy the inflammatory RCM parameters, such as inflammatory lesions (papule and pustules) and exocytosis/dermal inflammation, were significantly reduced, whereas the number of comedos and infundibula with hyper-reflecting borders, characterized by accumulation of keratin, improved in 6–8 weeks, in parallel with an increased number of normal follicles.

Infundibula hyper-keratinization, detectable by RCM as bright circles surrounding the pilosebaceous follicles, may represent the early event leading to microcomedo development. According with Cunliffe et al., in fact, the hyper-proliferation of ductal keratinocytes and alterations of their physiologic turnover and desquamation induced by hormonal/growth factor, lead to the accumulation of corneocytes inside the pilosebaceous duct causing ductal obstruction, responsible to initiate the events that induce the comedo development (16).

At the end of the study period, the reduction in the RCM number of Papules, Pustules, Comedones and infundibula with hyper-reflecting border was counterbalanced by the increase in the number of regular infundibula with normal reflecting border. In particular, the ratio between the number of normal infundibula and the number of hyper-reflecting infundibula had an approximately fourfold increase (1.47 at baseline to 6.93 after 6 weeks of therapy). From these data, we can suggest that the use of a topical product containing antimicrobial agents and retinoid exert its action on the two separate components of the acne lesions with different time-course. The former rapidly act on the inflammatory

components, which result diminished early after only 2 weeks of treatment, whereas the retinoid slowly affects the hyperkeratotic component, with a visible effect on the infundibula structure only after 6 weeks. This different pace, with visible benefits on the skin redness and papules and pustules, can induce a lower adherence to the prescription, not enabling to the other substances to effectively act on the background pathogenic phenomena of acne, such as the infundibular hyperkeratosis, with early recurrences and patient's unsatisfaction as a consequence. On the other hand, it is important to stress the relevance to achieve the normalization of the infundibular structures to avoid or delay the formation of new lesions, keeping a long-term efficacy thorough topical product application, which may be beneficial also as co-adjuvant in systemic treatments.

The *in vivo* microscopic study of topical drug dynamics may enable to increase the knowledge on the microscopic phenomena occurring on the skin during the treatment, giving information on the correct modality for therapy prescription. Further studies on larger series and comparing different products are required to confirm these preliminary data. However RCM may represent a useful tool for an objective evaluation of acne treatment efficacy, mechanism of action and time-course.

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None.

Conflicts of interest

The authors have no conflict of interest to declare.

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