ORIGINAL RESEARCH ARTICLE

Am J Clin Dermatol 2011; 12 (2): 133-141
1175-0561/11/0002-0133/$49.95/0

Resveratrol-Containing Gel for the Treatment of Acne Vulgaris

A Single-Blind, Vehicle-Controlled, Pilot Study

Gabriella Fabbrocini, Stefania Staibano, Giuseppe De Rosa, Valeria Battimiello, Nunzio Fardella, Gennaro Ilardi, Maria Immacolata La Rotonda, Amelia Longobardi, Marialuisa Mazzella, Maria Siano, Francesco Pastore, Valerio De Vita, Maria Luisa Vecchione and *Fabio Ayala*

Department of Systematic Pathology, Division of Clinical Dermatology, University of Naples Federico II, Naples, Italy

Abstract

Background: Acne vulgaris is a complex, chronic, and common skin disorder of pilosebaceous units. The major pathogenic factors involved are ductal hyperkeratinization, obstruction of sebaceous follicles resulting from abnormal keratinization of the infundibular epithelium, stimulation of sebaceous gland secretion by androgens, and microbial colonization of pilosebaceous units by *Propionibacterium acnes*, which promotes perifollicular inflammation.

Aim: The aim of the study was to investigate the therapeutic effects of resveratrol, a natural phytoalexin produced by some spermatophytes, such as grapes and other plants, on acneic skin.

Methods: Resveratrol was incorporated in a carboxymethylcellulose-based gel. The chemical stability of resveratrol after storage at 4°C for 30 days was investigated by high-performance liquid chromatography (HPLC). The resveratrol-containing hydrogel was administered to 20 patients affected by acne vulgaris enrolled in this single-blind study. The resveratrol-containing formulation was applied daily as a solo treatment on the right side of the face for 60 days, while the hydrogel vehicle was applied to the left side of the face as a control. To objectively evaluate the results, a digital photographic database was used to collect images. The number and type of lesions were recorded for each patient, to compare the Global Acne Grading System (GAGS) score before treatment with that obtained at the end of the study. Moreover, with the innovative technique of follicular biopsy, areas of acneic skin were prepared for histopathology. The average area occupied by microcomedones at baseline was compared with that at the end of treatment.

Results: HPLC analysis demonstrated that resveratrol, upon incorporation into the gel, did not convert to its *cis*-isomer when stored at 4°C for 30 days. All patients were satisfied with the active treatment and none experienced adverse effects. Clinical evaluation showed a 53.75% mean reduction in the GAGS score on the resveratrol-treated sides of the face compared with 6.10% on the vehicle-treated sides of the face. These data were supported by histologic analysis, which showed a 66.7% mean reduction in the average area of microcomedones on the resveratrol-treated sides of the face. The comparison with the vehicle-treated side of the face (9.7% reduction) showed a clinically relevant and statistically significant decrease of lesions in areas treated with resveratrol-containing hydrogel.

Conclusion: This pilot study showed positive results for resveratrol gel in acne, and should be considered a valid starting point for further testing of the effectiveness of this molecule in different concentrations and formulations and in a larger group of patients.

Background

Acne vulgaris is a complex, chronic, and common skin disorder of pilosebaceous units that occurs predominantly on the skin of the face, the upper back, and the upper chest. This disease usually begins at the time of the sharp increase in androgen production that occurs during adolescence, but may affect individuals of any age.[1] In recent years, the multifactorial nature of acne has been elucidated but much remains to be learned. The major pathogenic factors involved are ductal

hyperkeratinization, obstruction of sebaceous follicles resulting from abnormal keratinization of the infundibular epithelium, stimulation of sebaceous gland secretion by androgens, and microbial colonization of pilosebaceous units by *Propionibacterium acnes*, which promotes perifollicular inflammation. Acne begins when the pilosebaceous ducts become plugged with keratinocytes to form comedones, sebum builds up and distends the follicles, and the anerobe *P. acnes* proliferates in the sebum. If the comedo ruptures into the dermis, inflammation results and a pustule or papule forms.[2]

Currently, inflammatory acne is treated with antibacterials (both oral and topical) or oral isotretinoin. Although widely used and often effective, antibacterials have a significant limitation, which is the development of resistant strains of *P. acnes*. Oral isotretinoin is by far the most effective treatment for severe inflammatory acne, routinely delivering dramatic improvements.[3-6] However, because of its established teratogenicity, isotretinoin use has been restricted. Furthermore, the recently publicized, controversial risk of depression/suicidal ideation has made isotretinoin use even more problematic.[7,8] Current topical treatment options for inflammatory acne are suboptimal. Rational development of new treatments for acne is based on a molecular description of the mechanisms involved in acne.

Overstimulation of the initiation of the preclinical inflammatory process or defective negative feedback regulation may be major reasons for the interruption of the normal cycling of the sebaceous follicle and be responsible for the initiation of the clinical inflammatory process in acne. Hereditary factors and excess androgen activity, for example, in puberty, may cause overstimulation, thus triggering sterile inflammatory phenomena. Neuroendocrinologic regulation and environmental factors, such as dietary lipids and smoking, have also been suggested to represent trigger mechanisms.[9] Hyperproliferation of the follicular epithelium leads to formation of the first acne lesions that can be found in normal-looking skin, the microcomedones (comedones with an area between 0.016 and 0.042 mm^2), characterized by the accumulation of abnormally desquamated corneocytes and excess sebum[10] and which are believed to be the precursor lesions of acne. The microcomedones may evolve into clinically visible comedones (blackheads and whiteheads) or inflammatory lesions (papules, pustules, or cysts). The very early stage of acne lesion development is associated with vascular endothelial cell activation and involvement of inflammatory events,[11] which corroborates the suggestion that acne may represent a genuine inflammatory disorder without involvement of bacteria in its initiation.

Recently, it has been demonstrated that, in inflammatory acne lesions, there is activation of nuclear factor-κB signaling (a transcription factor critical for upregulation of many proinflammatory cytokine genes). As a result, inflammatory cytokine genes (e.g. tumor necrosis factor [TNF]-α and interleukin [IL]-1ß) are activated. These primary cytokines cause inflammation by acting on endothelial cells to prepare adhesion molecules (e.g. intercellular adhesion molecule-1) to facilitate recruitment of all inflammatory cells into the skin. TNFα and IL-1ß also help to stimulate the proliferation of secondary cytokines, such as IL-8, which can cooperate in the movement of the inflammatory cells towards the increased concentration of these particular chemicals.[12]

P. acnes seems to be involved in both the initiation of acne lesions as a precursor of inflammation and in later events. *P. acnes* is a Gram-positive, non-motile rod, non-spore-forming, anerobic bacillus. As it is a member of the normal flora and is a harmless commensal, it is largely incapable of tissue invasion or serious infection. The organism metabolizes sebaceous triglycerides, consuming the glycerol fraction and discarding free fatty acids. As a consequence of *P. acnes* proliferation and metabolism in the obstructed follicles, *P. acnes* produces neutrophil chemoattractants. *P. acnes* also activates the complement system and is generally inflammatory when brought into contact with the immune system.[13] It has been shown that *P. acnes*, through activation of toll-like receptor 2 expressed in human monocytes, induces chemokine/cytokine synthesis in these cells.[14] These findings in combination with the expression of active toll-like receptors 2 and 4 and of CD14 in human keratinocytes have implicated *P. acnes* and toll-like receptors in acne inflammation. Recently, it has been shown that keratinocytes secrete selective human β-defensin-2 (hBD2) and IL-8 in response to *P. acnes* and that these molecules are potent chemotactic factors for leukocyte and neutrophil infiltration as well as for keratinocyte growth potential and differentiation at the site of *P. acnes* infections. This evidence shows that *P. acnes* is important for maintaining the inflammation process in acne.[15]

Because of the pathophysiology of the disease, the ideal treatment would be a single drug capable of suppressing the inflammatory response and also inhibiting keratinocytes and *P. acnes* proliferation. A potential candidate to fulfill these criteria is the phytoalexin resveratrol (3,4,5-trihydroxy-*trans*-stilbene). Resveratrol is a natural compound that exists in nature as *cis*- and *trans*-isomers. Most of the recorded health benefits are attributed to the *trans*-isomer.[16] The aim of this pilot cosmetic study was to evaluate the effects of a new topical formulation of *trans*-resveratrol in patients affected by inflammatory acne vulgaris.

Materials and Methods

Patients

The study group consisted of 20 patients (12 men and 8 women) with facial acne vulgaris involving both sides of their faces with nearly symmetric appearance, as determined by clinical criteria. Their age at entry to the trial ranged from 18 to 23 years.

At the time the study started, patients had been in therapeutic wash-out for at least 4 weeks for both topical and systemic acne therapies. The exclusion criteria were a history of allergy, diabetes mellitus, and severe systemic disease.

Materials

Trans-resveratrol, sodium carboxymethylcellulose, and polysorbate 60 were purchased from Sigma-Aldrich Corp. (St Louis, MO, USA). Analytic grade 96% ethanol, and high-performance liquid chromatography (HPLC) grade acetonitrile, methanol, and glacial acetic acid were obtained from Carlo Erba Reagenti (Milan, Italy). Glycerol (glycerin) was purchased from Polichimica (Bologna, Italy).

Methods

Resveratrol Analysis

Trans-resveratrol analysis and detection of *cis*-resveratrol were carried out using an HPLC system by Shimadzu (Kyoto, Japan), consisting of a LC-10AD pump equipped with a Luna C18 (250×4.6 mm, 5 µm) column (Phenomenex, Torrance, CA, USA), a Rheodyne 7725i injection valve, and a SPV-10A UV-vis detector set at the wavelength of 303 nm. The system was controlled by a SCL-10A VP System Controller (Shimadzu) connected with a computer. Chromatograms were acquired and analyzed by a Class VP Client/Server 7.2.1 program (Shimadzu). The analysis was performed with a mobile phase of methanol/water (50 : 50) with 0.5% volume in volume acetic acid in isocratic conditions at a flow rate of 1 mL/min. *Cis*-resveratrol was prepared from the *trans*-isomer by sunlight irradiation for 3 days, as reported elsewhere.[16]

Preparation of the Resveratrol-Containing Gel

The gel was prepared by dissolving 5 g of sodium carboxymethylcellulose and 10 g of 85% glycerol in 85 g of deionized water under stirring. Separately, in the absence of light, resveratrol was dissolved in deionized water at a final concentration of 0.01% weight in volume. Then, 1 g of this aqueous solution was incorporated into 10 g of the vehicle gel under stirring in the absence of light. The vehicle gel and resveratrol-containing gel were stored at 4°C.

The stability of *trans*-resveratrol in the gel was evaluated soon after preparation and after different time frames of storage at 4°C. For each analysis, 0.5 g of the preparation was taken from the sample and resveratrol was extracted with 5 mL of methanol; then, the suspension was centrifuged at 4°C for 30 minutes at 60×*g* and the supernatant was analyzed by HPLC.

Cyanoacrylate Follicular Biopsy

Cyanoacrylate follicular biopsy, also known as skin surface biopsy, is a simple noninvasive method that removes only dead tissue and is used to study the stratum corneum, the many types of pathology within this compartment, and a vast array of microorganisms that may colonize or invade the layer.[17]

It is the most reliable tool to sample the follicular contents of facial skin[18] such as hair and keratin. In the acneic or nearly acneic skin, keratin material may be particularly abundant (microcomedones) prior to the appearance of clinically evident comedones (macrocomedones). Cyanoacrylate follicular biopsy was performed by distributing three drops of cyanoacrylate evenly on a slide holder, which was then applied with a slight pressure to the chosen area of the face. After about 3 minutes, the slide was removed, stripping an area of skin surface with a slow and undulating movement and being careful not to damage the stratum corneum sticker with its comedones.

Ematossilin-eosin was used to stain each sample and the micrometric scale of the Zeiss optical microscope was used to determine the density of microcomedones, i.e. the number present per mm^2, and the area occupied by each microcomedone. From these calculations, we then determined the average area of microcomedones. The parameter used to test the treatment was the average area occupied by microcomedones before and after treatment.

Resveratrol Protocol and Patient Monitoring

At baseline (T_0), patients underwent a careful dermatologic examination and received accurate information about the study. All patients signed the informed consent, which contained the study's description, aims, methods, and possible adverse effects. The study was submitted to a cosmeceutic committee, according to Italian cosmeceutic rules.

In order to carry out a comparative analysis, three digital photographs (front view, right side of the face, left side of the face) were taken and gathered in a database. The severity of the lesions was evaluated using the Global Acne Grading System (GAGS)[19] that considers six sites (five facial, one truncal) by giving each a specific numerical factor based on the surface area

Table I. Global Acne Grading System (GAGS)

Localization	Factor per grade (0–4)	Grade
I = forehead	2 × grade	0 = no lesions
II = right cheek	2 × grade	1 = ≥1 comedo
III = left cheek	2 × grade	2 = ≥1 papule
IV = nose	1 × grade	3 = ≥1 pustule
V = chin	1 × grade	4 = ≥1 nodule
VI = chest and back	3 × grade	

of each and the distribution and density of pilosebaceous units. This factor is multiplied by the known grade to give the value of the local score. The sum of the local scores gives the global score (table I).

At the end of the study, patients were asked to rate the clinical results on a 10-point satisfaction scale (1 = lowest to 10 = highest) including compliance, smell, texture, results, and quality of life.

Before the treatment was started, the GAGS score was calculated and all patients with a score between 8 and 24 were included in the study. In order to obtain an objective evaluation of the efficacy of the treatment, the number of acne lesions before beginning treatment and at the end of the study was recorded for each patient.

Moreover, to better define clinical results and to correctly evaluate the efficacy of the therapy, for each patient, two cyanoacrylate follicular biopsies (on the right and left sides of the face) were executed. Furthermore, digital photographs were captured while performing cyanoacrylate follicular biopsies: this guaranteed the reproducibility of the technique and better diagnostic accuracy.

The study was single blinded; each patient received two identical containers, marked by a letter of reference: 'C' for the formulation containing the vehicle gel and 'R' for the medicated formulation. Each patient was instructed to apply the gel in container R to the right side of the face, and the gel in container C to the left side once a day, preferably in the evening. Patients were told to store the gels in the refrigerator at a stable temperature.

All patients attended the first follow-up visit 1 month later (T_1) and each had three digital photographs taken. By analyzing digital photographs collected at T_0, two follicular biopsies were obtained from the same sites of each patient's face. Each patient returned the questionnaire that was given out during the first visit and since no patient had adverse effects, treatment was continued and the patients were asked to return for the last control check after a further 30 days.

Therefore, the second and final follow-up occurred 60 days (T_2) after the baseline visit, with the same evaluations repeated.

Statistical Analysis

The digital photographic data were analyzed using a test for nonparametric data (Wilcoxon signed-rank test for paired data). The null hypothesis (H_0) was that the median of the differences is zero (P+ = P–) and the alternative hypothesis (H_A) was that the median of the differences is negative (P+ < P–), $\alpha = 0.001$. The result is given by computing the binomial probability.

Results

The first step of the study was the development of an HPLC method to allow the detection of both *trans*- and *cis*-isomers of resveratrol. The chromatographic analysis of the aqueous solution containing *trans*-resveratrol gave a well defined peak with the retention time of 6.4 minutes. The sunlight irradiation of the *trans*-resveratrol solution for 3 days resulted in two distinct chromatographic peaks that were attributed, based on a previous report,[20] to *trans*- and *cis*-resveratrol, respectively (data not shown).

The second step of the study was the preparation of a dermatologic formulation containing resveratrol in which *trans*-resveratrol was incorporated at the final concentration of 1 μg/g of preparation. The stability of resveratrol in the preparation was evaluated soon after preparation and after 1, 10, and 30 days of storage at 4°C by HPLC analysis. Figure 1 shows the chromatogram obtained from the analysis of the resveratrol-containing gel after 30 days of storage. In this figure, only the chromatographic peak attributed to *trans*-resveratrol was observed, while there were no peaks at a higher retention time, indicating the absence of *cis*-resveratrol. The chromatographic peaks with a retention time between 2 and 4 minutes (figure 1) were not attributed to resveratrol or to chemical compounds derived from it, because they were also found upon analysis of the vehicle gel.

It is noteworthy that, in this study, in order to avoid interference with resveratrol activity on the skin, preservatives were

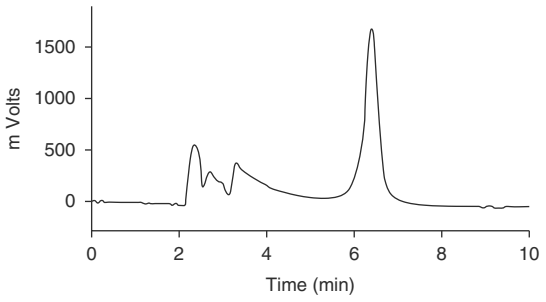

Fig. 1. Chromatogram from the analysis of the resveratrol-containing gel after storage at 4°C for 30 days.

Table II. Global Acne Grading System (GAGS) score before and after treatment with resveratrol and vehicle gels

Patient no.	Resveratrol-treated side			Vehicle-treated side		
	GAGS score before treatment	GAGS score after treatment	reduction (%)	GAGS score before treatment	GAGS score after treatment	reduction (%)
1	14	7	50	14	12	14
2	12	2	83	12	12	0
3	33	8	76	33	28	15
4	14	10	29	14	18	–28
5	16	12	25	16	14	12
6	9	6	33	9	15	–67
7	22	12	45	22	16	27
8	14	7	50	14	12	14
9	15	3	80	15	11	27
10	18	5	72	18	16	11
11	16	4	75	16	15	6
12	26	7	73	26	24	8
13	15	6	60	15	14	7
14	19	8	58	19	18	5
15	29	15	48	29	28	3
16	23	16	30	23	22	4
17	17	10	41	17	17	0
18	18	11	39	18	17	5
19	16	9	44	16	15	6
20	22	8	64	22	21	5
Mean	**18.40**	**8.30**	**53.75**	**18.40**	**17.25**	**6.10**

not added. Indeed, for time frames longer than 1 month, the presence of microbiologic contamination was sometimes observed. For this reason, although resveratrol transformation was not observed at the timepoints considered, the resveratrol-containing formulations were used for no more than 30 days, after which time new preparations were provided to the patients.

Data obtained using the GAGS at T_0, T_1, and T_2 showed an average 53.75% reduction in clinical lesions on the resveratrol-treated sides of the face compared with 6.10% on the vehicle-treated sides of the face (table II).

Data collected from digital photographs at times T_0, T_1, and T_2 and analyzed with the sign test for paired data ($p < 0.001$) showed that for the lesions on the resveratrol-treated side of the face the median of the differences in GAGS scores was negative, in contrast to what was observed on the vehicle-treated side of the face. Based on this result, it could be argued that the reduction in the degree of acne severity on the treated side of the face was statistically significant (figures 2 and 3).

The analysis of cyanoacrylate follicular biopsies showed a 66.7% mean decrease from baseline in the total area and a decrease in the density of microcomedones (area between 0.016 and 0.042 mm^2) on the resveratrol-treated side of the face, versus a 9.7% decrease on the vehicle-treated side of the face (table III). Furthermore, there was a total disappearance of macrocomedones in some patients (figures 4 and 5).

According to the patients' subjective self-evaluation, using the 10-point scale, the mean satisfaction rate was 8 for compliance, 9 for smell, 8 for texture, 8 for results, and 9 for quality of life.

Discussion

The etiology of acne is not yet fully clarified but it is widely accepted that its pathogenesis is multifactorial, with abnormal follicular differentiation and increased cornification, enhanced sebaceous gland activity and hyperseborrhea, and bacterial hypercolonization, as well as inflammation and immunologic host reactions being the major contributors.

New research into acne pathogenesis has identified several novel pharmacologic targets to interrupt the inflammatory

DOCKET ALARM

Explore Litigation Insights

Docket Alarm provides insights to develop a more informed litigation strategy and the peace of mind of knowing you're on top of things.

Real-Time Litigation Alerts

Keep your litigation team up-to-date with **real-time alerts** and advanced team management tools built for the enterprise, all while greatly reducing PACER spend.

Our comprehensive service means we can handle Federal, State, and Administrative courts across the country.

Advanced Docket Research

With over 230 million records, Docket Alarm's cloud-native docket research platform finds what other services can't. Coverage includes Federal, State, plus PTAB, TTAB, ITC and NLRB decisions, all in one place.

Identify arguments that have been successful in the past with full text, pinpoint searching. Link to case law cited within any court document via Fastcase.

Analytics At Your Fingertips

Learn what happened the last time a particular judge, opposing counsel or company faced cases similar to yours.

Advanced out-of-the-box PTAB and TTAB analytics are always at your fingertips.

API

Docket Alarm offers a powerful API (application programming interface) to developers that want to integrate case filings into their apps.

LAW FIRMS
Build custom dashboards for your attorneys and clients with live data direct from the court.

Automate many repetitive legal tasks like conflict checks, document management, and marketing.

FINANCIAL INSTITUTIONS
Litigation and bankruptcy checks for companies and debtors.

E-DISCOVERY AND LEGAL VENDORS
Sync your system to PACER to automate legal marketing.