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RESEARCH ARTICLE

Antimicrobial activity of topical agents against *Propionibacterium acnes*: an *in vitro* study of clinical isolates from a hospital in Shanghai, China

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Abstract **This study aimed to compare the antimicrobial activities of topical agents against *Propionibacterium acnes* isolated from patients admitted to a hospital in Shanghai, China. The minimal inhibitory concentrations of the cultured *P. acnes* were determined in accordance with the Clinical and Laboratory Standards Institute. Susceptibilities to clindamycin and erythromycin were compared in terms of gender, age, disease duration, previous treatment, and disease severity. A total of 69 *P. acnes* strains were isolated from 98 patients (70.41%). The susceptibility to triple antibiotic ointment (neomycin/bacitracin/polymyxin B) and bacitracin was 100%. The susceptibility to fusidic acid was 92.7%. The resistance rates to neomycin sulfate, erythromycin, and clindamycin were 11.7%, 49.3%, and 33.4%, respectively. The high resistance rate to clindamycin and erythromycin was significantly affected by gender, previous treatment, and disease severity rather than by age and disease duration. Topical antibiotics should not be used separately for long-term therapy to avoid multiresistance. The use of topical antibiotics should be determined by clinicians on the basis of clinical conditions.**

Keywords antimicrobial susceptibility/resistance; *Propionibacterium acnes*; topical antibiotics; *in vitro* study

Introduction

The pathogenesis of acne vulgaris is complex and thus poorly understood. Acne vulgaris is caused by the proliferation of intrafollicular *Propionibacterium acnes* [1]. This condition is treated with various antibiotics. For instance, topical antibiotics are used to inhibit *P. acnes* causing mild to moderate acne [2]. However, a significantly high percentage of *P. acnes* isolated from patients with acne is resistant to topical antibiotics, such as clindamycin and erythromycin, which are commonly administered drugs [3]. Therefore, topical antibiotics should not be recommended for use separately because of the risk of bacterial resistance and relatively slow onset of action [4].

Clindamycin or erythromycin combined with benzoyl peroxide may be preferred over clindamycin or erythromycin alone. Other topical antibiotics commonly used to treat acne vulgaris may be employed. Topical antibiotics, including triple antibiotic ointment (TAO; neomycin/bacitracin/polymyxin B) [5], fusidic acid (FA) cream [6], and mupirocin ointment [7], are also occasionally prescribed to treat acne vulgaris, but these ointments are not recommended. Their efficacies have been rarely compared. This study was conducted to compare the antimicrobial activities of various topical agents against *P. acnes* isolated clinically from patients admitted to a hospital in Shanghai, China.

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Materials and methods

Patients

We included 98 patients (36 females and 62 males; with a mean age of 21.32 ± 1.33 years (range: 14–36 years old)) with facial acne. The patients were recruited from the Department of Dermatology at Huashan Hospital of Fudan University between January 2015 and June 2015.

Laboratory studies

Culture and identification of P. acnes

We employed a randomized, open-label, single-center study in China. After facial acne lesions were cleaned with 75% ethanol, comedones, papules, and pustules were compressed with a comedone extractor. *P. acnes* isolates were inoculated in Brucella medium supplemented with defibrinated sheep blood and vitamin K and incubated in an anaerobic chamber (5% CO_2, 10% H_2, 85% N_2) for 48–72 h. After two purification cycles were completed, the cultured microorganisms were identified as *P. acnes* by using an API-20A system (bioMérieux, France).

Antibiotics

Mupirocin, fusidic acid, clindamycin, and erythromycin were purchased from Sigma (Sigma-Aldrich, USA). Neomycin sulfate, bacitracin, polymyxin B, and TAO were kindly provided by Zhejiang Reachall Pharmaceutical Co., Ltd. (Zhejiang, China). These antibiotics were dissolved in water.

Determination of susceptibility to antibiotics

The minimal inhibitory concentrations (MICs) of the eight antibiotics were determined on Brucella agar. Twelve different concentrations ranging from 128 mg/L to 0.06 mg/L with a 2-fold serial dilution were prepared. A standard inoculum of 10^5 colony-forming units per 1 μl was also prepared and delivered by a multipoint inoculator (Denley A40, Denley Ltd., Billingshurst, Sussex, UK). MICs were determined after the isolates were incubated in the anaerobic chamber at 35 °C for 48 h. The MIC of each antibiotic for each isolate was defined as the lowest concentration that does not yield growth. A standard strain of *P. acnes* (ATCC 6919) was used as a control sample.

The breakpoints used to define the susceptibility or resistance of anaerobes were determined in accordance with the recommendations of Clinical and Laboratory Standards Institute. Resistance to erythromycin and clindamycin was defined at a MIC of $\geqslant 8$ mg/L. According to the European Committee on Antimicrobial Susceptibility Testing, resistance to FA was defined at a MIC of $\geqslant 4$ mg/L. For mupirocin, the breakpoint was defined at a MIC of $\leqslant 8$ mg/L, and high-level resistance was established at a MIC of > 256 mg/L. For neomycin, the breakpoint was identified at a MIC of $\leqslant 10$ mg/L. For polymyxin B, the breakpoint was determined at a MIC of $\leqslant 2$ mg/L. For bacitracin, the breakpoint was set at $\leqslant 2$ mg/L. For TAO (3.5 or 5.0 mg neomycin, 5000 IU polymyxin B, and 400 U bacitracin/g), the susceptibility or resistance percentage was determined by the most active component, namely, neomycin, and the MIC of this antibiotic was used.

The susceptibility or resistance rates of clindamycin and erythromycin were further analyzed after the patients were stratified on the basis of gender, age, disease duration, previous treatment, and disease severity: (1) age, < 25 and $\geqslant 25$ years; (2) disease duration, < 2 and $\geqslant 2$ years; (3) previous treatment, a history of treatment with topical antibiotics or retinoids and systemic antibiotics or isotretinoin; and (4) disease severity, grades I to IV [8].

Statistical methods

Data were analyzed using SPSS version 16.0 for Windows. The effects of gender, age, and disease duration on the susceptibility rate for clindamycin or erythromycin was analyzed through one-way ANOVA. The effects of disease severity on the susceptibility rate were analyzed with Fisher's least significant difference test. $P < 0.05$ was considered statistically significant.

Results

Patients' demographics

Gram-positive *P. acnes* strains were isolated from 69 out of the 98 patients (70.41%). Of these 69 patients, 41 (59.42%) were males and 28 (40.58%) were females; 49 patients were younger than 25 years (20.00 ± 2.65 years) and 20 patients were older than 25 years (27.75 ± 3.09 years); 55 patients described a history of acne for less than 2 years (8.29 ± 6.13 months) and 14 patients reported a history of acne for more than 2 years (30.29 ± 7.28 months); and 39 patients were treated with topical antibiotics, systemic antibiotics, or retinoids and 30 patients did not receive any treatment before recruitment. According to Pillsbury criteria, acne was classified as grades I–IV in 5, 17, 26, and 21 patients, respectively.

MIC of the tested antibiotics to *P. acnes* strains isolated from facial acne

The susceptibility and resistance rates of topical antibiotics against *P. acnes* were determined (Table 1). At a breakpoint of $\leqslant 10$ mg/L, TAO susceptibility was 100% ($\leqslant 0.25$–8 mg/L MIC range). For bacitracin ($\leqslant 0.25$–2 mg/L MIC range), the susceptibility rate also reached 100%. The resistance rates were 11.7% for neomycin sulfate (1–16 mg/L MIC range), 49.3% for erythromycin ($\leqslant 0.06$ to > 128 mg/L MIC range), and 33.4% for clindamycin ($\leqslant 0.06$ to > 128 mg/L MIC range). In addition, 27.5% exhibited cross-resistance to clindamycin and erythromycin. The susceptibility rate to FA was 92.7% (0.25–2 mg/L MIC range), with 7.3% intermediate. *P.*

Table 1 Susceptibility and resistance rates (%) of topical antibiotics against *P. acnes*

Antibiotic name	Breakpoints		Number	% R	% I	% S	MIC_{50}	MIC_{90}	MIC Range
	S	R							
Neomycin sulfate	⩽10		69	11.7	0	88.3	8	16	1–16
Bacitracin	⩽2		69	0	0	100	0.5	1	⩽ 0.25–2
Polymyxin B	⩽2		69	100	0	0	128	>128	32 to > 128
TAO	⩽10		69	0	0	100	2	2	⩽ 0.25–8
Mupirocin	⩽8	> 256	69	100	0	0	>12	>512	> 512
FA	⩽1	⩾4	69	0	7.3	92.7	1	1	0.25–2
Clindamycin	⩽2	⩾8	69	33.4	11.6	55	0.5	>128	⩽ 0.06 to > 128
Erythromycin	⩽5	⩾8	69	49.3	0	50.7	64	>128	⩽ 0.06 to > 128

Susceptibility was defined on the basis of the following criteria: neomycin at ⩽ 10 mg/L; bacitracin at ⩽ 2 IU/ml; polymyxin B at ⩽ 2 IU/ml; TAO was based on the most active component, namely, neomycin as the MIC; mupirocin at ⩽ 8 mg/L and high-level resistance at > 256 mg/L; FA at ⩽ 1 mg/L; clindamycin at ⩽ 2 mg/L; and erythromycin at ⩽ 5 mg/L. R, resistance; I, intermediate; and S, susceptibility.

acnes exhibited complete resistance to polymyxin B (32 to > 128 mg/L MIC range) and mupirocin (> 512 mg/L MIC range).

Effects of gender, age, disease duration, previous treatment, and disease severity on the susceptibility or resistance rates for clindamycin and erythromycin

The susceptibility or resistance rates for clindamycin and erythromycin are shown in Table 2. Age and disease duration did not influence antibiotic susceptibility ($P > 0.05$). The antibiotic susceptibility of the females was significantly lower than that of the males ($P < 0.05$). The patients previously treated with oral antimicrobial agents for acne were less susceptible to clindamycin than those previously treated with topical retinoids. The patients previously treated with oral or topical antimicrobial agents for acne were less susceptible to erythromycin than those previously treated with oral or topical retinoids. The susceptibility rates were higher in the patients with mild to moderate acne than the rates in the patients with severe acne. The susceptibility rate for clindamycin was significantly different between grades I and IV and between grades II and IV. The susceptibility rate for erythromycin was also significantly different between grades I and IV.

Table 2 Susceptibility rates for clindamycin and erythromycin as a function of gender, age, disease duration, previous treatment, and disease severity

		Clindamycin (%)	Erythromycin (%)
Gender	Male (n = 41)	58.95±0.87	57.53±8.36
	Female (n = 28)	35.99±7.02	23.49±8.25
	P value	< 0.05*	< 0.05*
Age	< 25 years (n = 49)	55.27±7.77	47.18±10.76
	⩾ 25 years (n = 20)	60.32±5.50	54.76±14.87
	P value	0.862	0.781
Disease duration	< 2 years (n = 55)	56.53±13.65	51.07±13.56
	⩾ 2 years (n = 14)	50.00±10.00	50.00±10.00
	P value	0.816	0.968
Previous treatment	Oral antibiotics (n = 33)	41.82±6.29	39.09±5.53
	Topical antibiotics (n = 30)	53.33±5.77	40.00±10.00
	Oral isotretinoin (n = 16)	50.00±10.00	56.67±5.77*[3,4]
	Topical retinoids (n = 31)	61.52±7.84*[1]	65.15±5.01*[1,2]
Disease severity	I (n = 5)	66.67±28.87	66.67±28.87
	II (n = 17)	70.00±12.02	64.45±3.85
	III (n = 26)	53.71±3.21	42.13±4.01
	IV (n = 21)	31.19±2.30*[1,2]	36.19±7.19*[1]

*P<0.05. 1, comparison between treatment with topical retinoids and oral antibiotics, or severity of grades I and IV; 2, comparison between treatment with topical retinoids and topical antibiotics, or severity of grades II and IV; 3, comparison between treatment with oral isotretinoin and oral antibiotics; and 4, comparison between treatment with oral isotretinoin and topical antibiotics.

Discussion

Acne vulgaris is a common multifactorial skin disease and is mainly manifested as seborrheic lesions on the face and the upper torso. *P. acnes* is a Gram-positive anaerobic bacillus that colonizes the sebaceous glands. This microorganism is important in the pathogenesis of acne. The mixture of abnormally desquamated cells and excessive amounts of sebum in microcomedones create a suitable environment for *P. acnes* growth. In this environment, *P. acnes* produces pro-inflammatory mediators that cause inflammatory lesions. *P. acnes* interacts with the innate immune system to promote inflammation through at least four primary pathways: by activating Toll-like receptors, by triggering inflammasomes, by inducing matrix metalloproteinase production, and by stimulating antimicrobial peptide activity [9].

Compared with the vehicle alone, topical antibiotics for acne can significantly reduce the severity of inflammation. This finding is consistent with the results of various antimicrobial sprays. Thus, the use of topical antibiotics could be a part of acne treatments [10]. In 2003, 3–4 million topical antibiotics were prescribed by dermatologists [11]. Antibiotics have been prescribed for acne treatment for more than 40 years. The selection of antibacterial should consider acne severity, cost effectiveness, risk–benefit ratio, and potential for resistance [12]. *In vitro P. acnes* is considerably sensitive to a wide range of antibiotics, including macrolides, clindamycin, tetracycline, quinolone, penicillin, and cephalosporin. As the most widely used topical antibiotics, clindamycin and erythromycin exhibit bacteriostatic activity against *P. acnes*. They also elicit an anti-inflammatory effect by inhibiting the lipase production of *P. acnes* and the chemotaxis of leukocytes. *P. acnes* is also resistant to aminoglycoside, mupirocin, and metronidazole [13].

The first report of a resistant *P. acnes* strain isolated from patients with acne was published in USA in 1979. Since then, similar reports have been published in many countries. Importantly, a significant percentage of bacteria isolated from patients with acne are resistant to most common antibiotics, such as clindamycin, erythromycin, tetracycline, doxycycline, and minocycline, which are used for acne treatment [14]. Antibiotic resistance may occur after a short-term antibiotic treatment, and the resistance rate increases as the duration of antibiotic treatment is prolonged. A survey conducted in Europe indicates that *P. acnes* is resistant to clindamycin and erythromycin in 50% of patients with acne and to tetracycline in 20% of patients with acne. In general, resistance to erythromycin is the most common among antibiotics, and this condition is associated with cross-resistance to clindamycin [3]. With prolonged antibiotic treatment, the risk of *P. acnes* resistance is increased [10]. *P. acnes* resistance occurs in early stages when topical antibiotics are administered. Tanghetti [15] demonstrated that *P. acnes* resistance emerges 4 to 8 weeks after antibiotic therapy is administered and persists after antimicrobial agent treatment is terminated. In our study, the resistance rate of the isolated *P. acnes* was 49.3% for erythromycin (27.5% of which exhibited cross-resistance to clindamycin) and 33.4% for clindamycin. Age and disease duration were not associated with MIC. The MIC for erythromycin and clindamycin in females was significantly higher than that in males. This phenomenon might occur because females were more active in receiving treatment, including antimicrobial therapy, than males. The patients treated with oral antimicrobial agents were less susceptible to clindamycin than those previously treated with topical retinoids. The patients previously treated with oral or topical antimicrobial agents were less susceptible to erythromycin than those previously treated with oral or topical retinoids. The susceptibility to antibiotics in patients with mild to moderate acne was significantly higher than the susceptibility to antibiotics in those with severe acne. This finding indicated that the high level of exposure to antibiotics leads to an increased resistance rate to antibiotics, especially in patients exposed to systemic antimicrobial agents.

Neomycin sulfate, bacitracin, and polymyxin B sulfate have been used for topical prescriptions to prevent and treat common skin infections since 1956. Topical prescription is usually known as TAO. The susceptibility profiles for TAO have remained relatively unchanged since its discovery possibly because the components of TAO are not widely used parenterally in systemic infection treatment. In our study, clinical *P. acnes* isolates from acne lesions were all susceptible to TAO. Conversely, the isolates yielded relatively high resistance rates for clindamycin (33.4%) and erythromycin (49.3%). However, TAO is not recommended for acne treatment to reduce the risk of resistance.

Mupirocin reversibly binds to the isoleucyl transfer-RNA synthetase of bacteria. As a result, protein synthesis is inhibited. Mupirocin is bactericidal at concentrations suitable for topical application. However, resistance to mupirocin has emerged [16]. In our study, *P. acnes* was resistant to mupirocin. This observation suggested that mupirocin is not preferable for clinical acne treatment.

FA was first extracted from *Fusidium coccineum* in fermentation broth by Leo Pharma in 1962. The molecule contains a steroid-like structure but does not exhibit steroid activity. FA is often used as a topical treatment for skin and soft-tissue infections because of its high penetration and antimicrobial activity [17]. In our study, the susceptibility rate to FA (0.25–2 mg/L MIC range) was 92.7%, with 7.3% intermediate. The antibacterial activity of FA is superior to clindamycin and erythromycin, but its resistance rate from the nationwide data of New Zealand was increased [18] because of the increased use of FA in New Zealand communities. Although FA provides good

antibacterial activity to *P. acnes in vitro*, the wide use of FA is not recommended for acne treatment to reduce the risk of *P. acnes* resistance.

Our study is characterized by several limitations. For instance, we performed a single-center study and recruited few patients. Thus, our findings should be verified in further studies with a larger sample size. We did not also classify the clinical *P. acnes* strain on the basis of their biotypes or genotypes because these parameters might be closely related to the severity of acne or drug resistance of bacteria. Furthermore, *P. acnes* classification may not affect the MIC detection.

Our study provided experimental evidence for the use of topical antibiotics in acne treatment. Topical antibiotics should not be used alone for long-term therapy to prevent the development of multiresistant *P. acnes*. The selection of topical antibiotics should also be determined by clinicians on the basis of clinical conditions with evidence-based assessment.

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Compliance with ethics guidelines

Ying Ma, Nanxue Zhang, Shi Wu, Haihui Huang, and Yanpei Cao declare they have no conflict of interest. All of the procedures were performed in accordance with the ethical standards of the Institutional Review Board of Huashan Hospital, Fudan University and with the *Helsinki Declaration* of 1975, as revised in 2000. Informed consent was obtained from all of the patients before the study was conducted.

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