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ORIGINAL ARTICLE

Glycolic acid chemical peeling improves inflammatory acne eruptions through its inhibitory and bactericidal effects on *Propionibacterium acnes*

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ABSTRACT

Glycolic acid chemical peeling is effective for treating comedones, and some clinical data show that it also improves inflammatory eruptions. The purpose of this study was to identify the mechanism of glycolic acid chemical peeling to improve inflammatory acne. To assess growth inhibitory and bactericidal effects of glycolic acid on *Propionibacterium acnes in vitro*, we used an agar diffusion method and a time-kill method. To reveal bactericidal effects *in vivo*, we established an agar-attached method which correlated well with the ordinary swab-wash method, and we used the agar-attached method to compare the numbers of propionibacteria on the cheek treated with glycolic acid chemical peeling. Our results show that 30% glycolic acid (at pH 1.5, 3.5 and 5.5) formed growth inhibitory circles in the agar diffusion method, but the diameters of those circles were smaller than with 1% nadifloxacin lotion or 1% clindamycin gel. In the time-kill method, 30% glycolic acid (at pH 1.5 and 3.5) or 1% nadifloxacin lotion reduced the number of *P. acnes* to less than 100 CFU/mL within 5 min. In contrast, in 30% glycolic acid (at pH 5.5) or in 1% clindamycin gel, *P. acnes* survived for more than 4 h. Chemical peeling with 35% glycolic acid (at pH 1.2) decreased the number of propionibacteria on the cheeks of patients compared with untreated controls ($P < 0.01$). Our results demonstrate that glycolic acid has moderate growth inhibitory and bactericidal effects on *P. acnes*, and that chemical peeling with glycolic acid works on inflammatory acne via those effects.

Key words: acne vulgaris, agar-attached method, chemical peeling, glycolic acid, *Propionibacterium acnes*.

INTRODUCTION

Acne is a chronic inflammatory skin disease which is experienced by more than 90% of the population[1] and which affects emotional aspects of acne patients' quality of life.[2] The primary eruption of acne is a comedone, which is induced by abnormal cornification of the hair infundibulum and hypersecretion of sebum. *Propionibacterium acnes*, which prefers anaerobic and lipid-rich conditions, increases in number and induces inflammation.[3] In consequence, comedones develop into papules and pustules.[4] Severe inflammation sometimes causes cystic and/or nodular acne and forms atrophic or hypertrophic scars, which remain permanently.

Glycolic acid chemical peeling is accepted as a useful modality to treat acne.[5,6] It improves not only comedonal acne but also inflammatory acne.[7,8] The proposed mechanism of glycolic acid chemical peeling is usually thought to be the correction of abnormal keratinization in the infundibulum, which works directly on comedones and consequently improves inflammatory eruptions. However, experiments performed by Atzori *et al.*[7] and by our group[8] showed significant improvement of inflammatory eruptions from the first application. We assume that glycolic acid might have some bactericidal effects which improve inflammatory acne eruptions. The aim of our study was to characterize the bactericidal effects of glycolic acid *in vitro* and *in vivo*.

METHODS

Patients

Acne patients of all severities were enrolled for comparison of the agar-attached method with the swab-wash method. Patients with mild inflammatory acne, who had not received any oral or topical antibiotics for at least 1 month before participation, were enrolled for the chemical peeling experiments. These experiments were approved by the ethical committee of the Tokyo Women's Medical University, and each patient received a full explanation and signed a written informed consent form before the experiments.

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Materials

Glycolic acid (Sigma-Aldrich, St Louis, MO, USA) was dissolved in distilled water and the pH was adjusted with 1 N NaOH. Thirty percent glycolic acid (at pH 1.5, 3.5 and 5.5) was used for the *in vitro* study. One percent nadifloxacin lotion (Otsuka Pharmaceutical, Tokyo, Japan) and 1% clindamycin gel (Sato Pharmaceutical, Tokyo, Japan) were purchased. Commercially available materials for glycolic acid chemical peeling, including 35% glycolic acid (pH 1.2) and the neutralizer, were obtained from Tokiwa Pharmaceutical (Osaka, Japan). Anaerobic conditions were generated in small sealed bags with Anaeromeit-J (Nissui Pharmaceutical, Tokyo, Japan) which chemically absorbs oxygen. *P. acnes* (ATCC no. 6919) for *in vitro* study was purchased from the American Type Culture Collection (Rockville, MD, USA).

Growth inhibitory effects of glycolic acid *in vitro*

The agar diffusion method, which is the modified disk diffusion method, was used to assess growth inhibitory effects. Bacterial suspensions, which contained 1×10^6 CFU/mL *P. acnes*, were inoculated onto each Gifu Anaerobic Medium (GAM) plate. Eight-millimeter diameter holes were formed on each plate and 0.1 g of each tested material was placed in it. After incubation in anaerobic conditions at 37°C for 48 h, the diameter of each growth inhibitory circle was measured.

Bactericidal effects of glycolic acid *in vitro*

The time-kill method was used to estimate the bactericidal effects of glycolic acid. We incubated 1×10^6 CFU/mL *P. acnes* with each tested material for 5 min, 30 min, 60 min and 4 h. These samples were then diluted with 0.9% sodium chloride, and inoculated onto agar plates. After incubation in anaerobic conditions at 37°C for 48 h, the numbers of surviving colonies were counted.

Establishment of the agar-attached method

To measure the number of *P. acnes* on the cheeks of each subject, we used an agar-attached method, which is a modified stamp method, to count bacteria on the skin surface. We attached an agar plate (Petan-Check; Eiken Chemical, Tokyo, Japan) on the cheek for 5 s, and then incubated it in anaerobic conditions at 37°C for 48 h, after which the numbers of colonies on each plate were counted.

To confirm the reliability of the agar-attached method for counting *P. acnes* on the skin of acne patients, we compared it with the swab-wash method, which is ordinarily used to evaluate propionibacteria related with acne symptoms. For the swab-wash method, 4 cm^2 of each patient's cheek adjacent to the place where the sample for the agar-attached method was taken, was scrubbed with a sterile swab moistened with phosphate-buffered saline (pH 7.4) containing 0.05% Tween-20 (Sigma-Aldrich). The swab-tip was broken off into the wash fluid, and mixed vigorously, after which the wash fluid was serially diluted with distilled water. One hundred microliters of the diluted and undiluted wash fluids were spread over the surface of the GAM plates, and after incubation in anaerobic conditions at 37°C for 3 days, the numbers of colonies were counted. Logarithms of the numbers of colonies obtained from the swab-wash method and the agar-attached method were calculated, and their correlations were assessed.

Evaluation of the bactericidal effects of glycolic acid chemical peeling on *P. acnes in vivo*

After washing the entire face of each subject with soap, microorganisms were obtained from both cheeks using the agar plate method. A randomly selected side of the cheek of each patient was treated with 35% glycolic acid (pH 1.2) for 2 min or less until erythema appeared, then the reaction was stopped with a neutralizer. After washing the entire face with enough water and cooling for 2 min with a paper mask soaked with icy water, microorganisms on the skin surface were obtained again in the same way. All agar plates were incubated in anaerobic conditions at 37°C for 48 h. The numbers of colonies were then counted and decreasing rates of colonies were calculated to statistically compare untreated and treated sides with glycolic acid chemical peeling using the paired Student's *t*-test.

RESULTS

Growth inhibitory effects of glycolic acid *in vitro*

Growth inhibitory circles were observed in all plates treated with 30% glycolic acid, and the lower pH of 30% glycolic acid showed the greater inhibitory effect (Fig. 1). The averages of triplicate results of zone-of-inhibition diameters are summarized in Figure 2. The clindamycin gel and the nadifloxacin lotion showed larger zone-of-inhibition diameters than all 30% glycolic acid solutions.

Bactericidal effects of glycolic acid *in vitro*

The effects of glycolic acid on *P. acnes* growth are summarized in Table 1. The numbers of *P. acnes* were reduced to less than 100 colony-forming units/mL within 5 min when treated with 30% glycolic acid at pH 1.5, 3.5 or with 1% nadifloxacin lotion. In contrast, 30% glycolic acid at pH 5.5 or 1% clindamycin gel did not elicit any apparent decrease in colony counts, meaning that those two materials did not have a bactericidal effect.

Comparison between the swab-wash method and the agar-attached method

Thirty-two patients were enrolled in this part of the study. The logarithms of numbers of colonies obtained from their cheek skin using the swab-wash method and the agar-attached method are shown in a scatter graph (Fig. 3). The correlation rate was 0.72 ($P < 0.01$), which is an excellent correlation and confirmed the reliability of the agar-attached method.

Effects of glycolic acid chemical peeling on *P. acnes in vivo*

Nine female acne patients, aged 25–35 years, were enrolled in this part of the study. There were no apparent differences in the numbers of colonies of propionibacteria taken from the left or right sides of the faces before chemical peeling. After the glycolic acid chemical peeling, the colonies on the treated sides dramatically decreased compared with the untreated sides. Representative results are shown in Figure 4. Each treated side had much lower numbers of colonies compared with the untreated side, and as a consequence, the decreases on the treated sides were statistically higher than on the untreated sides ($P < 0.01$) (Fig. 5). Furthermore, we picked up seven of these colonies from patients' agar plates,

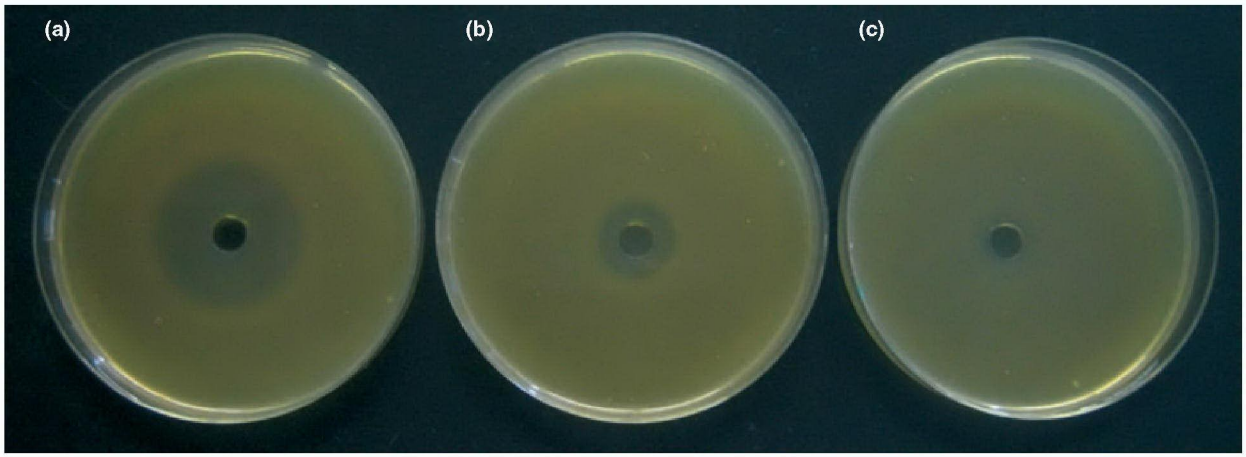

Figure 1. Agar diffusion method. All plates with 30% glycolic acid formed growth inhibition circles to *Propionibacterium acnes*. (a) pH 1.5, (b) pH 3.5, (c) pH 5.5.

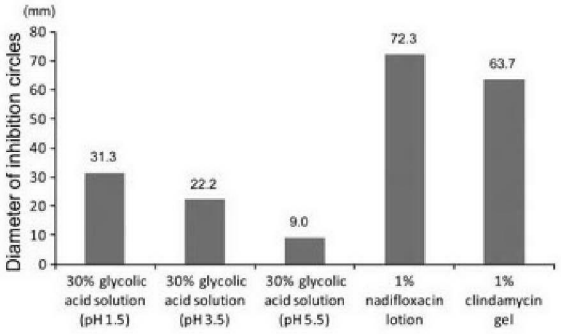

Figure 2. Growth inhibitory effect of glycolic acid. Solutions of 30% glycolic acid (pH 1.5, 3.5 and 5.5) formed zones of inhibition with diameters smaller than those elicited by clindamycin gel or nadifloxacin lotion.

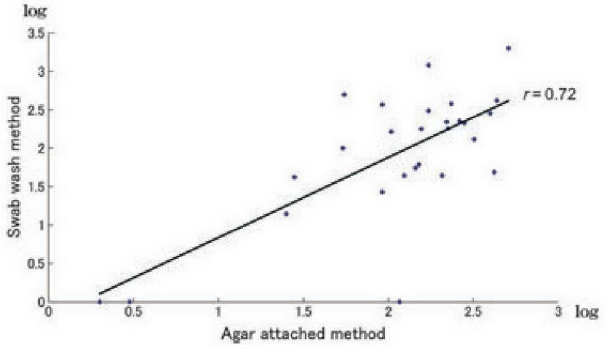

Figure 3. Correlation between the swab-wash method and the agar-attached method. A scatter graph of logarithms of numbers of *Propionibacterium acnes* obtained using the swab-wash method and the agar-attached method shows a significant correlation of 0.72 ($P < 0.01$).

and confirmed that all of them are *P. acnes* using Gram staining, catalase test and Rap ID ANA II system (AMCO, Tokyo, Japan).

DISCUSSION

Our clinical study on the efficacy of 30% glycolic acid chemical peeling for acne vulgaris revealed the rapid improvement not only of comedones but also of inflammatory eruptions, which prompted us to study the antibacterial effect of glycolic acid.

First of all, we performed an *in vitro* study to check the antimicrobial effects of glycolic acid. The agar diffusion method indicated that all of the examined glycolic acids have growth inhibitory effects

Table 1. Bactericidal effect of glycolic acid. The numbers of *Propionibacterium acnes* were reduced to <100 CFU/mL within 5 min after treatment with 30% glycolic acid (at pH 1.5 or 3.5)

	5 min	30 min	60 min	4 h
30% glycolic acid solution (pH 1.5) (CFU/mL)	<100	<100	<100	<100
30% glycolic acid solution (pH 3.5) (CFU/mL)	<100	<100	<100	<100
30% glycolic acid solution (pH 5.5)	1.1×10^6	3.3×10^5	3.3×10^5	3.2×10^5
1% nadifloxacin lotion (CFU/mL)	<100	<100	<100	<100
1% clindamycin gel	4.4×10^6	3.8×10^5	3.3×10^5	2.2×10^5

CFU, colony-forming units.

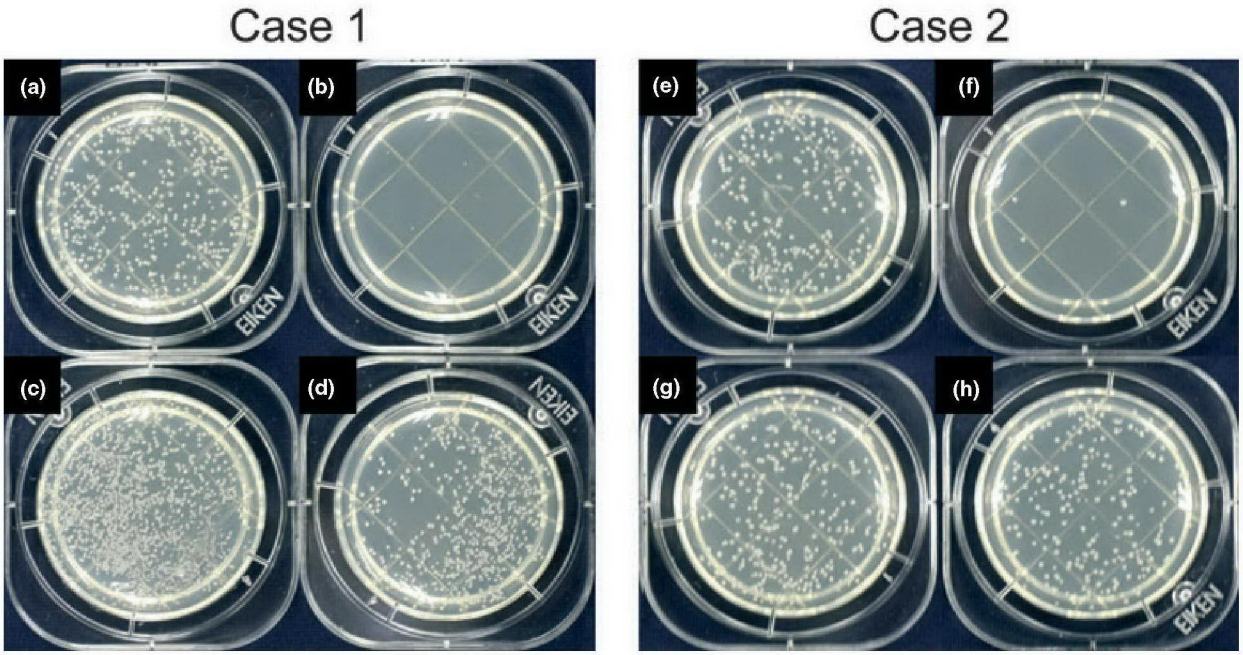

Figure 4. Bactericidal effect of glycolic acid chemical peeling. Representative results showing the bactericidal effects of glycolic acid chemical peeling. (a,e) Treated sides before the experiment; (b,f) treated sides after the experiment; (c,g) untreated sides before the experiment; (d,h) untreated sides after the experiment.

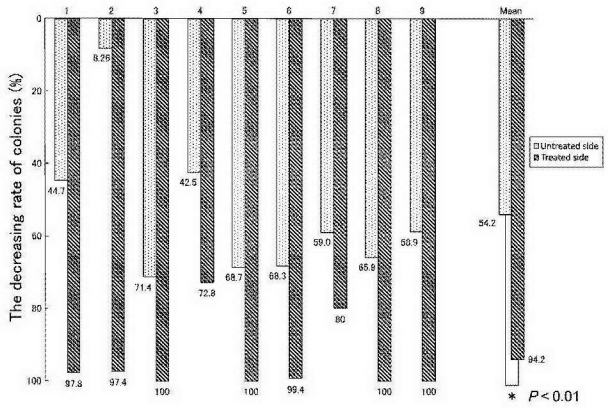

Figure 5. Decreasing rate of colonies after glycolic acid chemical peeling. The bars show the decreasing rates of colonies of both sides of the faces of all of nine cases and their mean. The decreasing rate of the treated side was statistically higher than the untreated side ($P < 0.01$).

on *P. acnes*, but its effects are milder than the effects of topical antibiotics. The time-kill method revealed that 30% glycolic acids (at pH 1.5 and 3.5) have a bactericidal effect. In this study, the clindamycin gel did not show any bactericidal effect on *P. acnes*. Clindamycin works on bacteria by binding preferentially to the 23 S subunit of the bacterial ribosome and inhibiting bacterial protein synthesis.[9] As a consequence, the mechanism of action of clindamycin is not bactericidal, but is bacteriostatic. On the other hand, nadifloxacin, which inhibits the configuration of supercoiled DNA by DNA gyrase, has both growth inhibitory and bactericidal effects. Based on these studies, we confirmed the effects of glycolic acid on *P. acnes*, and lower pH of glycolic acid has more growth inhibitory and bactericidal effects, although we still do not know how it works.

Next, we estimated the antimicrobial effects of glycolic acid *in vivo*. The swab-wash method is generally used to count colonies of propionibacteria on the skin surface[10] to determine the numbers of propionibacteria quantitatively on the skin of acne patients. This method requires complicated steps. You have to make special wash-fluid, to collect samples, to dilute them, to sow them on agar plates, to culture them in anaerobic condition and to count the numbers of colonies. On the other hand, the newly established agar-attached method requires only three steps to attach the plate on the cheeks, to culture them in anaerobic condition and to count the colonies. To determine the amount of *P. acnes* in infundibulum is important and the agar-attached method estimates only propionibacteria on the surface. Our experiments revealed that the agar-attached method is reliable because it correlates well with the swab-wash method which has already been established to measure the quantities of microbacteria in acne patients. The agar-attached method is much easier and more convenient than the swab-wash method.

Our results using the agar-attached method clearly show that 35% glycolic acid (pH 1.2) chemical peeling reduces the number of propionibacteria on the cheeks of acne patients. These results might come from the effects of acidic condition, but the most important point is that glycolic acid in practical concentration is safe and effective for inflammatory acne and comedonal acne.

In consequence, glycolic acid has antimicrobial effect on *P. acnes in vitro* and reduces the numbers of *P. acnes* on the cheeks of acne patients *in vivo*. Recently, reports of antibiotic-resistant *P. acnes* are increasing.[11–14] To prevent antibiotic-resistant bacteria, authorities recommend reducing the widespread use of antibiotics, not to use topical and oral antibiotics simultaneously and to use benzoyl peroxide (BPO). But BPO has some skin irritation and is not permitted for acne in some countries including Japan. Our data suggest that glycolic acid could be one alternative to BPO to reduce antibiotic-resistant *P. acnes*, especially when BPO is not available.

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