Effect of 30% Supramolecular Salicylic Acid Peel on Skin Microbiota and Inflammation in Patients with Moderate-to-Severe Acne Vulgaris

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ABSTRACT

Introduction: Thirty-percent supramolecular salicylic acid (SSA), a modified salicylic acid preparation, is a safe and effective treatment for moderate-to-severe acne vulgaris (AV). However, its mechanism of action remains unclear. We aimed to analyze the role of 30% SSA peels on skin microbiota and inflammation in patients with moderate-to-severe AV.

Methods: A total of 28 patients were enrolled and received 30% SSA peels biweekly for 2 months. The Global Acne Grading System (GAGS) score, skin water content, transepidermal water loss (TEWL), pH, and sebum levels were assessed. Skin microbial samples and perilesional skin biopsies were obtained at the onset and 2 weeks after treatment completion. Samples were characterized using a high-throughput sequencing approach targeting a portion of the bacterial 16S ribosomal RNA gene.

Results: After treatment, patients showed a significant improvement in their GAGS score and skin barrier indicators ($P < 0.05$). The GAGS score was positively associated with both the sebum concentration ($R = 0.3$, $P = 0.027$) and pH ($R = 0.39$, $P = 0.003$). Increased expression of caveolin-1 and decreased expression of interleukin (IL)-1a, IL-6, IL-17, transforming growth factor beta, and toll-like receptor 2 were observed in the skin tissue after treatment. The richness and evenness of the cutaneous microbiome decreased after treatment and the *Staphylococcus* proportion decreased significantly ($P < 0.05$), whereas the *Propionibacterium* proportion tended to decrease ($P = 0.066$).

Conclusions: On the basis of analyses of the skin barrier and microbiota, we speculate that the 30% SSA peel may have a therapeutic effect in patients with moderate-to-severe AV by improving the skin microenvironment and modulating the skin microbiome, thus reducing local inflammation.

Keywords: Inflammation; Microbiota; Moderate-to-severe acne vulgaris; Skin; Supramolecular salicylic acid

Xinyi Shao and Yangmei Chen contributed equally to this work.

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Key Summary Points

Why carry out this study?

While 30% supramolecular salicylic acid (SSA) is known to be a safe and effective treatment for acne vulgaris (AV), its mechanism of action is unknown.

This study aimed to clarify the effects of 30% SSA on the skin microbiota and inflammation in patients with AV.

What was learned from the study?

Species diversity and proportions were affected with 30% SSA treatment; *Staphylococcus* species decreased significantly, and *Propionibacterium* species tended to decrease

Caveolin-1 expression increased and transforming growth factor beta, toll-like receptor 2, and interleukin (IL)-1a, IL-6, and IL-17 expression decreased significantly in the skin tissue after treatment.

INTRODUCTION

Acne vulgaris (AV) is a chronic inflammatory disorder of the pilosebaceous unit that affects approximately 85% of adolescents and young adults [1–3]. Moderate and severe AVs are characterized by papules, pustules, cysts, and nodules, which can appear as disfiguring scars and need a relatively long treatment course. The etiopathogenesis of AV is multifactorial and involves follicular hyperproliferation, increased sebum production, inflammation, and microbial overgrowth [4]. Recently, studies have confirmed that patients with acne harbor an altered skin microbiome, and a more significant dysbiosis is found in patients with severe acne [5–7]. The loss of skin microbial diversity and the activation of innate immunity may lead to

chronic inflammation [8]. However, this role and its level of contribution remain unclear [9].

Patients with moderate-to-severe acne are often treated with topical and/or systemic isotretinoin and antibiotics. Combination therapy comprising chemical peels, phototherapy, and lasers, together with traditional therapy, can reduce the side effects such as skin irritation and redness, antibiotic resistance, and photosensitivity [10–12].

Salicylic acid (SA) is an o-hydroxybenzoic acid that acts against both non-inflammatory and inflammatory lesions in AV. Therefore, SA peels are widely used in the treatment of active AV [13–15]. However, SA has low water solubility. Supramolecular SA (SSA) is a new formulation that increases its solubility and reduces the side effects on the skin [16–18].

In a previous study, we found that 30% SSA was a safe and effective treatment for AV and could reduce sebum production [19]. However, very few studies have explored the role of SSA in the treatment of skin microbiota and inflammation in patients with moderate-to-severe AV. Therefore, we conducted a clinical trial to clarify the effects of 30% SSA on the skin microbiota composition and inflammation in patients with moderate-to-severe acne.

METHODS

Patients

Overall, 28 patients diagnosed with moderate-to-severe AV and 13 healthy controls with no skin problems were recruited from Chongqing, China, between September 2020 and April 2022.

The inclusion criteria were as follows: (i) patients aged > 18 years with AV; (ii) patients with a minimum of 30 acne lesions (comedones, inflammatory papules, or pustules); and (iii) patients presenting with Pillsbury II–IV facial AV.

The exclusion criteria were as follows: (i) patients with a history of any topical or oral medication use within 3 months of the baseline study; (ii) patients with other types of dermatoses, such as atopic dermatitis, rosacea,

melasma, or contact dermatitis; and (iii) patients who were breastfeeding or pregnant. This is a before–after case series, and the study was approved by the ethical review board of the First Affiliated Hospital of Chongqing Medical University, China. Written informed consent for participation in the study and publication of photographs was obtained from each participant before enrollment, adhering to the principles of the Declaration of Helsinki.

Clinical Evaluation

Investigator Evaluation
The severity of AV was evaluated objectively using the Global Acne Grading System (GAGS) [20] before administration (V0) and 8 weeks (V56) after the start of treatment. Side effects in the targeted areas were evaluated by investigators at each visit.

Patients' Self-Assessment
At the fifth visit, patients completed a questionnaire that included questions on their acne improvement. The patients ranked their acne improvement as 0 (no improvement or worse), 1 (mild improvement), 2 (moderate improvement), and 3 (obvious improvement).

Evaluation of the Epidermal Barrier Function

Facial images and red areas were measured at each visit using the VISIA-CR imaging system (Canfield Scientific, Fairfield, NY). The VISIA skin analysis system assesses the severity of red areas and provides a score ranging from 0% to 100%, with a higher score indicative of fewer red areas. The epidermal barrier function was evaluated at the first and fifth visits. Skin hydration was assessed using the Corneometer CM 825 (Courage + Khazaka Electronic GmbH, Cologne, Germany), transepidermal water loss (TEWL) was measured using the Tewameter TM 300 (Courage + Khazaka Electronic GmbH), and the skin pH and casual sebum level were measured using the Skin-pH-Meter PH 905 and the Sebumeter 815 (both Courage + Khazaka Electronic GmbH), respectively. All parameters of the epidermal barrier function were measured in the forehead, nose, left cheek, right cheek, and chin. Finally, the average values of the five sites were used in this study.

Skin Microbial Sample Collection

Skin samples of patients and healthy controls were collected before treatment administration (V0) and at 8 weeks (V56). All participants were asked to avoid washing their face and applying any topical agent 24 h prior to the examination. Samples were collected from a 9-cm^2 acne area on the cheek of each patient by rubbing the skin with a sterile swab in horizontal and vertical directions 50 times (lasting approximately 30 s). The swabs were immediately stored at $-80\,^{\circ}$C for subsequent DNA extraction. Each 9-cm^2 sampled area was identified using standardized photography to ensure that the same area was sampled at each follow-up visit. Microbiota sampling was conducted by the same investigator (XYS) during all visits.

Skin Biopsy Sample Collection

Perilesional skin biopsy samples were taken at V0 and V56 using a 2 mm punch biopsy. We chose a unified biopsy skin position in patients before and after treatment. The biopsied skin tissues were fixed with 4% paraformaldehyde, embedded in paraffin, and sectioned (4-μm-thick sections). Immunocytochemical staining was performed as previously described [21, 22]. The expressions of interleukin (IL)-1a, IL-6, IL-17, transforming growth factor beta (TGF-β), toll-like receptor-2 (TLR-2), and caveolin-1 were examined using immunohistochemistry. The sections were then observed and imaged under a microscope.

30% SSA Treatment

Patients received a 30% SSA (Broda; Borenda Biochemical Technology, Shanghai, China) peel at an interval of 2 weeks for a total of 8 weeks (a total of four peeling sessions were performed). Sensitive areas of the face, such as the lateral and medial canthi, oral commissures, lips, and

alar nasi grooves, were protected by applying a thin layer of petrolatum. Using a cotton tip applicator, a coat of the 30% SSA peeling agent was applied to patients' faces, moving from the forehead and advancing to the cheeks, chin, glabella, nose, and perioral area. Once white crystallization appeared, patients were asked to wash their faces with water and pat their faces dry, immediately followed by the topical use of a mask (Broda; Borenda Biochemical Technology).

DNA Extraction and 16S Ribosomal RNA Gene Polymerase Chain Reaction Amplification and Sequencing

Total genomic DNAs from swab samples were extracted using the OMEGA Soil DNA Kit (M5635-02; Omega Bio-Tek, Norcross, GA, USA), following the manufacturer's instructions. The V3–V4 region of the bacterial 16S ribosomal RNA (rRNA) genes was amplified using polymerase chain reaction (PCR) (initial denaturation at 98 °C for 5 min; 25 cycles of denaturation at 98 °C for 30 s, annealing at 53 °C for 30 s, and extension at 72 °C for 45 s; final extension of 5 min at 72 °C) using primers 338F (5′-ACTCCTACGGGAGGCAGCA-3′) and 806R (5′-GGACTACHVGGGTWTCTAAT-3′). PCR reactions were performed in 24 μL solution containing 5 μL of buffer (5×), 0.25 μL of fast *Pyrococcus furiosus* DNA polymerase (5 U/μL), 2 μL (2.5 mM) of deoxyribonucleoside triphosphates, 1 μL (10 μM) of each primer, 1 μL of DNA template, and distilled and deionized water. PCR amplicons were purified with Vazyme VAHTSTM DNA Clean Beads (Vazyme, Nanjing, China) and quantified using the Quant-iT PicoGreen dsDNA Assay Kit (Invitrogen, Carlsbad, CA). After the individual quantification step, amplicons were pooled in equal amounts, and paired-end 250-bp sequencing was performed using the Illumina MiSeq platform with MiSeq Reagent Kit v3 (Illumina, San Diego, CA) at Shanghai Personal Biotechnology Co., Ltd. (Shanghai, China).

Sequence-Based Microbiota Analysis

Sequencing data were mainly analyzed using QIIME2 and R packages (v3.2.0; R Foundation, Vienna, Austria). Observed relative abundances were estimated by dividing the observed number of 16S rRNA amplicon reads by the total number of reads per sample. Microbiota alpha diversity, representing microbial diversity within an individual sample, such as the Chao1 richness estimator, Shannon diversity index, Simpson index, and Pielou's evenness, were calculated using the operational taxonomic units (OTU) table in QIIME2. Beta diversity analysis was performed to investigate the structural variation of microbial communities across samples using UniFrac distance metrics and visualized via principal coordinate analysis. Taxa abundances at the OTU levels were statistically compared among samples or groups using MetagenomeSeq analysis. Pearson's correlation analysis was performed to assess the correlation between the skin microbes and the skin barrier parameters.

Statistical Analysis

The statistical analysis of the clinical and sequencing data was performed using R 4.1.1 (R Foundation for Statistical Computing). Data are statistically described in terms of mean ± standard deviation (mean ± SD), median and range, or frequencies and percentages. Comparisons were performed using Wilcoxon's signed-rank test for paired samples. Bacterial populations at different taxonomical levels (genus and phylum) were compared before and after the 30% SSA treatment using Wilcoxon's rank-sum test for paired observations. Statistical analysis of skin microbial differences before and after treatment was performed using R 4.1.1 (R Foundation for Statistical Computing). Pearson's correlation analysis was performed to assess the correlation between skin microbes and the skin barrier parameters. $P < 0.05$ was considered statistically significant.

Table 1 Background characteristics of healthy controls and patients with acne vulgaris

	Acne $n = 28$	Healthy controls $n = 13$	Overall P value
Sex (F/M)	15/13	9/4	0.84
Age (years)	24.60 ± 2.71	25.46 ± 2.99	
BMI (kg/m²)	22.13 ± 3.59	20.35 ± 2.54	
Duration (months)	48.84 ± 40.80	0	NA

BMI body mass index, *F* female, *M* male

RESULTS

Baseline Characteristics of Patients and Healthy Controls

A total of 30 patients were enrolled, and 28 completed the trial. Two patients were excluded owing to noncompliance with the treatment schedule. The skin samples and clinical characteristics of 13 controls were collected at baseline. The baseline characteristics of the patients and healthy controls are shown in Table 1.

Treatment with 30% SSA Peel Decreased the GAGS Score

VISIA-CR (Canfield Scientific Inc.) testing revealed a good improvement in acne, and acne lesions were significantly alleviated after 56 days of treatment on both sides (Supplementary Fig. S1). At the end of the eighth week of therapy, there was a significant decrease in the GAGS score ($P < 0.001$) (Fig. 1a).

The peel was well tolerated by all patients. Post-peel burning and a stinging sensation were the most common adverse effects noted in 25 patients. Post-peel erythema occurred in three patients. Post-inflammatory hyperpigmentation, blistering, crusting, scaling, hypertrophic scarring, or keloid formation were not observed in any patients. The results of patients' self-evaluation showed that 42.9% of patients' scores were 2 (moderate improvement) and 57.1% of patients' scores were 3 (obvious improvement) (Table 2). All patients were satisfied with the improvement in their acne lesions.

Treatment with 30% SSA Peel Improved Skin Barrier Function

After treatment with 30% SSA peels, we observed an important difference in the indicators of the skin barrier function (Fig. 1b–f). The pH, sebum, TEWL, skin water content, and red areas were significantly improved post-treatment compared with pre-treatment ($P < 0.001$).

Treatment with 30% SSA Peel Decreased the Alpha Diversity of the Skin Microbiome

We analyzed the diversity in the skin microbiomes of patients with AV and healthy participants. The alpha diversity methods determined that the skin bacterial communities diverged significantly between the samples from the faces of healthy participants and those with AV (Simpson diversity index: $P = 0.016$, Chao1 diversity index: $P = 0.019$).

The alpha diversities of the skin microbiomes of the 56 samples before and after the 30% SSA peel treatment were analyzed on the basis of the estimated (Chao1) richness value and the Shannon and Simpson diversity values. The three analyses resulted in similar outcomes, revealing that the skin microbiome prior to the 30% SSA treatment had the highest alpha diversity, followed by the skin microbiome after treatment (Fig. 2a–d). The Shannon and Simpson indices were higher before the treatment than after ($P < 0.05$). We also used unweighted UniFrac distance metrics to perform a principal coordinate analysis of the skin samples. This analysis did not reveal any significant differences before and after the 30% SSA peel treatment.
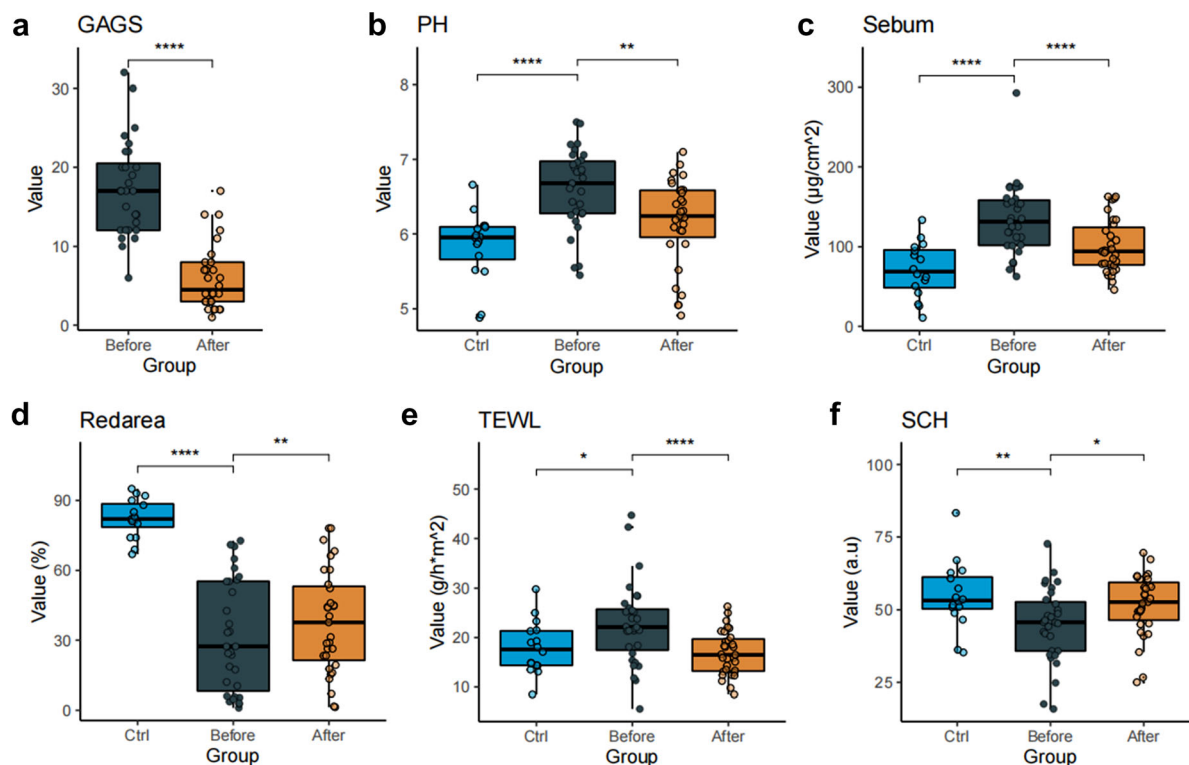
Fig. 1 a Reduction in the GAGS score after treatment with 30% SSA. **b–f** The epidermal barrier function in healthy controls and patients before and after 30% SSA treatment. *GAGS* Global Acne Grading System, *SSA* supramolecular salicylic acid

Table 2 Patient self-assessment scores on the fifth visit

	Self-assessment score				Mean ± SD
	0 (no improvement or worse)	1 (mild improvement)	2 (moderate improvement)	3 (obvious improvement)	
Patients (*n*)	0	0	12	16	2.51 ± 0.51

SD standard deviation

Treatment with 30% SSA Peel Decreased the Abundance of *Staphylococcus* and *Propionibacterium*

The results of sequencing showed that the skin microbiomes of both patients with AV and the healthy controls were dominated by four bacterial phyla (Proteobacteria, Actinobacteria, Firmicutes, and Bacteroidetes) and five genera (*Sphingomonas, Propionibacterium, Pseudomonas, Halomonas,* and *Staphylococcus*). However, the relative abundance of *Staphylococcus* had an increasing trend in patients with AV ($P = 0.012$).

Meanwhile, we found the ten most important bacterial families in relative abundance; the dominant phyla were Proteobacteria, Actinobacteria, Firmicutes, Bacteroidetes, and Chloroflexi, accounting for > 97% of bacteria. The relative abundance of *Staphylococcus* was significantly decreased after treatment in samples collected from the face ($P < 0.05$, Fig. 2e).
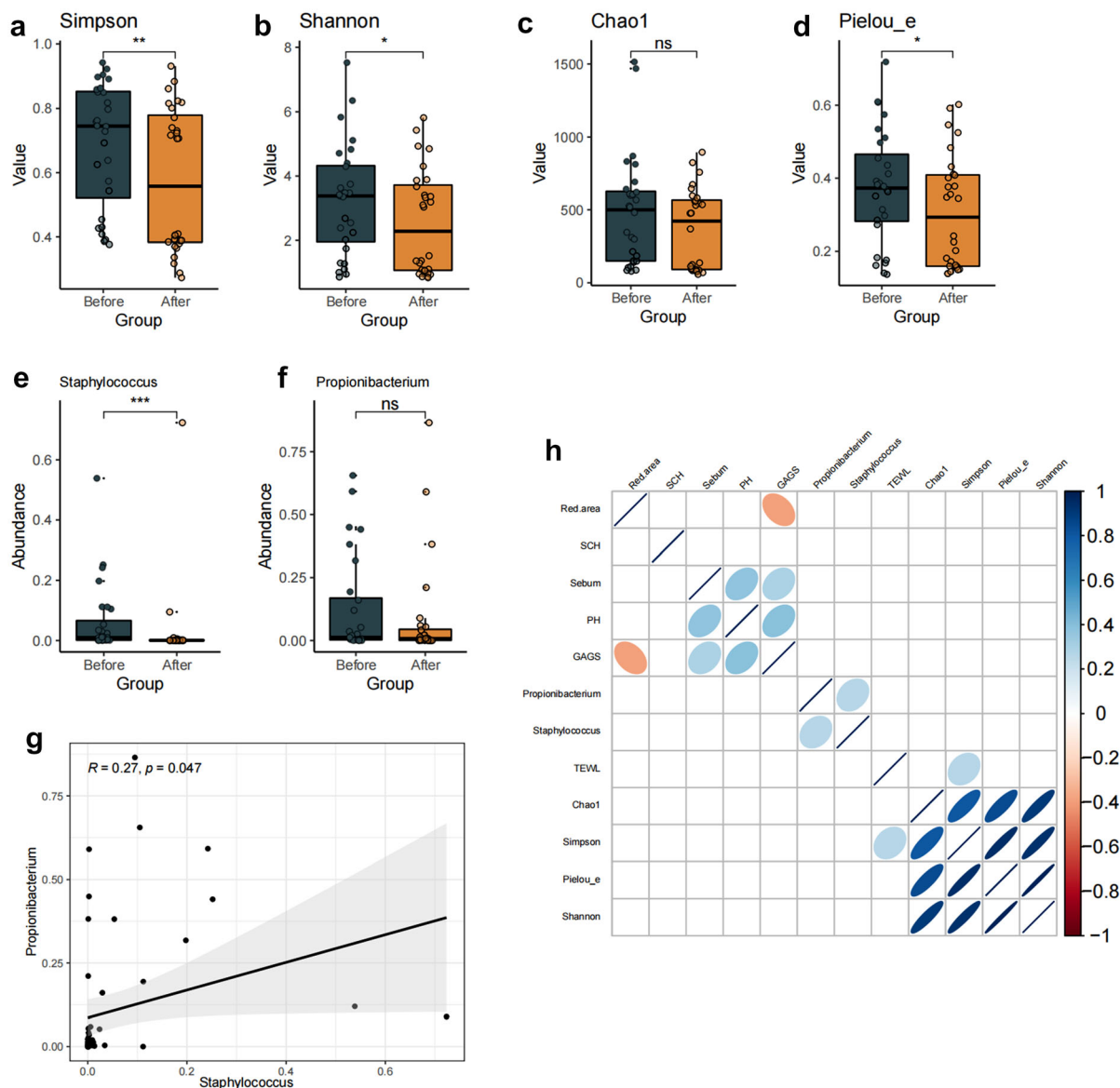
Fig. 2 a–d Boxplots of the alpha diversity of the skin microbiome before and after 30% SSA peel treatment. **e–f** Boxplots of *Staphylococcus* and *Propionibacterium* on the skin surface before and after treatment. **g** Analysis of *Staphylococcus* and *Propionibacterium*. **h** Correlation between *Staphylococcus*, *Propionibacterium*, and the skin biophysical parameters (pH, skin water content, sebum, red area, TEWL, GAGS score). *SSA* supramolecular salicylic acid, *GAGS* Global Acne Grading System, *TEWL* transepidermal water loss

Additionally, there was a trend toward a decrease in the relative abundance of *Propionibacterium* after 30% SSA treatment, but there was no significant difference in the abundance before and after 30% SSA treatment ($P = 0.066$, Fig. 2f).

Moreover, the results of the correlation matrix (Supplementary Fig. S2) of the major bacterial genera showed that the abundance of *Staphylococcus* was positively correlated with that of other genera and significantly positively correlated with that of *Propionibacterium* ($R = 0.47$) (Fig. 2g).
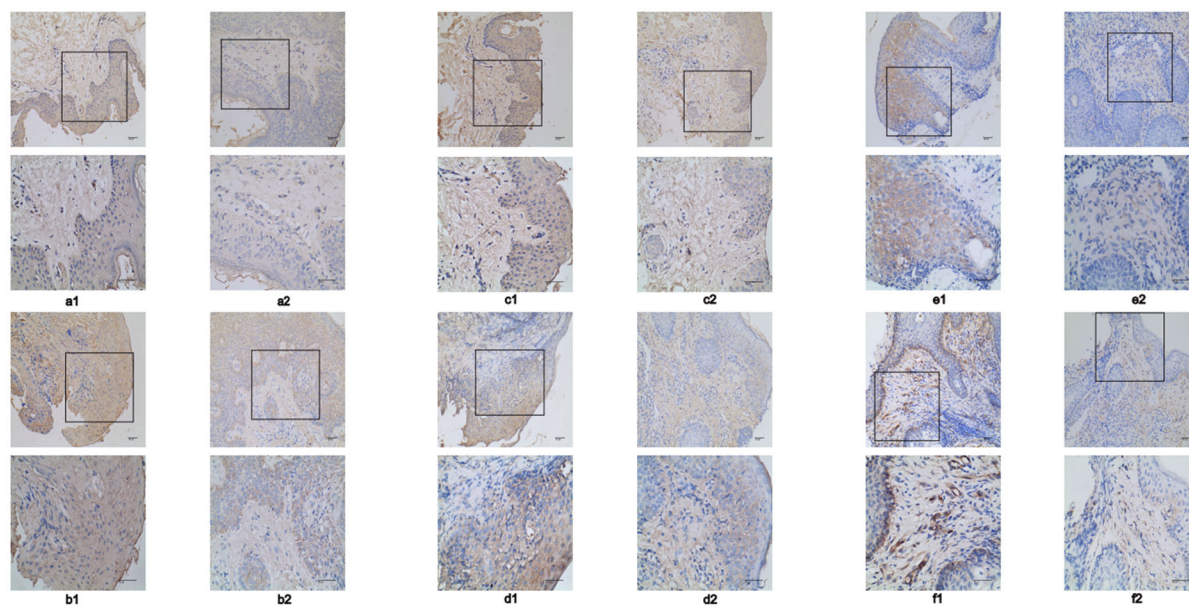
Fig. 3 a1–a2 IL-1a, IL-6, IL-17, TGF-β, TLR-2 expression with intense staining before 30% SSA treatment; IL-1a with weaker staining after 30% SSA treatment (IHC, ×200; IHC, ×400). **b1–b2** IL-6 expression with intense staining before 30% SSA treatment; IL-6 with weaker staining after 30% SSA treatment (IHC, ×200; IHC, ×400). **c1–c2** IL-17 expression with intense staining before 30% SSA treatment; IL-17 with weaker staining after 30% SSA treatment (IHC, ×200; IHC, ×400). **d1–d2** TGF-β expression with intense staining before 30% SSA treatment; TGF-β with weaker staining after 30%

SSA treatment (IHC, ×200; IHC, ×400). **e1–e2** TLR-2 expression with intense staining before 30% SSA treatment; TLR-2 with weaker staining after 30% SSA treatment (IHC, ×200; IHC, ×400). **f1–f2** Caveolin-1 expression with weaker staining before 30% SSA treatment; caveolin-1 with intense staining after 30% SSA treatment (IHC, ×200; IHC, ×400). *IHC* immunohistochemistry, *SSA* supramolecular salicylic acid, *IL* interleukin, *TGF-β* transforming growth factor beta, *TLR-2* toll-like receptor 2

Treatment with 30% SSA Peel Reduced Tissue Expression of Inflammatory Factors

There was a significant difference before and after the 30% SSA peel treatment in terms of changes in the skin tissue with respect to IL-1a, IL-6, IL-17, TGF-β, and TLR-2 expression levels, all of which had a significantly lower staining intensity after the treatment (Fig. 3a–e). However, an increasing trend in staining intensity in the skin tissue was observed for caveolin-1 after treatment (Fig. 3f).

Treatment with 30% SSA Peel Regulated Skin Microbiome by Improving the Epidermal Barrier

We analyzed the correlation between the diversity of the microbiome and the epidermal barrier functions and showed a positive correlation between the Simpson diversity value and TEWL ($R = 0.27$, $P = 0.047$) (Fig. 4a). The correlations between *Staphylococcus*, *Propionibacterium*, and the skin biophysical parameters (skin pH, skin water content, sebum, red area, TEWL, and the GAGS score) were further investigated (Fig. 2h). The abundance of *Staphylococcus* was negatively correlated with the skin water content ($R = -0.44$, $P = 0.018$). Sebum secretion was positively correlated with the GAGS score ($R = 0.3$, $P = 0.027$) and skin pH ($R = 0.38$, $P = 0.004$). In addition, the GAGS score was positively associated with the skin pH ($R = 0.39$, $P = 0.003$) (Fig. 4b–e). Other parameters showed no significant correlations ($P > 0.05$).
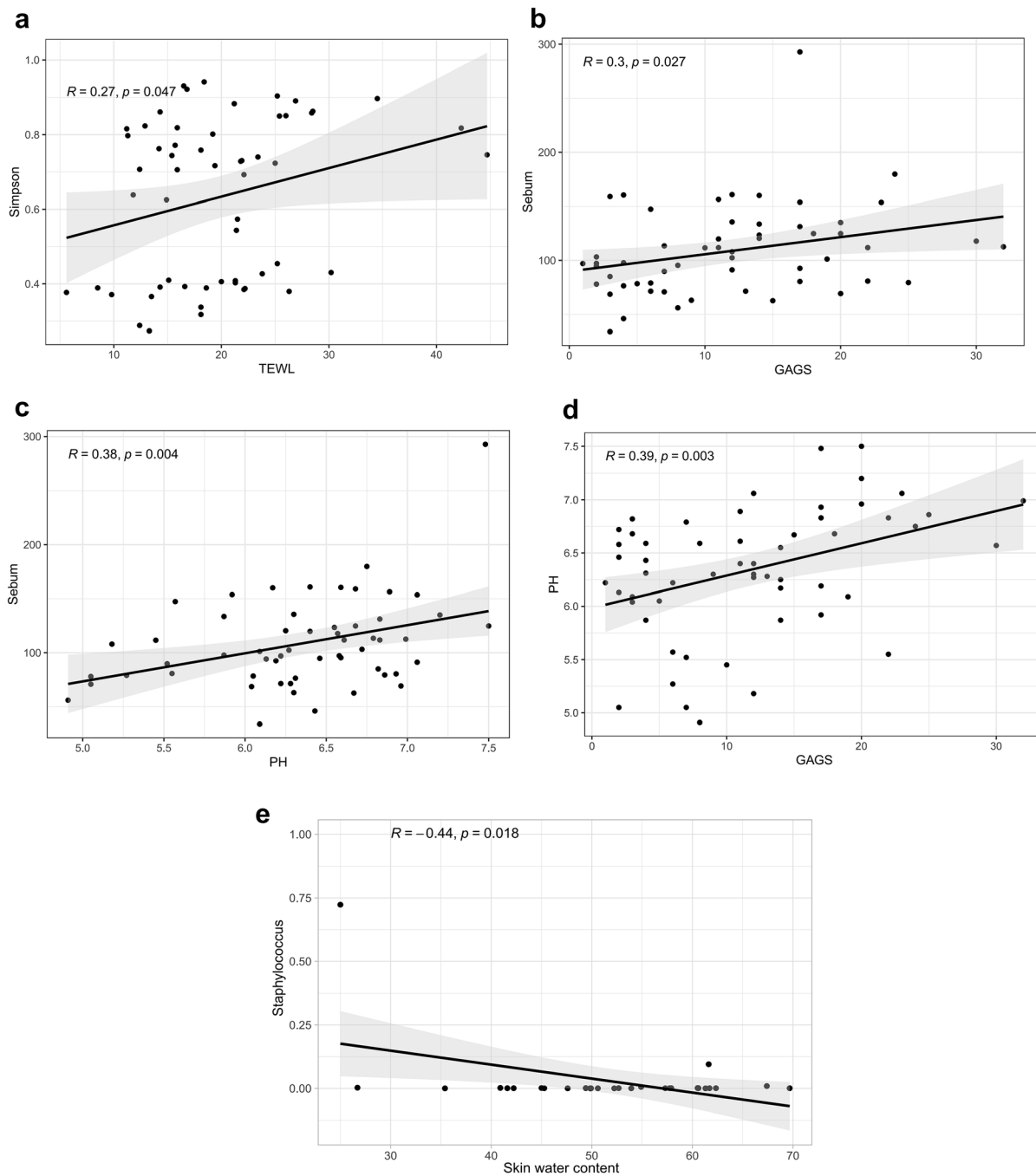
Fig. 4 a Correlation between the Simpson index and TEWL ($R = 0.27$, $P = 0.047$). **b** Correlation between the GAGS score and sebum concentration. **c** Correlation between pH and sebum concentration. **d** Correlation between the GAGS score and pH. **e** Correlation between the skin water content and abundance of *Staphylococcus*. *GAGS* Global Acne Grading System, *TEWL* transepidermal water loss

DISCUSSION

SA peeling is an established, safe, and easy treatment option for AV. Its mechanism for acne treatment includes suppressing the activated protein kinase/sterol regulatory element-binding transcription factor 1 pathway in the sebocytes to reduce sebum production and inhibiting the activity of the nuclear factor kappa B (NF-κB) to control inflammation [23]. Traditional synthetic SA has poor solubility in water. To increase its solubility, it is often dissolved in an organic solvent (i.e., ethanol). However, alcoholic solutions cause skin irritation, which may lead to poor compliance in patients. SSA is a water-soluble complex that delivers SA without alcohol or other solvents, is stable in water, and reduces irritation to the skin. Meanwhile, the effects of a 30% SSA peel are better than those of other concentrations of SSA peels in vitro [24, 25]. In this study, patients with moderate-to-severe AV who were treated with 30% SSA had significantly fewer lesions and lower GAGS scores, suggesting that the treatment was effective. Nevertheless, the mechanism of action of 30% SSA peel treatment remains unclear.

The skin is a physical barrier between the body and the environment, preventing the colonization of pathogens [26]. Changes in the skin microenvironment are closely related to the occurrence and development of many skin diseases [27]. A previous study suggested that the secretion of sebum and TEWL were increased in patients with AV more than in healthy controls [26]. However, the correlation between the skin surface pH and the severity of AV remains controversial [28–30]. In the present study, pH and sebum and TEWL concentrations were significantly decreased, indicating improvement in the skin barrier. Further, Pearson's correlation analysis suggested that the sebum concentration and skin pH were positively correlated with the GAGS score ($R = 0.3$, $P = 0.027$; $R = 0.38$, $P = 0.0041$). This might have resulted from the slow release properties and high permeability of SSA [18]. SSA achieves maximum efficacy in low pH and reduces irritation of the skin. Additionally, the bidirectional regulation of keratinocytes by SSA may play a significant role in the improvement of skin barrier function. At high concentrations of SSA, the upper layer of the stratum corneum is exfoliated owing to the dissolution of desmosomes and a decrease in corneocyte adhesions. However, SSA can increase epidermal thickness at lower concentrations by activating basal keratinocytes [31, 32]. The results suggested that 30% SSA treatment improved moderate-to-severe AV by improving the skin microenvironment.

Human skin is colonized by a wide variety of microbes, which play an important role in skin homeostasis. The dysbiosis of the skin microbiome is implicated in the protection and pathogenesis of various diseases [33, 34]. There is evidence to suggest that *Propionibacterium* and *Staphylococcus* contribute significantly to the pathogenesis of AV [35–37]. The over-colonization of *Propionibacterium acne* triggers the immune response in sebocytes, keratinocytes, and monocytes [38]. In addition, the proportion of *Staphylococcus* increases with the severity of AV [38–41]. In this study, we found that patients with moderate-to-severe AV showed increased amounts of *Staphylococcus* and *Propionibacterium* compared with healthy controls ($P = 0.013$ and $P = 0.0015$, respectively), suggesting that the two genera could be possible biomarkers of moderate-to-severe AV and that their overgrowth may be related to the pathogenesis of acne. A recent study suggests that *Propionibacterium* and *Staphylococcus* are associated with disease flares; *P. acne* may produce a factor or provide an environment that promotes *Staphylococcus* biofilm formation, and an unbalanced equilibrium between *P. acne* and *Staphylococcus epidermidis* contributes to the development of AV [38]. Our study found that the abundance of *Staphylococcus* decreased significantly and that of *Propionibacterium* tended to decrease after SSA treatment. Moreover, the abundance of *Propionibacterium* decreased with the reduction in the abundance of *Staphylococcus* ($R = 0.27$, $P = 0.047$). Our findings add further evidence to corroborate that propionibacteria and staphylococci interact with each other and suggest that 30% SSA may play a therapeutic role by regulating the skin

microbiome in patients with moderate-to-severe AV.

Inflammation plays an important role in the onset, development, and resolution of AV [42]. Inflammation may be associated with changes in the skin surface pH and disturbance of the stratum corneum, allowing microorganisms to stimulate the production of pro-inflammatory cytokines. With the reduction in skin bacteria, the production of pro-inflammatory cytokines is also reduced, thereby improving inflammation and acne severity. Virulent *P. acnes* is one of the most important factors that induce an inflammatory response in acne and activates TLR-2 and TLR-4 in keratinocytes and sebocytes, leading to the activation of signaling cascades and the production of pro-inflammatory cytokines. Subsequently, IL-1, IL-6, IL-17, caveolin-1, and TGF-β cytokines induce innate immunity [43–50]. By analyzing the immunohistochemistry results of the perilesional skin biopsy in patients with moderate-to-severe AV, we found that the levels of inflammatory biomarkers, such as IL-1a, IL-6, IL-17, TGF-β, and TLR-2, were significantly decreased after 30% SSA peel treatment. In addition, the secretion of caveolin-1 in the same area was upregulated after treatment. Thus, 30% SSA treatment significantly reduces the production of pro-inflammatory cytokines in the skin of patients with moderate-to-severe acne and improves local skin inflammation.

Different microenvironments may play a role in the growth or inhibition of microorganisms [26, 30, 51]. In addition, an increase in the skin surface pH leads to impaired barrier function, disturbances in the skin microbiome, and inflammation [34]. Hyperseborrhea favors *P. acnes* overgrowth and biofilm formation, promotes subsequent inflammation, disturbs follicular barrier function, and induces comedogenesis [52]. The sudden changes, together with the activation of innate immunity, might lead to chronic inflammation [53, 54]. In the above studies, 30% SSA affected the skin microenvironment and skin microbiota in patients with moderate-to-severe acne. In addition, it may alleviate the disease by reducing the level of local skin tissue inflammation. Combined with previous studies, we speculated that 30% SSA may improve the skin microenvironment in patients with moderate-to-severe AV, inhibit the colonization of *Propionibacterium* and *Staphylococcus*, regulate the skin microbiota, reduce the production of local pro-inflammatory cytokines, and treat moderate-to-severe AV. A potential limitation of our study is the small sample size and that no metagenomic sequencing was performed.

CONCLUSIONS

Our study demonstrated that 30% SSA treatment can improve the GAGS score of patients with moderate-to-severe AV. On the basis of further analysis of the skin barrier and microbiota, we speculate that 30% SSA may exert its therapeutic effect by improving the skin microenvironment and modulating the skin microbiome, thereby improving local inflammation. Our findings provide a novel insight into the therapeutic rationale of 30% SSA treatment for moderate-to-severe AV.

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Compliance with Ethics Guidelines. This study was approved by the Ethics Committee of the First Affiliated Hospital, Chongqing Medical University (Ethical code number: 2022-066). Written informed consent was obtained from each participant before enrollment, and this study adhered to the Principle of the Declaration of Helsinki. Photography consent was also obtained from each participant before enrollment.

Data Availability. The datasets used and/or analyzed during the current study are available from the corresponding author upon reasonable request.

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