Original paper

Topical application of bacteriocins from *Bacillus subtilis* promotes *Staphylococcus aureus* decolonization in acneic skin and improves the clinical appearance of mild-to-moderate acne

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Abstract

Introduction: Patients with mild-to-moderate acne are frequently colonized by *Staphylococcus aureus* on their skin, which alters microenvironmental skin conditions and exacerbates disease symptoms. Bacteriocins produced by *Bacillus subtilis* may act as antimicrobial peptides against Gram-positive bacteria.

Aim: To investigate whether topical application of bacteriocins from *B. subtilis* could serve as a potential strategy for promoting *S. aureus* decolonization from acneic skin.

Material and methods: The research product was a cream formulation containing 1% bacteriocins from *B. subtilis*. First, we conducted a 60-day pilot study on the effect of topically applied bacteriocins from *B. subtilis* on the absolute abundance of *S. aureus* in 12 patients with mild-to-moderate acne. Second, we designed an 8-week, uncontrolled, open-label, multicentre clinical study to investigate whether the topical application of bacteriocins from *B. subtilis* reduces the number of inflammatory and non-inflammatory lesions, as well as Global Acne Grading Scale (GAGS) scores, in 373 patients with mild-to-moderate acne.

Results: At the microbiological level, quantitative PCR showed a decrease in the absolute abundance of *S. aureus* in acne areas after topical application of the research product for 60 days (–38%, $p < 0.001$). In the clinical study, the number of inflammatory and non-inflammatory lesions was found to decrease at 8 weeks by 59% ($p < 0.001$) and 58% ($p < 0.001$), respectively, compared with baseline. A 56% decrease was observed for GAGS scores.

Conclusions: Topical bacteriocins from *B. subtilis* can promote *S. aureus* decolonization in acneic skin, ultimately improving the clinical appearance of mild-to-moderate acne.

Key words: bacteriocins, acne, *Staphylococcus aureus*, Global Acne Grading Scale.

Introduction

Acne vulgaris is a common chronic skin disorder of the sebaceous follicles, which can affect up to 50.9% of women and 42.5% of men throughout their 20s and may continue to occur throughout adulthood [1]. The disease pathogenesis is complex and involves interplays between hyperplasia of the sebaceous glands, the subsequent formation of microcomedones associated with hyperkeratinisation of the follicular wall, and the induction of inflammatory reactions in keratinocytes and sebocytes [2, 3]. Apart from *Cutibacterium acnes*, which can perpetuate the pathogenetic process of acne through the induction of proinflammatory and chemotactic molecules

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[4, 5], acneic skin is frequently colonized with *Staphylococcus aureus*. Using a high-throughput sequencing approach, Dreno *et al.* [6] found that Staphylococci were more abundant on the surface of comedones, papules, and pustules than on non-lesional skin. Taheri *et al.* [7] also reported that light emitted from digital screens may promote the proliferation of *S. aureus*, which may in turn be associated with acne pathogenesis. Jusuf *et al.* [8] were able to identify *S. aureus* growth in both non-inflammatory and inflammatory acne lesions. Additionally, both antibiotics and dietary supplements containing probiotics may reduce the *S. aureus* carriage rate in facial acneic skin [9, 10].

Bacteriocins are bacterially produced, ribosomally synthesized antibacterial peptides secreted for defence against the growth of closely related bacterial species [11, 12]. Bacteriocins produced by Gram-positive, aerobic, and endospore-forming *Bacillus subtilis* – including subtilin and subtilosin – are active against many strains of gram-positive bacteria, including *S. aureus* [13]. They mainly restrict the growth of pathogenic bacteria by promoting pore formation on the target cell surface. Given their safety and stability [12], we reasoned that topically applied bacteriocins from *B. subtilis* could have therapeutic potential in acne by promoting *S. aureus* decolonization.

Aim

Two distinct investigations were therefore undertaken. First, we conducted a 60-day pilot study on the effect of topically applied bacteriocins from *B. subtilis* on the absolute abundance of *S. aureus* in the skin of 12 patients with mild-to-moderate acne. Second, we designed an 8-week, uncontrolled, open-label, multicentre clinical study to investigate whether the topical application of bacteriocins from *B. subtilis* may reduce the number of inflammatory and non-inflammatory lesions, as well as *Global Acne Grading Scale* (GAGS) scores, in patients with mild-to-moderate acne.

Material and methods

Materials

The topical cream tested in this study contained bacteriocins from *B. subtilis* (1% weight/weight; Biodue S.p.A., Tavarnelle Val Di Pesa, Italy) in a moisturizer base.

Procedures

Patients with active facial acne of mild-to-moderate severity (*Global Acne Grading Scale* [GAGS] score from 1 to 30) [14, 15] were eligible for inclusion. Subjects were excluded if they had previously received oral retinoids, oral antibiotics, tretinoin, or benzoyl peroxide. In addition, patients with diabetes mellitus, endocrine disease, or severe physical illnesses and those who were currently using oral contraceptives, implantable contraceptives, or steroids were excluded [16]. All participants involved in the microbiological and clinical investigations were asked to withdraw any topical product 14 days before the beginning of the studies. Moreover, they were not allowed to use any topical intervention throughout the entire study period. The study participants were instructed to apply the topical cream over acne areas twice per day (morning and evening) for 60 days in the microbiological study and for 8 weeks in the clinical study. There were no known protocol deviations during the study. Both investigations were approved by the local Ethics Committee (identifier: 2021/14E) and were in accordance with the tenets of the Helsinki Declaration. Written informed consent was obtained from all participants.

Microbiological study

The microbiological study was aimed at assessing whether topical application of the cream containing bacteriocins from *B. subtilis* was able to promote *S. aureus* decolonization in acne areas. A total of 12 patients (6 males and 6 females; age range: 22–35 years) were recruited from Italian private practices. The sample size for this pilot study was chosen based on feasibility and costs. Skin swab specimens from facial acne areas before and after 60 days of topical treatment (paired samples) were obtained by trained personnel. Quantitative real-time PCR for absolute *S. aureus* quantitation was carried out as previously described [17]. In brief, total nucleic acid from acneic skin swab specimens was amplified and the DNA concentration was quantified using the CFX96 Real-Time System (Bio-Rad, Hercules, CA, USA). Standard curves for the absolute abundance values of *S. aureus* in collected specimens were constructed using seven 10-fold dilutions (from 4 ng/µl to 4 fg/µl) of *S. aureus* USA300 [18].

Clinical study

This 8-week, uncontrolled clinical study had an open-label design. We did not conduct a power analysis to determine sample size; instead, we used a convenience sample. A total of 373 Caucasian patients with mild-to-moderate acne (139 males and 234 females, mean age: 22.0 ±6.4 years) were included. All participants were enrolled from Italian private practices and dermatology clinics. Clinical assessments were performed at baseline and 8 weeks thereafter. The primary endpoint of the clinical study was the changes in the number of inflammatory and non-inflammatory lesions, as well as GAGS from baseline to the end of the study. GAGS considers 6 locations on the face and chest/upper back, with a factor for each location based roughly on surface area, distribution, and density of pilosebaceous units [14]. The severity was graded as mild if the score was 1–18, moderate with scores from 19 to 30, severe with scores from

31 to 38, and very severe with scores of more than 38 [15]. The secondary endpoints were tolerance and overall satisfaction with the topical cream. Tolerance was assessed by asking patients about any signs or symptoms of local (burning, itching, or stinging sensation) or systemic adverse reactions. The overall satisfaction was rated on a 4-point scale, as follows: excellent, good, average, or poor.

Statistical analysis

Data are presented using descriptive statistics. Paired Student's *t*-tests were used to compare pre- and post-treatment data in both investigations. All analyses were carried out in SPSS (version 20.0; IBM, Armonk, NY, USA), and statistical significance was determined by a 2-tailed *p*-value < 0.05.

Results

Microbiological study

The results of the pilot microbiological study revealed that the absolute abundance of *S. aureus* in facial acne areas in the 12 subjects who underwent paired skin swab sampling decreased after 60 days of treatment with topically applied bacteriocins from *B. subtilis*. Based on quantitative real-time PCR results, the mean absolute abundance of *S. aureus* was 2024 ±338 fg/µl at baseline and 1254 ±214 fg/µl at 6 months, i.e. a 38% decrease ($p < 0.001$).

Clinical study

All patients ($n = 373$) successfully completed the study. The number of inflammatory lesions significantly decreased by 59% ($p < 0.001$, paired Student's *t*-test) after 8 weeks of treatment with the topical cream; the number of inflammatory lesions was 10.89 ±8.61 at baseline and 4.39 ±5.92 at 8 weeks. Similarly, the number of non-inflammatory lesions significantly decreased by 58% ($p < 0.001$, paired Student's *t*-test) after 8 weeks of treatment with the topical cream; the number of non-inflammatory lesions was 13.36 ±8.60 at baseline and 5.60 ±4.39 at 8 weeks. The mean GAGS score was 11.98 ±9.21 at baseline and 5.17 ±4.22 at 8 weeks, i.e. a 56% decrease ($p < 0.001$, paired Student's *t*-test). Representative images of a patient before (left panel) and after (right panel) 8 weeks of treatment with bacteriocins from *B. subtilis* are shown in Figure 1. The treatment was well-tolerated, and none of the patients reported burning, itching, or stinging sensation after topical application. No patient discontinued treatment due to adverse local or systemic effects. The overall satisfaction with the topical cream was rated as excellent by 188 (50.4%) patients, good by 168 (45%) patients, average by 15 (4%) patients, and poor by 2 (0.6%) patients.

Discussion

The results of our microbiological and clinical studies indicate that topically applied bacteriocins from *B. subtilis* – a safe bacterial-derived ingredient [13] – may decrease the number of both inflammatory and non-inflammatory skin lesions as well as GAGS scores in patients with mild-to-moderate acne. Our results suggest that the clinical effects of bacteriocins from *B. subtilis* may be related to the capacity to promote *S. aureus* decolonization in acne areas. Notably, the treatment approach was safe and was associated with good satisfaction levels.

After 60 days of topically applied bacteriocins from *B. subtilis*, we found a statistically significant 38% decrease in the absolute abundance of *S. aureus* in the acneic skin. An important strength of our microbiological study is that we focused on the absolute bacterial abundance as the main outcome. Growing evidence suggests that the absolute abundance is not only paramount for species competition within microbial communities but can also be a key regulator of *S. aureus* virulence [19]. In this scenario, investigating the modifications in absolute rather than relative abundance is likely to have major clinical implications. In an era of increasing emphasis on the risks of resistance to antibiotic treatment [20], the microbiological activity described in our study makes the topical application of bacteriocins from *B. subtilis* an excellent option for rapid decolonization of acneic skin from *S. aureus*.

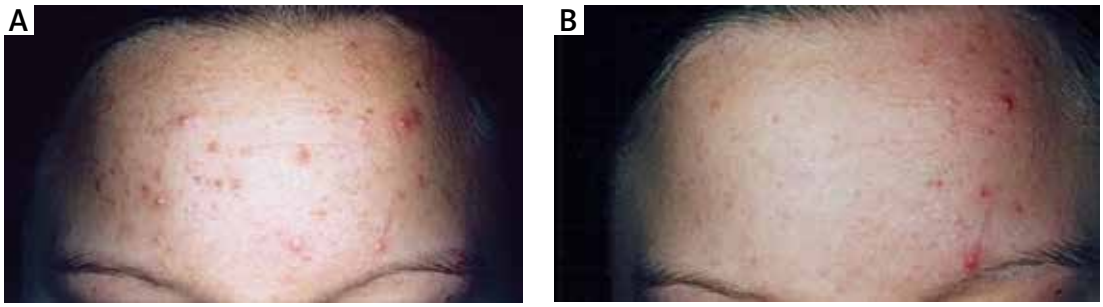

Figure 1. Representative images of a patient before (**A**) and after (**B**) 8 weeks of treatment with bacteriocins from *Bacillus subtilis*. A significant improvement in the clinical appearance of acne was evident (baseline: number of inflammatory lesions = 14; number of non-inflammatory lesions = 19; after 8 weeks: number of inflammatory lesions = 3; number of non-inflammatory lesions = 7)

We thus speculate that the observed clinical effects of topically applied bacteriocins on the number of inflammatory and non-inflammatory lesions as well as on GAGS scores may be mediated by a reduction in the absolute abundance of *S. aureus* in acne areas. Although our data require confirmation in large placebo-controlled trials, our proof-of-concept evidence suggests that bacteriocins from *B. subtilis* may serve as a topical strategy to improve the clinical appearance and the severity of acne, potentially representing an alternative to avoid the harmful effects of systemic antibiotic therapy on the microbiota of other body sites.

The main limitations of our study include the exclusive focus on Caucasian patients, the lack of a placebo arm, and the fact that the microbiological study was performed only in a subset of participants ($n = 12$). Currently, our results cannot be considered as a basis for treatment recommendations. Patients with mild-to-moderate acne should be treated on an individual basis according to each patient's characteristics based on the results of well-designed clinical trials. Our study did not compare the effects of topically applied bacteriocins with those of topical antibiotics. Finally, we specifically focused on *S. aureus* as the main bacterial species of interest. It is plausible that a decrease in *S. aureus* abundance could lead to changes in the skin bacterial community (including *C. acnes*), which could in turn reduce local cutaneous inflammation, although this possibility needs to be proven by further investigations.

Conclusions

The results of our microbiological and clinical studies indicate that topically applied bacteriocins from *B. subtilis* can promote *S. aureus* decolonization in acneic skin, ultimately improving the clinical appearance of mild-to-moderate acne. Further studies are necessary to determine the optimal dose and treatment duration with topical bacteriocins in acne. Future research directions include examining how the whole skin microbiota of acneic patients would change in response to topical application of bacteriocins, and determining if bacteriocins extracted from bacteria other than *B. subtilis* (e.g. the probiotic strain *Lactobacillus salivarius* LS03 [21]) might be useful for clinical use in acneic patients.

Conflict of interest

This study was partly funded by Biodue SpA. The funding sponsor had no role in the design of the study; in the analysis or interpretation of data; in the writing of the manuscript, and in the decision to publish the results.

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