New Approach in Acne Therapy

A Specific Bacteriocin Activity and a Targeted Anti IL-8 Property in Just 1 Probiotic Strain, the L. salivarius LS03

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Goals: The aim of this research was to assess the antibacterial activity of *Lactobacillus salivarius* LS03 (DSM 22776) against *Propionibacterium acnes* and its anti-inflammatory properties by inhibiting *P. acnes*-induced interleukin-8 (IL-8) release.

Background: Acne is the most common skin disease, causing significant psychosocial problems for those afflicted. Currently available agents for acne treatment, such as oral antibiotics, have limited use. Thus, development of novel agents to treat this disease is needed. In the generation of inflammatory lesions, proliferation of *P. acnes* in the obstructed follicles is critical. The administration of beneficial microorganisms represents a promising approach for treating several skin alterations and can have many favorable effects.

Study: For the inhibition assay, *P. acnes* was spread on Propionibacter Isolation Agar Base plates, and LS03-soaked disks were placed directly on the agar surface. Peripheral blood mononuclear cells, isolated from healthy volunteers, were preincubated with phytohemagglutinin 1 μg/mL for 1 hour and stimulated with the probiotic strains for 24 hours to simulate an in vitro IL-8 release model. The IL-8 concentration in the supernatants was analyzed in duplicate using ELISA Kit.

Results: *L. salivarius* LS03 exerted a significant inhibitory capacity against the target pathogen strain. This antagonistic activity was primarily ascribable to the feature of LS03 strain of secreting active bacteriocins against *P. acnes*. Concerning the IL-8 analysis, 3 different *L. salivarius* strains were able to inhibit the release of this chemokine by 10% to 25%.

Conclusions: *L. salivarius* LS03 probiotic strain could be an alternative treatment to antibiotic/anti-inflammatory therapy in subjects presenting acne vulgaris.

Key Words: acne, interleukin-8, inflammation, probiotic strain, antimicrobial activity

(*J Clin Gastroenterol* 2018;52:S78–S81)

In recent years, significant progress has been made in the understanding of the pathophysiological mechanisms of acne and the role of *Propionibacterium acnes.*[1]

Acne vulgaris is the most common skin disease, causing significant psychosocial problems for those afflicted.[2] It is very common, affecting almost 80% of adolescents and young adults aged 11 to 30 years.[3,4] Acne is frequently associated with depression, anxiety, and other psychological ailments.[5] Accordingly, mental health impairment scores are higher among acne patients compared with a number of other chronic, nonpsychiatric medical conditions.[6]

The pathophysiology of acne includes hyperseborrhea, abnormal follicular keratinization, and *P. acnes* proliferation in the pilosebaceous unit. Found primarily on the face and upper trunk (chest and back), these specialized follicles have protruding sebaceous glands associated with them.[7,8] Excessive shedding of epithelial cells from the follicle walls combined with increased amounts of sebum produced by associated sebaceous glands are 2 important factors that contribute to follicular obstruction.[9–11] This obstruction leads to the formation of microcomedos, which most probably represent the precursor lesions of acne. The microcomedo can progress into either a noninflammatory comedo or become inflamed and present as a papule, pustule, or nodule.[2]

P. acnes are gram-positive, anaerobic bacteria that are part of the normal skin biota.[12] *P. acnes* seems to play a central part in the development of acne lesions both early and late in the pathophysiological process.[13] In the generation of inflammatory lesions, proliferation of *P. acnes* in the obstructed follicles is believed to be critical.[14] This is based on the in vitro evidence that *P. acnes* stimulates monocytes to produce proinflammatory mediators and chemotactic factors via Toll-like receptor 2 and protease-activated receptors, and time-tested clinical observations that antibiotic-inhibiting *P. acnes* improve inflammatory acne.[11,15] *P. acnes* secretes lipases, metalloproteases, chemotactic factors, and porphyrins. All interact with molecular oxygen, generating toxic, reactive oxygen species and free radicals, causing keratinocyte damage.[15] Furthermore, this bacterium contributes to the development of retentional lesions by increasing the proliferation of keratinocytes and the expression of proteins implicated in the differentiation of keratinocytes. A recent study found that cell-free extracts of *P. acnes*, through a Toll-like receptor 2-dependent signaling pathway, were capable of upregulating secretion of proinflammatory cytokines by activating NF-κB and p38 MAP kinases' pathways in human SZ95 sebocytes.[16] In the light of the central role of *P. acnes* in the pathogenesis and persistence of acne vulgaris, antibiotic use directed against this bacterium has been the backbone of acne treatment for over 50 years.

Interleukin-8 (IL-8), also known as CXCL8, is a CXC-type chemokine often associated with inflammation and whose activity is increased by oxidative stress; for this reason, it is considered a key parameter in localized inflammation. IL-8 has been identified as the leading proinflammatory mediator in acne.[17] It is a potent chemotactic factor that predominantly exerts its effects on neutrophils.[18]

From the Biolab Research S.r.l., Novara, Italy.
Supported by Probiotical S.p.A.
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DOI: 10.1097/MCG.0000000000001053

In addition, androgens are believed to contribute to the pathogenesis of acne by influencing the growth of follicular corneocytes.[2]

Acne is unmistakably not primarily an infectious disease, and merely killing *P. acnes* might improve this condition, even if not necessarily resulting in disease resolution or cure.[19]

Currently available agents for acne treatment, such as topical and oral antibiotics, have limited use. This is mainly attributable to the steady increase of antibiotic resistance, with many countries facing the evidence that >50% of *P. acnes* strains are resistant to topical macrolides.[2] The overall incidence of *P. acnes* resistance increased from 20% in 1978 to 62% in 1996. A correlation has been shown between the emergence of resistant *P. acnes* and antibiotic use.[20] Outstandingly, countries with low resistance levels have restricted antibiotic use to treat acne, which emphasizes the need to reduce their use at a global level.[21,22] Resistant *P. acnes* strains can emerge quickly, for example, topical clindamycin monotherapy results in an increase in resistant *P. acnes* count to >1600% of baseline values by week 16.[23] Data from Hong Kong provide evidence of a connection between the development of antibiotic-resistant *P. acnes* biotypes and increased age, a longer duration of acne, and a longer duration of antibiotic treatment.[24] Oral antibiotics still have a role in the pharmacological approach to moderate-to-severe acne, but only with a topical retinoid, benzoyl peroxide, or their combination, and ideally for no longer than 3 months.[25]

One of the biggest challenges of recent years has been the attempt to treat dermatitis and, in particular, acne with natural methods, avoiding massive topical use of soaps and topical or systemic oral antibiotic treatments. Thus, development of novel agents to alleviate this disease is needed.

On the basis of evidence that as many as 40% of acne patients have hypochlorhydria, Stokes and Pillsbury[26] theorized that insufficient stomach acid may induce the relocation of colonic bacteria toward distal portions of the small intestine, also profoundly modifying the normal intestinal microbiota. Hypochlorhydria has been confirmed in recent years to be a significant risk factor for small intestinal bacterial overgrowth (SIBO).[5] SIBO has been connected with increased intestinal permeability, whereas rectification of SIBO by antimicrobial treatment helps to restore the normal intestinal barrier.[27] Although the frequency of SIBO in acne vulgaris has not been precisely quantified yet, a recent study reports that SIBO is 10 times more predominant in subjects with acne rosacea compared with healthy controls.[28] Probiotic oral administration has also proven beneficial in the reduction of SIBO.[29]

On the basis of the aforementioned, administration of beneficial microorganisms represents a promising approach for treating several skin alterations and can have many favorable effects.

The aim of this research was to assess the antibacterial activity of *Lactobacillus salivarius* LS03 (DSM 22776) against *P. acnes* and also its anti-inflammatory properties by inhibiting *P. acnes*-induced IL-8 release.

MATERIALS AND METHODS

Bacterial Strain Growth Conditions

The *Lactobacillus* strain was grown overnight in De Man, Rogosa, and Sharpe (MRS) broth (Difco, BD, MD) at 37°C.

Disk Diffusion Method

The different concentrations of *L. salivarius* LS03 culture were evaluated at different pH values: pH 4 and pH 7 (modified with NaOH). *P. acnes* (ATCC 11827) was spread on Propionibacter Isolation Agar Base plates (HiMedia Laboratories) and LS03-soaked disks were placed directly on the surface of the agar. The plates were incubated under microaerophilic conditions at 37°C for 48 hours, after which the diameters of the inhibition zones were measured in millimeters. In this study, the MRS broth was used as a negative control. The plate inhibition technique experiments were carried out in duplicate, and the mean values of growth inhibition zones around the disks were measured using a ruler.[30]

Peripheral Blood Mononuclear Cell Isolation

Peripheral blood mononuclear cells (PBMCs) were isolated either from fresh buffy coats obtained from the local blood bank or from the peripheral blood of normal volunteers as described.[31] Briefly, after obtaining the necessary consent from the donor, a fresh buffy coat was mixed gently with an equal volume of 2.5% Dextran T500 and left for 40 minutes for erythrocyte sedimentation. Ten millileters of leukocyte-rich supernatant was recovered and layered over 5 mL of Ficoll-Histopaque and centrifuged for 30 minutes at 350*g* at 4°C. The PBMC-rich ring was recovered and washed twice in phosphate-buffered saline. PBMCs were then resuspended in RPMI medium supplemented with 100 U/mL penicillin, 100 μg/mL streptomycin, and 2 mM L-glutamine, and allowed to adhere at 37°C on 24-well plates (2×10^6 cells/well). Purified monocyte/macrophage populations were obtained by adhesion (90 min, 37°C, 5% CO_2), with nonadherent cells (mainly lymphocytes) being removed by gentle washing; cell viability (trypan blue dye exclusion) was usually >98%.

IL-8 Release In Vitro Cellular Model

PBMCs were preincubated with phytohemagglutinin (PHA) 1 μg/mL for 1 hour and stimulated with probiotic strains for 24 hours to simulate an in vitro IL-8 release model. After 24 hours, samples were centrifuged, and supernatants were collected and kept at −80°C until analyzed. The content of IL-8 in the supernatants was analyzed, in duplicate, using kit ELISA (EBioscience, Human IL-8 ELISA Ready-Set-Go, 2nd Generation). The human IL-8 ELISA research-use-only kit was employed for the quantitative determination of IL-8 in samples using 96-well plates and a microplate reader.[32]

Statistical Analysis

The statistical analysis of results was conducted using the *t* test for paired data, with differences regarded as significant if the *P*-value was below 0.05.

RESULTS

Inhibition Assay

As can be seen from Figure 1, the strain *L. salivarius* LS03 has exerted a surprising and superior inhibitory capacity against the target pathogen strain.

The results also showed that no inhibition zone was observed in association with a negative control (MRS).

This antagonistic activity was predominantly attributable to the feature of LS03 strain of secreting active bacteriocins against *P. acnes*.

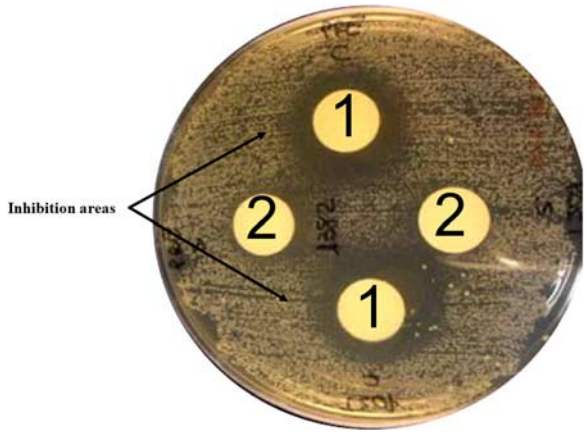

FIGURE 1. Inhibitory activity of *Lactobacillus salivarius* LS03 in a representative plate of *Propionibacterium acnes* and LS03-soaked disks. 1, active cell culture; 2 = MRS, negative controls. MRS indicates De Man, Rogosa, and Sharpe.

IL-8 Release Evaluation

This study suggested that 3 different *L. salivarius* probiotic strains (Fig. 2) are able to inhibit the release of IL-8 produced by PHA-activated PBMCs significantly from 10% to 25%. In particular, LS01 (DSM 22775), LS02 (DSM 20555), and LS03 (DSM 22776) were able to reduce PHA-activated IL-8 production in a PBMCs model by 17%, 16%, and 27%, respectively.

DISCUSSION

Experimental and human studies have revealed that a variety of physiological and psychological stressors—extremes of temperature, confinement, academic examination, crowding, and acoustics—can unsettle normal intestinal microbiota.[33,34] Most remarkable among these stress-induced changes are reductions in lactobacilli and bifidobacteria species.

In addition, there have been clues that intestinal permeability may be amplified in acne vulgaris.[5] Gut microbes may enhance the presence of circulating endotoxins in acne vulgaris patients, indicating that intestinal permeability is a potential issue for many acne patients.

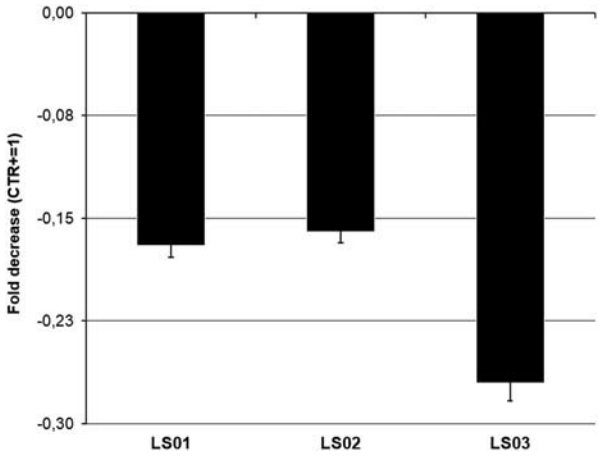

FIGURE 2. Interleukin-8 release in human monocytes/macrophages. Cells were pretreated for 1 hour with phytohemagglutinin and then stimulated by probiotic strains (prokaryotic cells/eukaryotic cells ratio: 1:1) for 24 hours.

Some studies have suggested the usefulness of selected probiotic administration in subjects affected by acne vulgaris.

An Italian study involving 40 patients has involved the supplementation of an oral formulation containing 250 mg freeze-dried *L. acidophilus* and *Bifidobacterium bifidum* as an adjuvant to standard care. The probiotic-supplemented group showed better clinical outcomes, but even better tolerance and compliance with antibiotics.[35]

A recent investigation involving 56 acne patients recognized that consumption of a *Lactobacillus*-fermented dairy beverage improved the clinical score of acne over 12 weeks. Specifically, the probiotic drink efficiently reduced the total lesion count in association with a relevant reduction in sebum production. Even if the addition of lactoferrin (an anti-inflammatory milk protein) to the probiotic product increased the efficacy of inflammatory lesion attenuation, the benefits recorded with the probiotic drink alone offer further sustenance to the opinion that probiotics have an adjuvant role to play in acne therapy.[36]

Furthermore, *P. acnes* growth has been shown to be quietened by certain bacteria-secreted substances, such as antimicrobial peptides and organic acids, from various bacterial strains.

A recent in vitro study has shown that *Bifidobacterium* strains, isolated from stool samples of healthy donors, can hinder the growth of *P. acnes*. In particular, *Bifidobacterium adolescentis* SPM0308 and *Bifidobacterium longum* SPM1207 reduced the viability of *P. acnes* by 84% and 75%, respectively.[37]

Our results have shown that *L. salivarius* LS03 is able to exert a significant antimicrobial activity against *P. acnes* in conjunction with an anti-inflammatory effect. A noteworthy inhibition of the release of IL-8 by PBMCs was shown as well by all the 3 *L. salivarius* tested, namely LS01, LS02, and LS03.

The use of these probiotic strains, with such an incisive action in the inhibition of IL-8, is of fundamental importance for limiting the proinflammatory action of this chemokine in the inflammatory site and the infection by *P. acnes* as well. IL-8 is often increased by oxidative stress, making it a key parameter in localized inflammation; for this reason, it has been identified as the main proinflammatory mediator in acne.

In the light of the literature discussed, our in vitro results suggest that especially *L. salivarius* LS03 probiotic strain could be a valid alternative treatment to antibiotic/anti-inflammatory therapy in subjects presenting acne vulgaris. Anyway, future experimentations in humans will be needed to more completely investigate the activity of this beneficial bacterium.

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