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ARTICLE

The effect of *Bacillus subtilis* mouth rinsing in patients with periodontitis

S. Tsubura · H. Mizunuma · S. Ishikawa · I. Oyake · M. Okabayashi · K. Katoh · M. Shibata · T. Iizuka · T. Toda · T. Iizuka

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Abstract *Bacillus subtilis* is an effective probiotic product for prevention of enteric infections both in humans and animals. We hypothesized that a mouth rinse containing *Bacillus subtilis* should adhere to and colonize part of the oral bacteria on periodontal tissue. The rinsing ability of Extraction 300E (containing *Bacillus subtilis:* E-300) was compared with that of a mouth wash liquid , Neosteline Green (benzethonium chloride; NG) that is commonly used in Japan. Compared with NG rinsing, E-300 rinsing resulted in a marked change in the BANA-score. The mean BANA values (score ± SD) over the course of the study from 0 to 30 days were 1.52±0.51 ($p \leq 0.1$) and 0.30±0.47 ($p \leq 0.01$) for E-300, and 1.56±0.51 and 0.93±0.68 for NG, respectively. Gingival Index also had improvement, while probing pocket depth and bleeding on probing showed small improvements. Mouth rinsing with E-300 significantly reduced periodontal pathogens compared with NG. These results suggest that *Bacillus subtilis* is an appropriate mouth rinse for patients with periodontitis.

Introduction

Periodontitis is defined as a chronic disease caused by multiple factors and components, including oral bacteria and the host immune system [1]. To date, there is no comprehensive one-step treatment. In general, periodontitis has been treated by surgery (scaling and root planing, periodontal surgery) and/or chemical treatments (antibiotics, such as amoxicillin, tetracycline, and azithromycin) [2, 3]. However, the use of antibacterial drugs as a conventional method is associated with some risks, such as the production of drug-resistant bacteria and allergic reactions in the elderly [4].

On the other hand, it is well known that the use of mouth rinsing material has an effective and distinct role in the management of patients with periodontitis [5–7]. Furthermore, it has been reported that gastrointestinal tract "probiotics" may need some modifications or additions when used as oral probiotics [8]. For instance, oral

S. Tsubura (✉)
Tsubura Dental Clinic,
9-6 Enomachi, Utsunomiya,
Tochigi 320-0802, Japan
e-mail: tshu@ucatv.ne.jp

H. Mizunuma
Mizunuma Dental Clinic,
1490-2 Motegi, Motegimachi,
Tochigi 321-3531, Japan

S. Ishikawa
Ishikawa Dental Clinic,
1-8-4 Kanai, Nasukarasuyama,
Tochigi 321-0628, Japan

I. Oyake
Oyake Dental Clinic,
2815 Himuro, Utsunomiya,
Tochigi 321-3232, Japan

M. Okabayashi
Okabayashi Dental Clinic,
6-6 Hatsune, Nasukarasuyama,
Tochigi 321-0628, Japan

K. Katoh
Katoh Katsuma Dental Clinic,
1129-2 Ohta, Takanezawa, Shioya,
Tochigi 329-1217, Japan

M. Shibata
Shibata Dental Clinic,
271-15 Imaichi, Nikko,
Tochigi 321-1261, Japan

T. Iizuka · T. Toda · T. Iizuka
AHC Co., Ltd,
343-1 Kosogimachi, Maebashi,
Gunma 371-0831, Japan

probiotic bacteria should adhere to and colonize periodontal tissue, and they should become part of the biofilm.

The commercial product, Extraction 300E (E-300, AHC Co. Gunma, Japan), is prepared from the supernatant of cultured medium of *Bacillus subtilis* isolated from Japanese soil, and it has been used as a prevention of enteric infections for livestock or a control for human's skin-care substances. E-300 contains 0.9 g/100 ml of *Bacillus subtilis*, which is supposed to improve human immunologic activity. E-300 has been shown to activate macrophages and natural killer cells, stimulate the production of immature leucocytes, and induce interferon [9, 10].

Since *Bacillus subtilis* may provide some protection against infections caused by oral pathogens, the effect of mouth rinsing with E-300 on periodontal inflammation was examined.

Materials and methods

From May 2007 to April 2008, 54 non-smoker subjects (21 males, 33 females; mean age, 53.4 years; age range, 44–62 years) with chronic periodontitis and with no severe general diseases were selected. They also had at least 20 natural teeth, including at least eight sites with probing depth (PD)>4 mm on molar teeth. All participants were selected from patients referred to the authors' clinics. The study protocol was approved by the committee of Ethical Affairs of the Nippon Dental University and was conducted according to the principles outlined in the Declaration of Helsinki on experiments involving human subjects. The subjects gave their written informed consent before participating in the study. Participants received initial periodontal treatment, such as dental prophylaxis to remove all supragingival plaque, stain, and calculus. The test group rinsed with E-300, and the control group rinsed with a widely used material, Neosteline Green (NG), containing benzethonium chloride 0.2 g/100 ml.

After the initial periodontal treatment, mouth rinsing was done with three drops (0.1 ml) of E-300 or NG in water (20 ml) for 20 seconds every morning and evening for 1 month.

Tooth brushing was done three times per day without using any toothpaste, and none of the teeth received any treatment during this study. During the study, participants followed their usual dietary habits, but they were instructed to refrain from using any other commercial mouth rinses. Four anterior or premolar teeth that had a pocket deeper than 4 mm and had similar probing depths and gingival inflammation levels were selected for assessment of clinical parameters and subjected to microbial tests.

Clinical parameters

Probing pocket depth (PPD), bleeding on probing (BOP), and the gingival index (GI) were assessed for all participants at baseline, 14 days (2 weeks), and 30 days (1 month) after subgingival scaling and root planing (SRP). Each examiner performed all measurements on six sites per tooth, excluding third molars.

PPD and BOP were assessed using a manual periodontal probe (CP10SE; HuFriedy, Chicago, IL, USA). First, a periodontal probe with a 0.5-mm diameter tip was inserted into the gingival crevice and swept from the distal aspect to the mesial aspect of the tooth at a depth of about 1 mm and at an angle of about 60 degrees, while in contact with the sulcular epithelium. Second, BOP was then recorded and scored as present/absent by running the probe 1–2 mm into the gingival crevice. Gingivitis of the buccal and lingual marginal gingival and interdental papillae of all scorable teeth was scored using the Loe–Silness GI on a 4-point scale from 0 (absence of inflammation) to 3 (severe inflammation) [11].

Microbial assessment

The BANA test (BANAMet LLC, USA) is a chair-side diagnostic system that is highly sensitive and specific for determining the presence of "red-complex" periodontal pathogens (*Porphyromonas gingivalis, Treponema denticola, and Tannerella forsythia*) [12]. Plaque was collected for BANA analysis from the same tooth sites that were tested for the clinical parameters at 0, 14, and 30 days. After incubation for 5 minutes at 35°C with Evan's black dye solution, naphthylamine released as a result of the presence of any one of the BANA-hydrolyzing bacterial species diffuses to form a permanent blue-black color. The relative intensity of the blue color (strong positive, positive, or negative) was assessed.

Statistical methods

E-300 and NG groups were compared with the mean baseline PD, BOP, GI, and BANA using an analysis of variance and Fisher's protected least significant difference test.

Results

Fifty-four participants were examined according to the protocol and all of them completed the study.

Clinical findings

The PPD and BOP scores (mean ± SD) on days 0, 14, and 30 are shown in Tables 1 and 2, respectively. E-300 had no demonstrable effect on PPD and BOP at 30 days

Table 1 Mean probing pocket depth (mm ± SD) of E-300 and NG

Group	*N*	Baseline	14 days	30 days
E-300	27	4.8±0.75	4.5±0.51	4.2±0.60
NG	27	4.7±0.72	4.0±0.65	3.5±0.51

compared with NG. The GI score (mean ± SD) on days 0, 14, and 30 are shown in Table 3. E-300 showed remarkable GI reduction, although there was no significant difference in GI score compared with NG at 30 days. The BANA scores on days 0, 14, and 30 are shown in Table 4. E-300 was significantly more effective on BANA scores ($p<0.01$) between 0 and 30 days compared with NG.

Discussion

In the last few decades, different applications have been found for *Bacillus subtilis* strains, including the industrial production of proteases, preparation of alkaline-fermented food, and as a probiotic product for the prevention of enteric infections both in humans and in animals [13, 14]. Probiotics are often regulated as dietary supplements and marketed for improving or maintaining health [15].

However, probiotics have been extensively investigated from the perspective of oral health for less than a decade. The literature from the past few years has shown that probiotic administration effectively reduces the number of *Streptococcus mutans*, suggesting a role for probiotics in caries prophylaxis [16]. Moreover, in 2007, Hatakka et al. reported that probiotics also reduced oral *Candida* counts in the elderly and might offer a new strategy for controlling oral yeast infections [17]. However, only a limited number of studies have examined the effectiveness of probiotics for periodontal diseases. In 2006, Krasse et al. showed that gingival bleeding and gingivitis decreased after administration of the probiotic *Lactobacillus reuteri* [18].

Table 2 Mean bleeding on probing (BOP[a]) (score ± SD) of E-300 and NG

Group	*N*	Baseline	14 days	30 days
E-300	27	1.6±0.51	1.0±0.65	0.5±0.51
NG	27	1.6±0.50	1.3±0.53	0.8±0.62

[a] BOP scoring: 0 is non-bleeding to slight bleeding, tooth site of 0–30%; 1 is moderate bleeding, tooth site of 31–60%; 2 is severe bleeding, tooth site of more than 60%

Table 3 Mean gingival index (GI[a]) scores (score ± SD) of E-300 and NG

Group	*N*	Baseline	14 days	30 days
E-300	27	1.9±0.70	1.1±0.62	0.7±0.62
NG	27	1.9±0.75	1.4±0.75	1.0±0.65

[a] GI scores: 0 is normal, 1 is slight gingival inflammation (no bleeding using instrument), 2 is moderate gingival inflammation (bleeding using instrument), 3 is gingival abscess, bleeding

In our study, we evaluated the effect of mouth rinsing with E-300 for patients with periodontitis as a probiotic product. Rinsing with E-300 produced marked changes in the GI and BANA test compared with NG. It is likely that E-300 could play a greater role in situations when improving the condition of the periodontal pocket and local immunocompetence at the oral mucous epithelium. E-300 appears to adjust oral flora, activate gingival fibroblast cells, and induce cytokine production.

Probiotic tablets were placed in the mouth for a few minutes to allow direct contact. These results suggest that E-300 has the capacity to control chronic or acute periodontitis, may be used for implant treatment, and has an effect on oral mucosal disease. The mechanism of the antibacterial effect of rinsing with E-300 is assumed to depend on the inhibition of proteases that originate from oral bacteria.

Therefore, more research is needed to identify appropriate effector strains for oral probiotics specifically designed to prevent and treat periodontal disease. The experimental protocol did not include any oral hygiene instruction before/at baseline; however, subjects in both groups might have systematically altered their oral hygiene regimens due to observation. Many factors, including attention bias, are known to contribute to perceived placebo effects in clinical trials.

E-300 may contribute to human health care not only with respect to oral problems but also with respect to control of host immunological conditions.

Table 4 Mean BANA-score[a] (score ± SD) of E-300 and NG

Group	*N*	Baseline	14 days	30 days
E-300	27	1.5±0.51	0.7±0.54	0.3±0.47[b]
NG	27	1.6±0.51	0.7±0.54	0.9±0.68

[a] BANA score: 0 is negative, 1 is positive, 2 is strong positive

[b] Significant difference from baseline, $p<0.01$

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