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ORIGINAL ARTICLE

Clinical changes in periodontal subjects with the probiotic *Lactobacillus reuteri* Prodentis: A preliminary randomized clinical trial

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Abstract

Objectives. The aim of this study was to assess the clinical effect of the administration of *Lactobacillus reuteri* Prodentis as a probiotic agent in the treatment of initial to moderate chronic periodontitis. Secondary objectives were to evaluate the patient 'compliance' factor and to observe the potential side-effects of the probiotic agent. ***Materials and methods.*** Twenty systemically healthy, non-smoking subjects with initial-to-moderate chronic periodontitis were enrolled in this 1-month double-blind, placebo-controlled, randomized clinical trial. Subjects were randomly assigned to receive tablets containing *Lactobacillus reuteri* Prodentis or placebo once a day for 30 days. Clinical parameters were collected at baseline and 30 days post-treatment. ***Results.*** Periodontal clinical parameters were improved in the test group after a 30-day intervention. The test group demonstrated a statistically significant reduction ($p < 0.05$) in all the periodontal parameters included in the study (plaque index, bleeding on probing and pocket probing depths), while the control group treated with placebo did not show any statistically significant change in periodontal parameters. ***Conclusions.*** These data indicate that oral administration of *Lactobacillus reuteri* Prodentis improved the short-term clinical outcomes in non-smoking patients with initial-to-moderate chronic periodontitis.

Key Words: *probiotics, lactobacillus reuteri prodentis, chronic periodontitis, randomized-controlled clinical trial*

Introduction

Periodontitis is a progressive, destructive disease that affects the supporting tissues of the teeth, including the alveolar bone [1]. The main pathogenic agents associated with periodontitis are *Porphyromonas gingivalis*, *Treponema denticola*, *Tannerella forsythia* and *Aggregatibacter actinomycetemcomitans* [2]. These bacteria have a variety of virulent characteristics allowing them to colonize the subgingival sites, escape the host's defense system and cause tissue damage [3].

Conventional periodontal treatment involves mechanical supra and subgingival debridement, which results in reductions of the total subgingival microbiota [4]. However, recolonization toward pre-treatment levels occurs within weeks [5] and re-establishment of a pathogenic microbiota occurs within months [6,7]. Thus, a life-long need for re-treatment arises, creating a serious socio-economic problem. Additionally, increasing levels of antibiotic-resistant bacteria favour the development of approaches that do not rely on antibiotics.

According to the World Health Organization in 2001, probiotic bacteria are defined as live micro-organisms which, when administered in adequate amounts, confer a health benefit on the host [8].

Beneficial bacteria have been extensively studied for their health-promoting effects. While the beneficial impact of probiotic bacteria is well established for certain gastrointestinal diseases [9], its possible role in combating oral infections is sparsely investigated [10,11].

Several clinical studies have already demonstrated the effectiveness of certain probiotics in the treatment of systemic and infectious diseases, such as acute diarrhoea and Crohn's disease [12] and in the treatment of cardiovascular diseases, urogenital infections, oropharyngeal infections and cancer [13,14].

The potential application of probiotics for oral health has recently attracted the attention of several teams of

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researchers suggesting that probiotics could be useful in preventing and treating oral infections, including dental caries [15,16], periodontal disease [17,18], halitosis [19] and infections by *Candida albicans* [20].

Various studies have reported the capacity of lactobacilli to inhibit the growth of periodontopathogens [21,22]. These observations suggest that lactobacilli residing in the oral cavity could play a role in the oral ecological balance.

In the past few years, probiotics have been investigated for periodontal health [23]. Teughels et al. [24] have shown the benefits of the application of beneficial bacteria, as an adjunct of periodontal treatment, demonstrating inhibition of the recolonization of pathogens in periodontal pockets and reduction of bleeding on probing.

Other clinical trials have demonstrated a reduced prevalence of moderate-to-severe gingival inflammation as well as an improved plaque index and probing depth in adults after regular use of probiotics chewing gums or tablets [25].

Recently, Shimazaki et al. [26] used epidemiological data to assess the relationship between periodontal health and the consumption of dairy products such as cheese, milk and yoghurt. The authors found lower probing depths and less loss of clinical attachment in individuals who consumed these dairy products.

However, data are still sparse and thus more information is needed on the colonization of probiotics in the oral cavity and their possible effects. Due to the globalization and increasing problems with antibiotic resistance, the alternative concept of probiotic therapy deserves further research in the field of periodontal health. With the number of bacteria-resistant diseases on the rise and the length of time it takes to develop new antibiotics, it might be time to consider another alternative in the treatment of periodontal disease.

The aim of this double-blind randomized placebo-controlled was to clinically evaluate the impact of probiotic therapy in non-smoking patients with initial-to-moderate chronic periodontitis.

Materials and methods

This study was a randomized placebo-controlled, parallel design, double-blind clinical trial with a 1-month follow-up. Ethical approval (C-40-ASA-08) was obtained from the Ethics Committee of Universitat Internacional de Catalunya, Barcelona, Spain and the study was conducted according to the principles outlined in the Declaration of Helsinki on experimentation involving human subjects.

Subjects and study schedule

Twenty-seven patients were assessed for their eligibility before entering the study. Of these, seven subjects were excluded; five because they did not meet the inclusion criteria and two because they refused to participate. Thus, 20 patients participated in the study. One patient from the placebo group was withdrawn because she had to take antibiotics during the study period.

Recruitment was carried out from January 2009 to July 2010. Data entry of the information and statistical analysis were performed by the end of January 2011.

Probiotic product

Gum PerioBalance® (Sunstar, Switzerland) is the first probiotic specifically formulated to fight periodontal disease. It contains a patented combination of two strains of *Lactobacillus reuteri* (ATCC 55730 and ATCCPTA 5289) specifically selected for their synergetic properties in fighting periodontopathogens.

Each tablet contains at least 2×10^8 living cells of *L.reuteri* Prodentis. Users as advised to use a tablet every day in the evening after brushing their teeth, to allow the probiotics to spread throughout the oral cavity and attach to the various oral surfaces.

Population screening

Subjects eligible for the study were identified from the population referred to the dental clinic of Universitat Internacional de Catalunya, Barcelona, Spain.

Subjects who fulfilled the study inclusion/exclusion criteria were provided with oral information and also a written information sheet related to the study protocol. All participants signed and they were invited to participate in the study. Informed consent was obtained from all the subjects to be entered in the study.

The inclusion criteria were: subjects aged 18 years or more, non-smokers with chronic periodontitis according to the criteria at the 1999 International Classification by Armitage [27], good general health, stated availability throughout the entire study period and willingness and capacity to comply with the protocol.

The exclusion criteria included: pregnant or lactating females, subjects who required antibiotic pre-medication for the performance of periodontal examination, people who had antibiotic or antiseptic treatments in the previous 3 months, use of all forms of nicotine (smoking, substitute gums, ...) and systemic diseases such as uncontrolled diabetes, cardiovascular disease and infectious diseases.

Randomization

Subjects were assigned in ascending order at the enrolment visit and were randomly assigned by a computer-generated table to receive one of the two treatments. A balanced random permuted block approach (5-unit block size) was used to prepare randomization tables in order to avoid unequal balance between the two treatments.

The randomization table was sent to the manufacturer (GUM, Sunstar) in Switzerland, which prepared the tablets containing *Lactobacillus reuteri* Prodentis and the placebo tablets with the same appearance, colour and shape.

The randomization code was not open until all data had been collected and analysed.

Investigator calibration

The single examiner (M.V) measured full-mouth pocket probing depths (PPD) and recessions. After completion of all measurements, the intra-examiner repeatability was assessed. The examiner showed 98.7% reproducibility.

Clinical examination

A total of 20 patients were randomized into either the test group or the placebo group. The subjects were asked to continue their normal dietary and oral hygiene habits during the study period and not to use any oral antimicrobial preparations (such as mouth rinses or breath fresheners) or antibiotics during the 30-day study period. None of them received any mechanical periodontal treatment during that same period.

A complete periodontal examination was conducted including a full medical and dental history, an intra-oral examination and a full-mouth periodontal probing. Registration of probing pocket depths (PPD), Plaque Index (PI) and bleeding on probing (BOP) was done.

Clinical parameters were assessed using a manual probe CP-15 UNC (Hu- Friedy Co., Chicago IL) by the calibrated examiner (MV) at six sites per tooth, excluding third molars.

The Plaque Index (PI) was recorded by assigning a binary score to each surface (1 for plaque present, 0 for absent) and calculating the percentage of total tooth surfaces that revealed the presence of plaque detected by the use of a periodontal probe [28].

Similarly, bleeding on probing (BOP) was calculated as a percentage of bleeding surfaces vs all surfaces according to Ainamo and Bay [28]. In this registration a bleeding point is considered when bleeding emerges within 10 s after probing.

Probing pocket depths (PPD) were measured in mm as the distance from the gingival margin to the location of the tip of a periodontal probe inserted in the pocket with moderate probing force [29]. PPD were recorded at six sites of every tooth present: mesio-buccal, mid-buccal, disto-buccal, disto-lingual, mid-lingual and disto-lingual.

After the initial examination the subjects were given either test or placebo product. Both active and placebo were identical in taste, shape and texture. The patients were instructed to dissolve the tablet in the mouth once a day, directly after dental hygiene procedures in the evening. The tablet should be dissolved in the mouth allowing it to spread throughout the oral cavity and to attach to the dental surfaces. They were also instructed not to change their oral hygiene regimens and not to take other probiotic products throughout the test period. Neither professional prophylaxis nor tooth-brushing instructions was performed during or before the experimental period.

The study or placebo product was taken daily for 30 days, after which the subjects were re-examined in the same way as in the baseline investigation.

Data management and statistical analysis

Data were entered into an Excel (Microsoft Office 2008) database. The database was subsequently locked, imported into SPSS 15.0 version for Windows, formatted and analysed.

The significance of differences between test and placebo groups in baseline was evaluated according to distribution. Using the independent samples *t*-test for age, PI, BOP, PPD 4–5 mm and PPD 6 mm (all of them normally distributed) and the χ^2 test for gender (categorical data).

The significance of the difference within each group before and after treatment was evaluated with the paired samples *t*-test if it's normally distributed or non-parametric Wilcoxon's sign-rank test in the other case. For parametric and non-parametric tests, a p-value < 0.05 was considered as statistically significant.

Results

Subject and clinical characteristics at baseline

The baseline characteristics of the 20 subjects who participated in the study are displayed in Table I. The mean age of the participants was 58.0 (51.4–64.7) years for the test group and 53.8 (44.3–63.1) years for the placebo group. Females accounted for 20% of the test group and 56% of the placebo group. None of these demographic parameters showed a statistically significant difference between groups.

The baseline examination revealed that the two study groups showed similar characteristics for percentage of pockets, plaque and bleeding levels, with no significant differences between the two groups (Table I).

Clinical measurements

Plaque index. Plaque scores decreased in the test group from baseline to 1 month and the difference was statistically significant. However, in the placebo group an increase in the mean values occurred suggesting a non-statistically significant ($p = 0.421$) increase of plaque accumulation among the placebo group during the study period.

Table I. Demographic and clinical characteristics at baseline. Mean values ± standard deviation.

	Test group (n = 10)	Placebo group (n = 9)	p-value
Age (95% CI)	58.0 (51.4–64.7)	53.8 (44.3–63.1)	0.403 (t-student)
Gender	F:20%, M:80%	F:56%, M:44%	0.130 (Chi^2)
Plaque index (%)	69.5 ± 16.95	62.9 ± 24.21	0.500 (t-test)
Bleeding on probing (%)	55.3 ± 16.39	40.0 ± 23.36	0.124 (t-test)
% Pocket probing depths 4–5 mm	50.1 ± 17.92	38.1 ± 16.37	0.146 (t-test)
% Pocket probing depths ≥ 6 mm	12.3 ± 16.13	13.7 ± 16.42	0.905 (Mann-Whitney test)

All p-values were greater than 0.05.

The mean PI (%) at baseline was 69.5 for the test group and 62.9 for the control group. At visit 2, after use of the probiotic agent, the mean PI was 52.5 for the test group and 67.4 for the control group. The change in PI between visit 1 and visit 2 for the test group was statistically significant ($p = 0.009$), demonstrating a reduction of the plaque index after the use of the probiotic agent; however, the control group demonstrated a non-statistically significant increase in PI after the use of the placebo tablets (Table II).

Bleeding on probing. The mean BOP (%) at baseline was 55.3 for the test group and 40.04 for the control group. At visit 2, after the use of the probiotic agent, the mean BOP was 29.3 for the test group, showing a statistically significant reduction ($p = 0.005$) in bleeding on probing after the use of the *Latobacillus reuteri* Prodentis (Figure 1), but the control group demonstrated a non-statistically significant increase in BOP with a mean BOP of 47.0 (Figure 2).

Pocket probing depths (4–5 mm). The percentage of sites (median) with PPD of a specific threshold at baseline and the changes in the percentage of pockets within groups (difference between baseline and 30 days) are reported in Table II.

The mean percentage of sites with PPD of 4–5 mm at baseline for the test group was 50.1 and 38.1 for the control group. After 30 days of treatment the mean PPD for the test group showed a statistically significant reduction ($p = 0.022$), with a mean of 40.4, and the control group increased the PPD mean to 45.3.

Pocket probing depths (≥ 6 mm). The mean percentage of sites with PPD ≥ 6 mm at baseline for the test group was 12.3 and 13.7 for the control group. After the study period the mean PPD for pockets ≥ 6 mm for the test group decreased to 7.5 with a p-value = 0.012, showing a statistically significant reduction after the administration of the probiotic agent (Figure 1). For the control group, the reduction in PPD between visit 1 and 2 was not statistically significant ($p = 0.889$), with a mean PPD of 13.4 (Figure 2).

The reduction of clinical parameters after administration of the probiotic agent is significantly superior to the variation after administration of the placebo.

Adverse events and compliance

At the 30-day follow-up visit, 10 subjects in the test group (100%) and nine subjects in the placebo group (100%) reported no adverse effects. The types of adverse effects they were asked were: stomach upset,

Table II. Summarizes the changes in clinical parameters (PI, BOP, PPD 4–5 mm and PPD ≥ 6 mm) for the test and placebo groups during the 30-day-period intervention. Mean values ± standard deviation before and after probiotic or placebo treatment.

Index/Treatment	Baseline	Day 30	p-value
Plaque index (%)	69.5 ± 16.95	52.5 ± 14.25	0.009* (t)
Probiotic	62.9 ± 24.21	67.4 ± 16.57	0.421 (t)
Placebo			
Bleeding on probing (%)	55.3 ± 16.39	29.3 ± 15.04	0.005* (W)
Probiotic	40.0 ± 23.36	47.0 ± 17.43	0.314 (W)
Placebo			
% sites with pocket probing depths 4–5 mm	50.1 ± 17.92	40.4 ± 17.76	0.022* (t)
Probiotic	38.1 ± 16.37	45.3 ± 10.38	0.121 (t)
Placebo			
% sites with pocket probing depths ≥ 6 mm	12.3 ± 16.13	7.5 ± 11.40	0.012* (W)
Probiotic	13.7 ± 16.42	13.4 ± 13.31	0.889 (W)
Placebo			

W, Wilcoxon test; t, t-student test.
*Statistically significant.

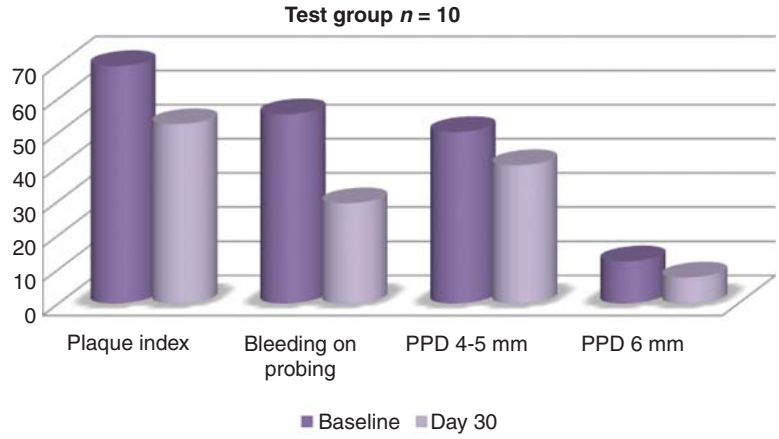

Figure 1. Variation of periodontal clinical parameters (%) before and after the probiotic treatment.

gastrointestinal disorder (diarrhea), metallic taste, headache and nausea/vomiting.

Compliance with the course of the intake of tablets and the number of pills not taken by the participants were also documented. All subjects returned the medication bottles. Nineteen subjects from both groups (100%) completed the course of tablets as indicated.

Discussion

The data indicated that the experimental therapy with probiotics resulted in clinically significant short-term improvements in clinical periodontal disease parameters.

This study is in accordance with previous studies that have demonstrated the potential benefit of using replacement therapies in patients with periodontal disease [17,18,24,26,30]. In contrast to our study, the population target of those studies was not patients with initial-to-moderate chronic periodontitis. A lack of evidence among this group of patients was considered in order to choose the experimental population.

The percentage of PPD reduction at 1 month for initial pockets of 4–5 mm was 19% after the probiotic treatment and 38% reduction from initial pockets of ≥6 mm. This is also consistent with the results of different studies [17,31].

Plaque index and bleeding on probing were decreased after treatment with probiotics in the test group. Our results are also comparable with the study of Twetman et al. [32], where *Lactobacillus reuteri*-containing chewing gums were used used in 42 healthy patients and they assessed effects on crevicular fluid volume, cytokines (interleukin-1β, interleukin-6, inteleukin-10 and TNF-α) levels and bleeding on probing. Bleeding on probing was significantly reduced.

In our study, a reduction of clinical indices was not observed in the placebo group subjects; however, a non-significant increase of clinical indices was reported. We considered it unlikely that this phenomenon was induced directly by the placebo tablets. The experimental protocol did not include any oral hygiene instruction before or at baseline, although subjects in both groups might have systematically altered their oral hygiene regimens due to observation. Many factors including attention bias contribute to perceive placebo effects in clinical trials, even in large-scale placebo-controlled RCTs [33].

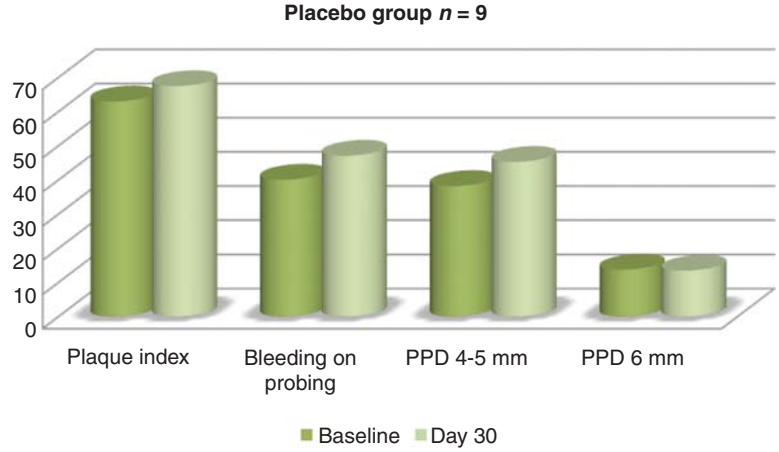

Figure 2. Variation of periodontal clinical parameters (%) before and after the placebo treatment.

Our randomized clinical trial results suggest that probiotics with *Lactobacillus reuteri* Prodentis may effectively improve the periodontal condition. These results are in accordance with previous studies that have demonstrated that periodontal parameters are improved by the use of probiotic agents. In the study by Krasse et al. [17], subjects with moderate-to-severe gingivitis were included. The subjects were given one of two different *L. reuteri* formulations or a placebo. Gingival index decreased significantly in all three groups and one of the *L. reuteri* formulations (LR-1) improved gingival index significantly more than the placebo group. We also investigated the effect of the probiotic lactobacilli on periodontal parameters and found that oral administration of *Lactobacillus reuteri* Prodentis improved the periodontal clinical parameters such as probing depths, bleeding on probing and plaque index. Shimauchi et al. [18] and colleagues evaluated the effect of probiotic intervention on the periodontal condition of subjects without severe periodontitis. A total of 66 volunteers received either *L.salivarius* WB21 containing tablets with xylitol or xylitol alone. Periodontal parameters were improved in both groups after 8 weeks. Any possible differences in smokers could not be verified in our study, since smokers were excluded from the study population.

With a similar study design, Vivekananda et al. [31] demonstrated recently the plaque inhibition, anti-inflammatory and anti-microbial effects of *Lactobacillus reuteri* Prodentis during non-surgical therapy and the maintenance phase of periodontal treatment. Thirty non-smoking patients with chronic periodontitis were included in the study. The study period was 42 days and the participants took *L. reuteri* Prodentis tablets or the corresponding placebo tablets twice daily from day 21 to day 42 in a split-mouth design protocol. At day 42 plaque index, gingival index, gingival bleeding index, pocket probing depths and clinical attachment level were significantly reduced by scaling and root planning plus administration of probiotic agent.

The selection of the 'best' probiotic for oral health is also a controversial issue [34]. We selected *Lactobacillus reuteri* as the probiotic for the present study because *Lactobacilli* are found in the oral cavity and have been shown to have varying ability to interfere with the growth of oral pathogens [35–40]. The selection of *Lactobacillus reuteri* in our study is also comparable with some other studies [17,31,32].

Limited information is available about appropriate probiotic dosing regimens and only a few dose-comparison studies have been undertaken [41]. In this study we prescribed one tablet of *Lactobacillus reuteri* Prodentis per day. The rationale is based on the study of Twetman et al. [32], where a dose–response relationship or a threshold level seemed to appear, but it would be too early to propose any clinical recommendations at this stage. The choice of dosage comes from an analysis of previous studies and the manufacturer's recommendation.

The fact that 100% of test subjects completed the full cycle of probiotics demonstrated that this dosage of one tablet per day allows for complete compliance from patients, which is critical from a clinical point of view. Compliance with the study protocol was judged acceptable based on interviews and by picking up the distributed tablet pots.

The issue of safety is of special concern due to the increased probiotic supplementation. None of the participants in our study presented with any adverse event. This result is in accordance with previous studies [42], which similarly did not identify any negative 'side effects' or tolerance problems associated with the consumption of *Lactobacillus reuteri*.

In conclusion, the findings of the present study have indicated that the use of probiotic *Lactobacillus reuteri* Prodentis in non-smoking patients with initial-to-moderate chronic periodontitis has resulted in significant improvements in clinical conditions compared with placebo. These observations are valid after 1 month of treatment.

Our results in this study suggest that a probiotic intervention with *Lactobacillus reuteri* Prodentis could be a useful tool for treatment of inflammation and clinical symptoms of periodontitis, especially in non-smoking subjects with initial-to-moderate chronic periodontitis. This approach may provide a valuable addition or alternative to the armamentarium of treatment options for periodontitis.

Limitations of the present study include the relatively small number of participants and the short-term study period. Therefore, further long-term studies including a large-scale RCT are necessary to determine the utility of probiotics as an alternative approach for the treatment and prevention of periodontal diseases. The best means of administering probiotics and the dosages needed for different preventive or therapeutic purposes are still under investigation.

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