Clinical Effects of *Lactobacillus rhamnosus* in Non-Surgical Treatment of Chronic Periodontitis: A Randomized Placebo-Controlled Trial With 1-Year Follow-Up

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Background: Probiotics are living microorganisms that provide beneficial effects for the host when administered in proper quantities. The aim of this double-masked placebo-controlled parallel-arm randomized clinical trial is to evaluate the clinical effects of a *Lactobacillus rhamnosus* SP1-containing probiotic sachet as an adjunct to non-surgical therapy.

Methods: Twenty-eight systemically healthy volunteers with chronic periodontitis were recruited and monitored clinically at baseline and 3, 6, 9, and 12 months after therapy. Clinical parameters measured included plaque accumulation, bleeding on probing, probing depths (PDs), and clinical attachment loss. Patients received non-surgical therapy, including scaling and root planing (SRP), and were assigned randomly to a test (SRP + probiotic, n = 14) or control (SRP + placebo, n = 14) group. The intake, once a day for 3 months, of an *L. rhamnosus* SP1 probiotic sachet commenced after the last session of SRP.

Results: Both test and control groups showed improvements in clinical parameters at all time points evaluated. However, the test group showed greater reductions in PD than the control. Also, at initial visits and after 1-year follow-up, the test group showed a statistically significant reduction in the number of participants with PD ≥6 mm, indicating a reduced need for surgery, in contrast to the placebo group.

Conclusion: The results of this trial indicate that oral administration of *L. rhamnosus* SP1 resulted in similar clinical improvements compared with SRP alone. *J Periodontol 2016;87:944-952.*

KEY WORDS

Chronic periodontitis; *Lactobacillus rhamnosus*; probiotics; root planing.

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Chronic periodontitis (CP) is an inflammatory process that affects the attachment structures of teeth. Periodontitis constitutes the second most frequent cause of tooth loss worldwide[1-3] and in the Chilean adult population,[4] where it affects >90% of adults.[5] Moreover, studies[6,7] performed in South and Central America have shown that the prevalence of severe disease was high (>30%) in those populations.

Conventional treatment modalities of periodontal disease include non-surgical and/or surgical management, with an emphasis on mechanical debridement. Improvements in clinical parameters are achieved when the levels, proportions, and percentage of sites colonized by different periodontal pathogens are effectively reduced after therapy and a new microbial community with higher proportions of host-compatible microorganisms is established.[8] However, mechanical debridement as a sole therapy is not always effective in improving clinical parameters.[9] Therefore, the association of mechanical debridement with systemic antibiotics has been introduced in the treatment of periodontal diseases.[10] These treatment modalities are aimed at eliminating the

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entire microbiota, irrespective of its pathogenicity. Because of the emergence of antibiotic resistance and frequent recolonization of treated sites with pathogenic bacteria,[11,12] there is need for new treatment paradigms in periodontal disease management.

Probiotics are defined as live microorganisms that, when administered in adequate amounts, confer a health benefit on the host.[13] Probiotics have been used to directly modify the resident oral microbiome[14] and proposed to modulate immune responses.[15] *Lactobacillus* constitutes the most common bacterial genus used as a probiotic.[16] *Lactobacillus rhamnosus* SP1, also known as *L. rhamnosus* GG, is a well-documented gut probiotic strain[17] with decades of safe use for improving the gastrointestinal health and immune modulation.[18]

Clinical human studies have shown improvements in periodontal parameters after the use of this probiotic.[19-25] Evidence suggests that probiotics could be beneficial during periodontal therapy because they may aid in the reduction of pathogenic bacteria[14] and/or serve as anti-inflammatory adjuncts.[15,26] However, a recent review concludes that more studies are necessary to evaluate the efficacy of probiotics in oral health maintenance[27] and to understand the mechanisms by which ingested bacteria target microbiome functions contributing to the prevention and management of major health concerns.[28] This study proposes that a treatment involving non-surgical therapy plus intake of a *L. rhamnosus* SP1-containing probiotic sachet may result in improved clinical effects compared with conventional mechanical therapy for CP.

MATERIALS AND METHODS

Patient Population and Clinical Criteria for Inclusion and Exclusion

This trial started in October 2013 and finished in March 2015. This double-masked placebo-controlled parallel-arm randomized clinical trial (RCT) was conducted in accordance with the Declaration of Helsinki of 1975, as revised in 2013. The Local Ethical Committee of the Faculty of Dentistry at University of Chile approved the clinical trial (Decision 2012/08). The protocol of the study was explained to all patients, and written informed consent was obtained after explanation of the purpose, nature, risks, and benefits of participating in this study.

Individuals seeking periodontal care or referred for periodontal care to the Diagnosis Center of the Faculty of Dentistry, University of Chile, were screened for the study. Forty-nine volunteers were examined initially, of which 28 were included in the present study (14 males and 14 females, aged 35 to 68 years; mean age: 49.8 years). Inclusion criteria for entry were as follows: 1) healthy, non-institutionalized male or female patients; 2) aged ≥35 years; 3) presence of a minimum of 14 natural teeth, excluding third molars; 4) presence of ≥10 posterior teeth; and 5) previously untreated periodontitis. Exclusion criteria were as follows: 1) having received any periodontal treatment before the time of examination; 2) suffering any systemic illness; 3) having received antibiotics or non-steroidal anti-inflammatory therapy in the 6-month period before the study; and 4) pregnancy and nursing. CP was defined as having at least five teeth with periodontal sites with probing depth (PD) ≥5 mm and clinical attachment loss ≥3 mm, 20% bleeding on probing (BOP), and extensive radiographically determined bone loss.[29]

Experimental Design: Clinical Trial

The sample size was calculated considering differences of at least 1 mm between groups for clinical attachment level (CAL) change in sites with initial PD ≥7 mm and assuming a standard deviation of 1.0 mm. Based on these calculations, it was determined that 14 individuals per group would be necessary to provide an 80% power with an α of 0.05.

Each participant was given a code number during the enrollment visit by the study coordinator (JG). Participants with CP were randomized by the study coordinator over the two treatment groups: 1) control (scaling and root planing [SRP] + placebo); or 2) test (SRP + probiotic). Randomization was computer generated, with allocation concealment by opaque sequentially numbered sealed envelopes. The study coordinator was responsible for allocation concealment. Eligible individuals were allocated randomly to the test and control groups according to sex, age, and smoking status after the basal examination, using a randomization table (JG). The test group patients used *L. rhamnosus* SP1 (2×10^7 colony forming units [CFU]/day)‖ for 3 months. The study product was tested against a placebo from the manufacturer of identical taste, texture, and appearance. The dose was one sachet taken orally every day. Identical sachets were presented to patients. Individuals were instructed to dissolve one sachet in water (150 mL) and ingest it once a day after brushing their teeth. Participants in both groups were instructed in oral hygiene regimens using a manual toothbrush. Non-surgical therapy involved SRP per quadrant performed at 1-week intervals in four to six sessions (by PC and CG). SRP was performed using an ultrasonic scaler¶ and hand instruments.# The patients started taking the probiotic or placebo after the last session of SRP. Periodontal supportive therapy was performed every 3 months (by PC and CG), with monitoring of individual compliance, medical history, and diet throughout the study period. Except for the study

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coordinator (JG), all study personnel and patients were masked to the study group assignment. Only after study completion was the designation of the different groups revealed. The study coordinator handed out the study materials.

Clinical Examination

Clinical examination was recorded at baseline and 3, 6, 9, and 12 months after therapy.

Periodontal clinical parameters were evaluated at six sites in all teeth, excluding third molars. These parameters included PD, dichotomous mesio-buccal, mid-buccal, disto-buccal, disto-lingual, mid-lingual, and mesio-lingual measurements of supragingival plaque accumulation,[30] and BOP at the base of the crevice. CAL was determined using the distance from the cemento-enamel junction (CEJ) to the free gingival margin (FGM) and the distance from the FGM to the bottom of the pocket/sulcus. From these two measurements, CAL (distance from the CEJ to the bottom of pocket/sulcus) was calculated. The assessment of the periodontal supporting tissue status was made with a first-generation manual periodontal probe.** At the time of recording depths, if necessary, measurements were approximated to the nearest whole millimeter. One calibrated examiner (AM) performed the evaluations and measurements of all patients. Calibration training was performed within successive days during which a group of 10 volunteers were examined. All examinations were repeated until an acceptable consistency was achieved, which was determined by an intraclass correlation coefficient of 0.80.

Outcome Variables

The primary outcome variable was change in CAL. Secondary outcome variables were changes in PD, plaque index (PI), and BOP, percentages of sites with PD ≥5, ≥6, and ≥7 mm, percentages of teeth with PD ≥5, ≥6, and ≥7 mm, and number of individuals with PD ≥5, ≥6, and ≥7 mm.

Subanalyses were performed on these outcome variables, taking into account the initial PD. A pocket was considered shallow if its initial PD was ≤3 mm, as moderate if its initial PD was between 4 and 6 mm, and deep if it was ≥7 mm.

In this study, risk for disease progression is used at a patient level according to Lang and Tonetti[31] as low (no more than four sites with PD ≥5 mm), moderate (five to eight sites with PD ≥5 mm), or high (at least five sites with PD ≥5 mm).

Compliance and Adverse Reactions

The participants received the sachets containing the probiotics or placebo at 1, 2, and 3 months and were called by phone each week to check for compliance. In each control visit or phone call, the clinical examiner (AM) inquired about general health changes, use of mouthrinses, use of probiotic products, and any adverse events.

Statistical Analyses

For all statistical evaluations, the patient was maintained as the unit of measurement. The compliance of parameters to the normal distribution was evaluated using Shapiro–Wilk test. The balancing of groups by age, sex, and smoking was tested by Mann–Whitney *U* test and Fisher exact test. Quantitative data were recorded as the mean value ± SD for all investigated parameters. Friedman test and McNemar test were used to compare intragroup parameters. The statistical significance was set at $P<0.05$ for both tests. The Bonferroni-corrected Wilcoxon signed-ranks test was used to evaluate the intragroup comparisons. Bonferroni-corrected Mann–Whitney *U* test and Fisher exact test were used to compare intergroup parameters. For the all Bonferroni-corrected tests, the statistical significance was set at $P<0.005$.

The statistical analysis was performed using a statistical package.††

RESULTS

Twenty-eights patients, 14 in the test group and 14 in the placebo group, were analyzed. All participants completed the study period, no adverse events were observed, and all participants were compliant with the study requirements.

The flowchart of the study is shown in Figure 1. The mean age was 52.7 ± 7.3 years for the test group and 46.9 ± 10.3 years for the control group. The proportion of males (seven) and females (seven) was equal in both groups. The number of smokers was four for the test group and two for the control group (Table 1). No significant differences in demographic and medical characteristics were found between groups ($P>0.05$). The clinical characteristics of the 28 individuals who participated in the study are shown in Table 1. No significant differences in baseline parameters were found between groups ($P>0.05$). The mean CAL, PD, BOP, and PI values for the baseline and 3-, 6-, 9-, and 12-month time points for both groups are presented in Table 1. Statistically significant intragroup differences were observed in the amount of full-mouth CAL and PI reduction ($P<0.05$). There was a significant PD reduction in the test group ($P<0.05$) and BOP reduction in the control group ($P<0.05$). However, multiple comparisons of intragroup measures showed that there were no differences ($P>0.005$) (Table 1).

Table 1 summarizes the reduction in PD, BOP, PI, and attachment gain. No statistically significant intergroup differences were observed ($P>0.005$).

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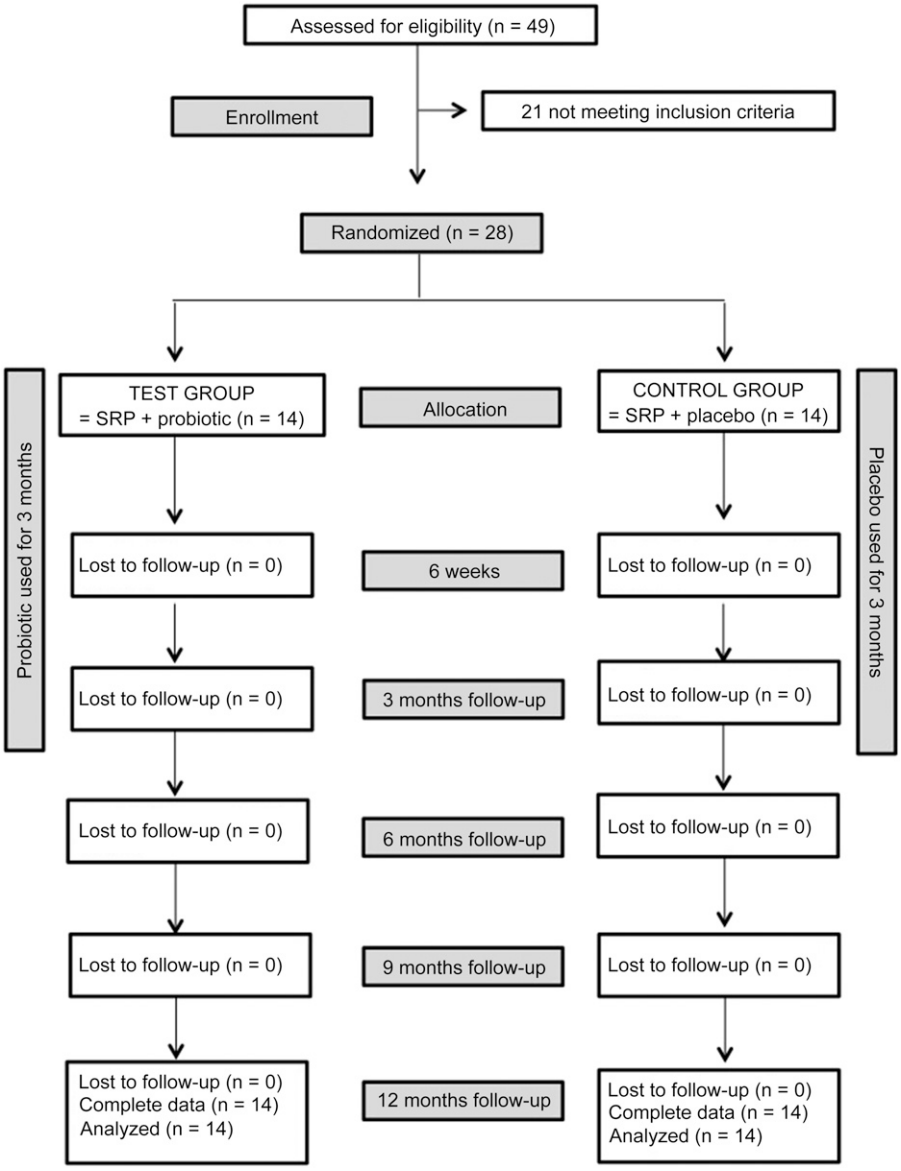

Figure 1.
Flowchart of the study design.

Additional subanalysis regarding the PD was performed for the baseline and 12- month time point (Table 2). There was a significant PD reduction in both groups (P <0.05). For both shallow sites and moderate pockets, there was a significant reduction in PD mean in both groups (P <0.05). Significantly lower percentages of sites and teeth with PD ≥5 mm in both groups (P <0.05) were observed. There were significantly fewer patients with PD ≥6 mm in the test group (P <0.05). Analyzing the data according to the individual risk profile for periodontal progression[31] revealed that almost 100% of patients had low risk for disease progression in 12 months. However, there were no differences between groups (P >0.05).

DISCUSSION

This double-masked placebo-controlled parallel-arm RCT evaluated the clinical effects of *L. rhamnosus* SP1 administered one time a day for 3 months plus non-surgical therapy in CP. The results of this study showed that periodontal clinical parameters improved in the test and control groups; however, there was a clinical benefit in the group using *L. rhamnosus* SP1 as an adjunct to SRP in PD reduction. Reductions in percentage of sites and number of teeth with PD ≥5 mm were greater in the test group. Importantly, only the test group had statistically significant reductions in the number of patients with PD ≥6 mm, a parameter commonly used to determine the need for periodontal surgery, and therefore the probiotic treatment decreased the need for a surgical phase after initial therapy.

To the best of the authors' knowledge, this is the first study to assess the adjuvant effects of an *L. rhamnosus* SP1 probiotic on periodontal therapy in patients with CP over a 1-year period. After 1-year follow-up, both groups showed sustained decreases in overall PD. However, patients in the SRP + probiotic test group showed a higher reduction in mean PD and higher reduction in percentage of sites with PD ≥5 mm. These data suggest that the beneficial effects of the probiotic seen at the initial follow-up visits were also sustained at 1 year. The results are in accord with other studies with a 1-year follow-up.[19,22] For instance, Tekce et al.[22] evaluated the effect of lozenges containing *Lactobacillus reuteri*‡‡ as an adjuvant to initial periodontal therapy in CP. The inclusion criteria were patients with CP with radiographically detected horizontal bone loss and the presence of at least two teeth with one site with PD of 5 to 7 mm and a gingival index (GI) ≥2 in each quadrant. At all time points after treatment, the measured clinical parameters were significantly lower in the group receiving SRP plus *L. reuteri*-containing lozenges compared with the

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Table 1.

Intragroup and Intergroup Comparisons of Clinical Parameters (mean ± SD or n)

	Test Group (n = 14)						Control Group (n = 14)					
	Baseline	3 Months	6 Months	9 Months	12 Months	*P*	Baseline	3 Months	6 Months	9 Months	12 Months	*P*
Age (years)	52.7 ± 7.3						46.9 ± 10.3					
Sex (males/females)	7/7						7/7					
Smokers	4						2					
CAL (mm)												
Overall	4.2 ± 0.9	3.8 ± 0.9	3.9 ± 1.2	4.0 ± 1.1	4.1 ± 1.0	**<0.001***	4.9 ± 1.3	4.2 ± 1.4	4.3 ± 1.6	4.8 ± 0.9	4.8 ± 1.3	**<0.001***
Difference with baseline		0.05 ± 0.1	0.3 ± 0.6	0.07 ± 0.5	0.07 ± 0.5			0.7 ± 1.3	0.7 ± 1.0	0.09 ± 0.8	0.09 ± 0.8	
PD (mm)												
Overall	2.7 ± 0.6	2.2 ± 0.6	2.1 ± 0.4	2.2 ± 0.5	2.1 ± 0.5	**0.02***	2.5 ± 0.3	2.1 ± 0.2	2.2 ± 0.2	2.1 ± 0.2	2.0 ± 0.2	0.13
Difference with baseline		−0.5 ± 0.2	−0.6 ± 0.3	−0.5 ± 0.3	−0.6 ± 0.3			−0.4 ± 0.4	−0.4 ± 0.4	−0.4 ± 0.4	−0.4 ± 0.4	
BOP (%)												
Overall	41.1 ± 16.3	28.2 ± 10.2	29.7 ± 10.5	28.3 ± 9.3	29.3 ± 12.7	0.11	33.8 ± 16.1	23.6 ± 14.8	27.9 ± 8.9	24.1 ± 11.4	25.4 ± 10.3	**0.002***
Difference with baseline		−12.9 ± 15.9	−11.3 ± 14.4	−12.8 ± 19.7	−11.7 ± 20.3			−10.3 ± 14.6	−5.9 ± 14.9	−9.8 ± 17.9	−8.3 ± 18.4	
Plaque accumulation (%)												
Overall	63.1 ± 18.5	31.2 ± 18.3	30.4 ± 16.1	29.7 ± 19.9	33.1 ± 21.3	**<0.001***	52.1 ± 20.7	26.5 ± 15.1	29.0 ± 14.5	31.2 ± 13.1	35.5 ± 11.4	**<0.001***
Difference with baseline		−32.0 ± 16.0	−32.7 ± 11.4	−33.5 ± 12.2	−30.0 ± 11.5			−25.6 ± 14.4	−23.0 ± 13.5	−20.9 ± 14.3	−16.6 ± 17.0	

Intragroup comparison by *Friedman test ($P < 0.05$) and intragroup multiple comparison by Bonferroni-corrected Wilcoxon signed-rank test ($P < 0.005$). Significant values are given in bold.
Intergroup comparison by Mann–Whitney *U* test ($P < 0.05$), Fisher exact test ($P < 0.05$), and intergroup multiple comparison by Bonferroni-corrected Mann–Whitney *U* test ($P < 0.005$).

Table 2.

PD Measures at Day 0 and 12-Month Follow-Up and Risk for Disease Progression Outcome Measures (mean ± SD)

	Treatment Group					
	Test Group (n = 14)		Control Group (n = 14)		$P^{\dagger}$	
Variable	Mean ± SD	Δ ± SD	Mean ± SD	Δ ± SD	for Mean	for δ
PD (mm)						
Overall						
Day 0	2.7 ± 0.6		2.5 ± 0.3		0.34	
12 months	**2.1 ± 0.5***	−0.6 ± 0.3	**2.0 ± 0.2***	−0.4 ± 0.4	0.48	0.26
Shallow sites						
Day 0	2.2 ± 0.2		2.1 ± 0.2		0.17	
12 months	**1.8 ± 0.2***	−0.3 ± 0.2	**1.7 ± 0.1***	−0.3 ± 0.3	0.41	0.76
Moderate pockets						
Day 0	4.3 ± 0.3		4.5 ± 0.4		0.60	
12 months	**2.8 ± 0.6***	−1.5 ± 0.4	**3.0 ± 0.5***	−1.4 ± 0.4	0.20	0.45
Deep pockets						
Day 0	7 ± 0		7.9 ± 0.8		0.12	
12 months	3.7 ± 1.5	−1.0 ± 1.7	4.7 ± 0.9	−1.0 ± 1.7	0.28	0.96
Sites with PD (%)						
≥5 mm						
Day 0	7.3 ± 10.6		5.8 ± 5.6		0.94	
12 months	**2.5 ± 5.4***	−4.9 ± 5.7	**1.8 ± 1.2***	−3.9 ± 5.1	0.21	0.65
≥6 mm						
Day 0	2.9 ± 6.1		1.8 ± 3.0		0.67	
12 months	0.7 ± 1.0	−2.2 ± 5.5	0.7 ± 1.2	−1.1 ± 2.8	1.00	0.40
≥7 mm						
Day 0	0.9 ± 1.7		1.0 ± 2.2		0.96	
12 months	0.1 ± 0.4	−0.8 ± 1.8	0.3 ± 0.5	−0.7 ± 2.3	0.36	0.90
Teeth with PD (%)						
≥5 mm						
Day 0	24.3 ± 26.1		18.5 ± 14.6		0.71	
12 months	**8.4 ± 15.8***	−15.9 ± 13.5	**6.9 ± 3.6***	−11.5 ± 12.3	0.17	0.50
≥6 mm						
Day 0	10.9 ± 20.7		4.5 ± 5.2		0.67	
12 months	2.9 ± 4.1	−8.0 ± 18.2	2.0 ± 2.8	−2.4 ± 4.4	0.73	0.70
≥7 mm						
Day 0	4.4 ± 8.8		2.6 ± 4.8		0.89	
12 months	0.5 ± 1.4	−4.0 ± 8.9	1.2 ± 1.9	−1.4 ± 5.3	0.36	0.66
Number of patients with PD						
≥5 mm						
Day 0	14		14		1.00	
12 months	10		13		0.29	
≥6 mm						
Day 0	11		7		0.35	
12 months	**6**†		6		1.00	
≥7 mm						
Day 0	4		4		1.00	
12 months	1		4		0.58	

Table 2. (continued)

PD Measures at Day 0 and 12-Month Follow-Up and Risk for Disease Progression Outcome Measures (mean ± SD)

Variable	Treatment Group				$P^{\dagger}$	
	Test Group (n = 14)		Control Group (n = 14)			
	Mean ± SD	Δ ± SD	Mean ± SD	Δ ± SD	for Mean	for δ
Number of patients according to risk for disease progression (Lang and Tonetti[31])						
Low	13/14		14/14		1.00	
Medium	0/14		0/14			
High	1/14		0/14			

Intragroup comparison by *Wilcoxon signed-rank test and †McNemar test. $P < 0.05$. Significant values are given in bold.
Intergroup comparison by Mann–Whitney *U* test and and Fisher exact test. $P < 0.05$.

group that received SRP plus placebo. Ince et al.[19] evaluated the effects on clinical and biochemical parameters of an *L. reuteri*-containing probiotic supplementation adjunctive to initial periodontal therapy also in patients with CP showing that lozenges containing *L. reuteri* may be a useful supplement in moderately deep pockets. The results showed a clinically relevant benefit for the patients as "risk for disease progression" outcome measures were significantly better when *L. reuteri* lozenges were used. The results from this study show that the use of an *L. rhamnosus* SP1 probiotic as an adjunct to initial therapy is beneficial, similar to the use of *L. reuteri* as reported in the cited studies.[19,22]

However, the selection of the "best" probiotic for oral health is a controversial issue.[32] *L. rhamnosus* SP1 was selected as the probiotic for the present study because it has been shown to have beneficial effects on the immune responses of children and adults.[33-36] The immune modulation caused by a gut probiotic *Lactobacillus* strain might help reduce the immune overreaction observed in periodontitis. *L. rhamnosus* GG has been studied in the form of lozenges on gingival inflammation in healthy subjects without periodontopathogens at baseline. A previous study reported a decrease in gingival inflammation without affecting the oral microbiota.[26]

Mode of administration, dosage, and frequency may also affect therapy outcomes.[37] In this study, the *L. rhamnosus* SP1 sachet application (2×10^7 CFU/day) was started immediately after the last session of root planing, one time a day for 3 months. Teughels et al.[23] used *L. reuteri*§§ lozenges twice daily for 3 months, 1×10^8 CFU/day, immediately after a full-mouth disinfection procedure. A similar method was used by Vivekananda et al.,[24] with the exception that patients started to use the probiotic lozenges 21 days after SRP and no additional disinfection of the oral cavity was performed. Inclusion criteria of the Teughels et al.[23] study included the presence of moderate to severe generalized CP, according to van der Velden.[38] As expected, Teughels et al. found significantly larger PD reductions, especially in deep pockets, and significantly lower percentages of sites and teeth with a residual PD ≥5 mm than Vivekananda et al.[24] However, the later study still reported significant intergroup differences in PI, GI, BOP (percentage), PD, and CAL, in favor of the use of *L. reuteri* probiotic lozenges.[24] It seems then that, despite different mode of administration, the use of certain strains of probiotic as adjunct to SRP shows a consistent beneficial effect across studies.[19,22-24]

The issue of safety is of also of special concern. However, none of the participants in this study presented with any adverse event. This result is in accordance with previous studies,[19,22-24,39] which similarly did not identify any negative side effects or tolerance problems associated with the consumption of *L. reuteri*. Moreover, patient compliance in this study was extremely high, which indicates that the use of a probiotic does not represent a burden to the patient. Despite high compliance, the main limitation of the present study is the relatively small number of participants. Nevertheless, some clinically relevant differences were observed in favor of the use of the probiotic sachet. Therefore, this study shows the feasibility and could serve as a basis for future studies conducted in larger cohorts.

CONCLUSION

Under the limitations of the present study, the adjunctive use of *L. rhamnosus* SP1 sachets during initial therapy resulted in similar clinical improvements compared with SRP alone.

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