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Original article

The effects of *Lactobacillus reuteri* probiotics combined with azithromycin on peri-implantitis: A randomized placebo-controlled study

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ABSTRACT

Purpose: The aim of this randomized placebo-controlled clinical study was to investigate the effects of a probiotic tablet containing *Lactobacillus reuteri* in peri-implantitis patients.
Methods: Subjects comprised 30 patients with mild to moderate peri-implantitis. A baseline clinical examination and microbiological assessment were conducted, followed by an antibiotics treatment (azithromycin, 500 mg, once a day for 3 days). Subjects were divided into probiotic and placebo groups. The clinical examination and bacterial sampling were performed 0, 4, 12 and 24 weeks after the intake of probiotics. The clinical examination included probing pocket depth (PPD), bleeding on probing (BOP), the modified plaque index (mPI), and modified bleeding index (mBI). The number of bacteria was assessed using the PCR-invader method. The Wilcoxon rank-sum test and Wilcoxon signed-rank test with Bonferroni corrections were used for data analyses.
Results: Although the number of bacteria decreased after the administration of azithromycin in both groups, they increased again thereafter. No significant difference was observed in bacterial numbers between the two groups. Although PPD in the probiotics group was significantly lower at 4 and 24 weeks than at 0 weeks ($p < 0.05$), a significant decrease did not occur in the placebo group. The mBI score at 24 weeks was significantly lower in the probiotics group than in the placebo group ($p < 0.05$). No significant difference was observed in BOP or mPI between the two groups.
Conclusion: These results suggested that probiotics prevent inflammation by affecting host responses rather than improving microbial flora in peri-implant sulci in peri-implantitis patients.

1. Introduction

Dental implants as a missing dentition treatment have a very high success rate and have become the gold standard treatment. However, according to a recent systematic review, the incidence of peri-implant mucositis and peri-implantitis is 19–65% and 1–47%, respectively, while the mean prevalence of peri-implant mucositis and peri-implantitis is 43% and 22%, respectively [1]. Assuming that the incidence will not change, the number of implants infected with peri-implant disease will increase with an increase in the number of implants placed in the future.

Previous studies on peri-implantitis treatments showed that supramucosal biofilm control is effective [2], BOP reductions are significantly higher with mechanical debridement using an air-abrasive device than with carbon curettes [3], and non-surgical antimicrobial photodynamic therapy stops bone resorption in moderate peri-implant defects, but not in severe defects [4]. On the other hand, the bacterial counts of *Pseudomonas aeruginosa*, *Staphylococcus aureus*, and *S. anaerobius* decreased one month after mechanical debridement using an air-abrasive device, while a marked difference was not observed after 6 months [5], and a laser did not provide any additional benefits over SRP alone [6]. Non-surgical treatments using an air-abrasive device and laser may be effective to some extent in regions readily reachable with instruments, such as the anterior and premolar regions, but instrument operability is poor, especially in the molar region. Therefore, these are applicable only for limited cases. Probing pocket depth (PPD) and bleeding on probing (BOP) were previously reported to be reduced, and bone regeneration was

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observed with surgical regenerative therapy using XenoGraft and a membrane [7]. Therefore, surgical therapies including regenerative therapy are considered to be effective for the treatment of peri-implantitis. However, success rates depend on the skill of the dentist as well as the physical condition of the patient, and, thus, difficulties are associated with the application of these treatments to all patients.

The combination of mechanical debridement and systemic antibiotics is known to be an effective treatment for periodontal diseases [8]. The systematic administration of antimicrobials for peri-implantitis contributed to reducing BOP and PPD by surface decontamination following open flap debridement [9]. However, the extensive use of systematic antibiotics results in bacterial resistance in the subgingival flora and frequent recolonization [10–12], and, thus, is not recommended for use on a regular basis.

Probiotics are defined as live microorganisms that confer health benefits on a host when administered at adequate amounts [13]. The influence of probiotics on the body is related to the bacterial flora in the intestine. A large number of immunocytes are present in the intestinal tract and protect the body from pathogens. The bacterial flora in the intestine and immunocytes interact with each other and influence the immune system. The activation of CD4+T cells in the small intestinal mucosa has been confirmed in probiotics groups [14], and the inhibition of *Helicobacter pylori* was previously achieved with the combined intake of probiotics and antibiotics [15]. The intake of probiotics has been shown to prevent and improve systemic diseases, include improvements in high blood pressure [16], decreases in blood sugar levels [17], and the prevention of arterial sclerosis [18]. The advantages of probiotics on oral health have also been demonstrated. The risk of high levels of *Streptococcus mutans* is known to be reduced by the application of probiotics to the oral cavity [19], periodontal treatments using probiotics are effective for gingivitis and periodontitis [20], the gingival index and amount of bacterial plaque in moderate or severe gingivitis patients are reduced by treatments using *Lactobacillus reuteri* [21], and *L. reuteri* has been shown to prevent the growth of *Porphyromonas gingivalis* and *Prevotella intermedia* and reduce the concentrations of cytokines related to inflammatory reactions [22]. The peri-implantitis-related microbiota resembles the pathogens of periodontal disease.

The microbial flora associated with the etiology of peri-implantitis consists of anaerobic Gram-negative bacteria such as *Aggregatibacter actinomycetemcomitans*, *Prevotella intermedia*, *Porphyromonas gingivalis*, *Treponema denticola*, and *Tannerella forsythia* [23–26]. A few studies have already examined the effects of probiotics on peri-implant mucositis. A decrease in cytokine concentrations including proinflammatory molecules and improvements in clinical parameters in peri-implant mucositis patients were observed after the intake of probiotics [27]. And, additional benefits were not obtained when probiotics was combined with mechanical debridement and oral hygiene in peri-implant mucositis patients [28]. However, the influence of probiotics on peri-implantitis currently remains unknown. The present study investigated the influence of probiotics on peri-implantitis by observing changes in the microbial flora in peri-implant sulci and clinical parameters over time. A double-blind, randomized, and placebo-controlled clinical trial was conducted using peri-implantitis patients who took a probiotic tablet containing *L. reuteri* for 24 weeks. The hypothesis of this study was that *Lactobacillus reuteri* probiotics combined with azithromycin in peri-implantitis patients would lead to improved clinical and microbiological outcomes compared with azithromycin alone.

2. Materials and methods

2.1. Study design

This was a randomized, placebo-controlled, double blind, clinical study to evaluate the effects of a probiotic tablet on clinical parameters and microbiological profiles in the peri-implant sulci of peri-implantitis patients for 6 months. The study was performed between March 2016 and December 2016 at 7 facilities including Kyushu Dental University Hospital. Out of 46 eligible patients with peri-implantitis, 30 gave their informed consent and were consecutively enrolled in this study. The numbers of the subjects from the 7 institutions were 15, 14, 6, 3, 3, 3, and 2, respectively. This research was approved by the Ethics Committee of Kyushu Dental University (approval number 15-11) and followed the guidelines of the amended Declaration of Helsinki. This trial was also registered with the University Hospital Medical Information Network (UMIN) (study ID: UMIN000025813). All clinical examiners and patients were blinded until the end of the study.

Selection criteria for subjects were patients with mild to moderate peri-implantitis and (1) PPD of more than 4 mm and less than 7 mm, (2) bleeding or suppuration on probing (+), and (3) marginal bone loss >2 mm (as established from periapical X-rays). [29]

Exclusion criteria were: (1) patients with uncontrolled systematic disease, (2) patients who took antibiotics within 3 months, (3) pregnant or breast feeding women, (4) patients with acute symptoms around implants, (5) patients allergic to antibiotics, (6) patients who had to take antibiotics due to other diseases during the study, and (7) patients who did not receive surgical or non-surgical therapy within 6 months before the study initiation.

Sample size was calculated for the primary outcome, change in probing depth (PPD). In a randomized clinical trial in which probiotic *Lactobacilli reuteri* was administered against periodontal disease, the difference in change in the probing pocket depth (PPD) between the probiotic and placebo groups was 0.82 mm [30]. Based on this 0.82-mm difference between the study groups, the sample size was calculated to be approximately 15 subjects each in the test and placebo groups with a power of 0.80 and α at 0.05. Simple randomization was carried out using a computer software program (Microsoft Excel) that generates random numbers to assign participants. This was performed by one of the authors who had little involvement with assessment and held the randomization code until all data had been collected and all analyses had been performed. No subjects dropped out throughout the study period after randomization. The characteristics of patients in both groups are shown in Table 1. There was no significant difference between the two groups regarding age, oral hygiene, or duration after implant insertion.

Table 1
Baseline characteristics of the subjects.

	Probiotics	Placebo
Number	n = 15	n = 15
Age (y)	68.80 ± 7.46	65.87 ± 8.84
Male:female	3:12	5:10
Smoker	n = 3	n = 1
Implant location (max:man)	6:9	5:10
Oral hygiene (times/day)	2.73 ± 0.59	2.60 ± 0.63
Duration after implant insertion (years)	8.25 ± 4.16	6.04 ± 2.80

Allocation to the probiotics group or placebo group was conducted using the RAND function in Microsoft Excel. max, maxilla. man, mandible. y, year. d, day. There was no significant difference between the two groups regarding age, oral hygiene, or duration after implant insertion.

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