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学位論文要旨

岡山大学大学院医歯薬学総合研究科

教育研究分野 予防歯科学	身分 大学院生	氏名 ZHANG YIXUAN

論文題名 Effects of *Bacillus subtilis* on Periodontitis in a Mouse Model

マウスモデルにおける枯草菌（*Bacillus subtilis*）による歯周炎への影響

論文内容の要旨（2000字程度）

Introduction

Gut microbiota may influence the progression of periodontitis. Several studies have highlighted the potential role of gut microbiota in periodontal disease via the gut–alveolar bone axis. However, whether modulation of gut microbiota can suppress the progression of established periodontitis remains unclear. Strategies for modulating gut microbiota include probiotics, prebiotics, postbiotics, antibiotics, and fecal microbiota transplantation. *Bacillus subtilis* (BS) is an emerging probiotic that has been widely investigated for its immunomodulatory properties, as well as its potential to support gut barrier integrity and regulate intestinal microbiota. Therefore, we investigated whether BS supplementation attenuates periodontitis progression through modulation of the gut microbiota and improvements in intestinal morphology.

Methods

A total of 24 C57BL/6J mice (6 weeks old) were divided into four groups: control (C), periodontitis (P), BS, and BS-treated periodontitis (n = 6 per group). Periodontitis was induced by ligature placement for 7 days. BS (1.0×10^9 CFU/mL) or 0.9% saline was orally administered for 18 days. After euthanasia on day 25, intestinal tissues were collected for histopathological evaluation. Fecal samples for microbiome analysis and blood samples for biochemical analysis were also collected. All animal experiments were approved by the Animal Care and Use Committee, Okayama University (OKU-2023464). Maxillary bone samples were stained with methylene blue and analyzed for alveolar bone loss by measuring the distance between the cementoenamel junction and the alveolar bone crest (CEJ-ABC). The morphology of the small and large intestines was evaluated using ImageJ Software. Microbiome analysis was performed using next-generation 16S rDNA sequencing. To assess microbial diversity, alpha diversity indices (Shannon and Simpson) were calculated to measure species richness and evenness within each group. Beta diversity was evaluated using principal coordinate analysis (PCoA) based on Bray-Curtis dissimilarity to compare microbial community structure between the P and BSP groups. Serum vitamin D levels were measured using blood samples. Statistical analyses were performed using one-way or two-way analysis of variance (ANOVA), as appropriate, followed by Tukey's post hoc test for multiple comparisons. Results were considered statistically significant at $P < 0.05$.

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Results

The distance from the cementoenamel junction to the alveolar bone crest was significantly greater in the P group (0.343 ± 0.043 mm) than in the C group (0.243 ± 0.046 mm, $P < 0.01$). By contrast, a significant reduction in alveolar bone loss was observed in the BSP group (0.209 ± 0.056 mm) compared with the P group ($P < 0.001$). The BSP group (59.517 ± 11.750 ng/mL) showed significantly higher serum vitamin D levels than the other groups ($P < 0.001$). Histopathological analysis revealed no significant difference in intestinal crypt depth (ICD) between the P and BSP groups in either the small or large intestines ($P > 0.05$). ICD (small intestines) in the BSP group was significantly higher than that in the C and BS groups ($P < 0.05$). IVH (small intestine) in the BSP group was significantly larger than those in the other groups ($P < 0.01$) (Fig. 2c). The ratio of villus height to crypt depth (IVH/ICD) (V/C) (small intestine) in the P group was significantly smaller than that in the BSP group ($P < 0.001$) (Fig. 3d). Analysis of the 16S rDNA sequencing results revealed a significant difference in the alpha diversity index (Simpson) ($P = 0.048$), and PCoA demonstrated significant differences in microbial community structure between the P and BSP groups ($P = 0.044$). However, no significant differences were observed in the alpha diversity index (Shannon) ($P > 0.05$). Heat tree analysis comparing the P and BSP groups showed differences in butyrate-producing bacterial genera, particularly *Ligilactobacillus* and *Turicibacter*. Differential abundance analysis further indicated a significant increase in *Ligilactobacillus* and a decrease in *Turicibacter* in the BSP compared with the P group.

Discussion

The administration of BS significantly suppressed the progression of alveolar bone loss in a mouse model of periodontitis. Small intestinal morphology was improved in the BSP group compared with the P group. BS supplementation was associated with increased serum vitamin D levels and alterations in the abundance of butyrate-producing bacteria, including *Ligilactobacillus* and *Turicibacter*, in the gut microbiota. These findings suggest that BS treatment may be associated with concurrent changes in intestinal morphology and vitamin D levels. In addition, differences in gut microbiota composition were observed between the P and BSP groups. Therefore, this study provides new insights into the potential role of BS in suppressing the progression of alveolar bone loss under inflammatory conditions. In conclusion, oral administration of BS was associated with suppression of alveolar bone loss in a mouse model of ligature-induced periodontitis.