Abstract

Periodontitis affects the development of atherosclerosis,

and sword bean extract suppresses it:

-the study using atherosclerosis model mice and in vitro-

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Abstract

【Introduction】

It has been well known that relationship between periodontitis (PD) and atherosclerosis (AS). A clinical study by Stelzel et al. (2002) discovered that *P. gingivalis* DNA was found high frequently (88.5%) in the aortas of patients connected to a heart-lung machine, which indicated that a link between periodontopathogens entering the cardiovascular system. In addition, a preclinical study demonstrated that oral infection of *P. gingivalis* for ApoE$^{-/-}$ mice promoted AS development. This study has proposed the new possibility for the prevention of AS using the oral application of sword beans extract (SBE) based on the our reported evidences (Nakatsuka et al. 2014) The current series of studies, thus, aimed at evaluating the effect of SBE on AS and PD progression.

【Materials and Methods】

1. *in vivo*

Six-weeks-old male ApoE-/- mice (n=6) were randomly divided into 6 groups as follows; Group 1. Control (Con), Group 2. *P. gingivalis* induced PD (*P. g*), Group 3. Ligature induced PD (Lig), Group 4. SBE application (SBE), Group 5. *P. g* + SBE, Group 6. Lig + SBE. All the mice fed high fat diet. In *P. g* and *P. g* + SBE groups, the mice were orally inoculated by *P. gingivalis* (1.0×10^9 CFU/mL) in 5%CMC with PBS. In Lig and Lig + SBE groups, PD was induced by tying 5-0 silk ligature around the molars. In SEB groups, SBE was orally administered to mice (2 mg/ml). The present study was approved by Committee of Ethics on Animal Experiments at the Health Sciences University of Hokkaido (approved number 20-028). Bone resorption of the maxillary second molar was quantified. The areas from the CEJ to alveolar bone crest (ABC) were considered as bone resorption. The amount of bone resorption was calculated using Image J software. The maxilla was decalcification with EDTA (PH 7.4). Sections (5 μm) were cut and stained with Hematoxylin and eosin stain for histological assessment. Frozen tissue sections of the aorta were prepared and stained with H.E. staining and Oil Red O staining solution to assess plaque formation. Total RNA was isolated from mice heart including the aortic root and reverse transcription was performed. Then, cDNA was generated and RT-PCR was performed for analysis of IL-6, TNF-α, iNOS mRNA expression.

2. *in vitro*

RAW264.7 cells used in this study were cultured in DMEM supplemented with 10% fetal bovine serum and 1% penicillin/streptomycin at 37℃ and 5% CO_2. The cells were cultured in the presence or absence of *P. gingivalis* LPS and SBE. Gene expression of IL-6, TNF-α, and iNOS were examined by RT-PCR. And NO production was assessed using Griess reagent kit.

3. Data analysis

Data analysis was performed with JMP software. Statistical analysis was performed by one-way analysis of variance (ANOVA) followed by Tukey's multiple-comparison tests. Statistical significance was set at $p < 0.05$.

【Result】

1.*in vivo*

Bone resorption in the PD-induced mouse were significantly greater than the Con group, although the Lig group were significantly higher in bone resorption than the *P. g* group. The Lig + SBE group were significantly lower in bone resorption than Lig group. Histological assessment showed that the Lig group and the Lig + SBE group had epithelial thickening and alveolar bone resorption. In analysis of cross section of aortic area, the plaque area in the *P. g* group and Lig group were significantly higher than the Con group. The *P. g* + SBE and the Lig + SBE group were significantly lower in the plaque area than the *P. g* and the Lig group. Gene expression of TNF-α and iNOS in the *P. g* group significantly higher than in the Con and *P. g* + SBE groups.

2. *in vitro*

IL-6, TNF-α, and iNOS expression were significantly higher in the LPS group than in the Con and LPS + SBE groups. The NO production in LPS group was significantly higher than that in the Con and LPS + SBE groups.

【Discussion】

In this study, AS was promoted in the presence of PD. It can be considered that local inflammation affected the systemic inflammation. SBE suppressed the alveolar bone resorption, iNOS expression in the heart and atherosclerotic plaque formation. This was because SBE suppressed the mRNA expression of IL-6, TNF-α, and iNOS in macrophages in vitro, and suppressed the production of NO, in addition to the antibacterial and anti-inflammatory effects.

【Conclusion】

Within the limitation of this studies, it was demonstrated that periodontal infection can promote the plaque formation in the aortic and SBE has potential to prevent from the progression of the plaque due to its anti-inflammatory effects.