学　位　論　文　要　約

Anti-inflammatory effect of glycyrrhizin with *Equisetum arvense* extract
(グリチルリチン酸とスギナ抽出物による抗炎症効果)

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Objectives

Periodontitis is an infectious/inflammatory disease with progressive bone destruction, and inflammatory cytokines such as tumor necrosis factor α (TNF-α), which are produced as a host defense response, play an important role in the onset and progression of the disease. Therefore, appropriate control of inflammatory cytokines is necessary to prevent the onset of periodontitis and to inhibit the progression of the disease.

Glycyrrhizin (GL) has been widely used as an ingredient with anti-inflammatory properties and inhibits TNF-α production by lipopolysaccharide (LPS) derived from periodontal pathogenic bacteria, but there is a maximum dose for the use of GL. In the present study, I first explored plant extracts that enhance the anti-inflammatory effect of GL and elucidated the mechanism of enhancement. Next, I investigated the inhibitory effect of *Equisetum arvense* extract (EA), which enhanced anti-inflammatory effect of GL in the exploratory study, on osteoclastic bone resorption.

Methods

Exp. 1: Screening of plant extracts that enhance the anti-inflammatory effect of GL and elucidation of the mechanism of enhancement

The effects of extracts from six different plants (*Crataegus oxyacantha*, *Salvia officinalis*, *Equisetum arvense*, *Hamamelis virginiana*, *Paeonia lactiflora* and *Betula alba*) on GL-suppressed TNF-α expression levels in THP-1 macrophages stimulated with LPS were examined. Next, the inhibitory effect of GL with EA supplementation on TNF-α expression in junctional epithelium (JE) of LPS-induced periodontitis rat model was examined. The effects of GL and/or EA on LPS induced signal pathways were also analyzed by Western blotting.

Exp. 2: Investigation of the effect of EA on alveolar bone destruction

LPS-induced periodontitis model rats were used to examine the inhibitory effect of EA on the formation of osteoclasts along the alveolar bone margin. Moreover, effects of EA on the expression of osteoclastogenesis-related factors in cloned stromal-cell line from mouse bone marrow (ST2 cells) stimulated by LPS was analyzed by Real time PCR.

Results

Exp. 1: Screening of plant extracts that enhance the anti-inflammatory effect of GL and elucidation of the mechanism of enhancement

Screening experiments demonstrated that EA had the strongest additive effect on the suppression of TNF-α expression by GL at both mRNA and protein levels. In addition, LPS-induced periodontitis rat model showed that GL with EA supplementation significantly downregulated immunoexpression of TNF-α in JE, the front-line epithelium directly exposed to plaque-derived irritants such as LPS. Furthermore, signal pathway analysis showed GL downregulated the production of TNF-α by suppressing nuclear factor-kappa B (NF-κB) p65 phosphorylation, but not c-Jun N-terminal kinase (JNK) or p38 phosphorylation. In contrast, EA decreased JNK phosphorylation but not NF-κB p65 or p38 phosphorylation. The combination of EA and GL effectively attenuated LPS induced phosphorylation of NF-κB p65 and JNK.

Exp. 2: Investigation of the effect of EA on alveolar bone destruction

The number of osteoclasts formed along the alveolar bone margin in the LPS-induced periodontitis model rat was significantly reduced by EA administration to the same level as that in the Control group. Immunohistochemistry of osteoclastogenesis related factors showed that RANKL expression in the periodontal ligament was increased and OPG expression was decreased in the LPS group. In the LPS/EA group, the increased expression of RANKL was suppressed, while the expression of OPG was recovered, and rather enhanced than that in the Control group.

Conclusions

These results indicate that GL and EA additively suppressed TNF-α expression via suppression of LPS-induced phosphorylation of NF-κB p65 and JNK, respectively. In addition, it is indicated that EA has a novel function in regulating alveolar bone destruction by suppressing the LPS-stimulated increase in RANKL and inflammatory cytokine expression, restoring the suppression of OPG expression, resulting in decreasing osteoclastogenesis. This study suggests the possibility of developing a new periodontitis prevention/treatment product by combined GL and EA.