OR-I-3. The inhibitory effect of chemically modified tetracycline-8 on IL-6 production and Osteoclast Formation by *Actinobacillus actinomycetemcomitans* lipopolysaccharide

Woong Taek Han*, Young Joon Kim
Department of Periodontology, School of Dentistry, Chonnam University

Introduction

Chemically modified tetracyclines (CMTs) are able to regulate cytokines involved in bone resorption such as interleukin (IL)-1, TNF-α, and IL-6. However, the regulation of osteoclastogenesis by CMTs still remains unclear. In addition, there were few reports of whether the modulation of cytokines related to bone resorption such as IL-6 and macrophage colony stimulating factor (M-CSF) in periodontal ligament fibroblasts by CMTs would affect osteoclast formation.

The purpose of this study was to evaluate the ability of CMT-8 to inhibit IL-6 and M-CSF and osteoclast formation when stimulated by *Actinobacillus actinomycetemcomitans* (A.a) lipopolysaccharide (LPS) in co-cultures of RAW 264.7 cells and mouse periodontal ligament fibroblasts.

Materials and methods

1. Materials

CMT-5 and CMT-8 were obtained from CollaGenex Pharmaceuticals (U.S.A) and doxycycline was obtained from Dong-Gook Pharmaceuticals (Korea)

2. Lipopolysaccharide (LPS) preparation

The procedures for LPS isolation from *A. actinomycetemcomitans* (A.a) was as previously described by Wilson ME.

3. IL-6 Immunoassay

4. Gene expression assessed by polymerase chain reaction

Semi-quantitative PCR was performed. Semi-quantitative differences of IL-6 and M-CSF expression were normalized by GAPDH.

5. Osteoclastogenesis assays

TRAP staining was performed as instructed from the commercial kit (Sigma, U.S.A). TRAP-positive cells from each well were counted under light microscopy.

6. Statistical analysis

Results

IL-6 production was inhibited by SB203580, CMT-8, and doxycycline when mPDL fibroblasts were stimulated with A.a LPS. Whereas, IL-6 production was not inhibited with CMT-5. A.a LPS-induced M-CSF mRNA expression was also reduced to 34%, 22% by CMT-8 and doxycycline. However, CMT-5 and SB20358 had little effect on A.a LPS-induced M-CSF mRNA expression. IL-6 mRNA expression increased approximately 2.2-fold with A.a LPS when normalized to untreated control whereas, A.a LPS-induced IL-6 mRNA expression was reduced to 41% by SB203580. A.a LPS-induced IL-6 mRNA expression was also reduced to 32% by CMT-8. CMT-5 had little effect on A.a LPS-induced IL-6 mRNA expression. A.a LPS and M-CSF significantly induced TRAP-positive cell formation. Treatment with SB203580 and CMT-8 reduced the numbers of TRAP-positive cells formed in co-culture. p38 MAP kinase signaling pathway was activated by A.a LPS in mPDL fibroblasts. The p38 inhibitor, SB203580 decreased the phosphorylation level of p38 MAP kinase whereas, CMT-8 did not affect the phosphorylation level of p38 MAP kinase.

Conclusion

These results suggest that CMT-8 suppress IL-6 and M-CSF gene expression in mouse periodontal ligament fibroblasts and inhibit A.a LPS-induced TRAP-positive cell formation in co-culture. Therefore, CMT-8 have many beneficial effect in the inhibition of alveolar bone resorption.