Inhibition of Osteoclast Formation and Bone Resorption by Gymnasterkoreayne F from *Gymnaster koraiensis*

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INTRODUCTION

Osteoclasts are multinucleated cells that are derived from the hematopoietic myeloid/monocyte lineage via the action of M-CSF and receptor activator of NF-κB ligand (RANKL) (1). Binding of RANK to RANK activates and/or induces expression of the transcription factors NF-κB, AP-1, and NFAT2, which are important for osteoclast differentiation (1, 2). Fusion-induced osteoclast formation is critical for osteoclast development. Previous studies have shown that dendritic cell-specific transmembrane protein (DC-STAMP)-deficient mice develop osteopetrosis due to the defect in fusion process during osteoclast development (3), suggesting DC-STAMP is an essential molecule for osteoclastic cell-cell fusion. Several plants have been used for the treatment of osteoporosis in Asian countries. Gymnasterkoreayne F (GK-F) is methanol extract isolated from the leaves of *Gymnaster koraiensis*, which is a plant endemic to Korea. It has been reported that GK-F has the inhibitory activity against NFAT transcription factor. In this study, we examined the effect of GK-F on osteoclast development. We find that GK-F inhibits osteoclast differentiation from primary precursor cells without cytotoxicity. In addition, GK-F suppresses multinucleated osteoclast fusion from mononuclear osteoclasts. Furthermore, GK-F inhibits cytoskeletal organization leading to the impaired bone resorptive activity of osteoclasts.

METHODS

Osteoclast cultures: Whole bone marrow cells were isolated from C57/BL6 mice and cultured with RANKL and M-CSF in α-MEM containing 10% FBS (4). Osteoclasts were stained for tartrate-resistant acid phosphatase activity. To determine actin ring formation, bone marrow macrophages were cultured on bone with RANKL and M-CSF. Cells were fixed and stained with rhodamine-phalloidin.

Bone resorption assay: Osteoclasts were removed from the bone slices. After a brief rinse, the slices were incubated with 20 μg/ml peroxidase-conjugated wheat germ agglutinin for 30 min. After washing in PBS, 3,3′-diaminobenzidine was added onto the bone slices.

RESULTS

Osteoclasts are multinucleated cells that have a unique role for bone degradation and responsible for several bone diseases such as osteoporosis. Regulation of osteoclast formation has been considered an effective therapeutic approach to the treatment of osteoporosis.

To screen the natural compounds which inhibit osteoclast formation, we examined the effect of the extracts isolated from several medicinal plants on osteoclastogenesis. We found that Gymnasterkoreayne F derived from *Gymnaster koraiensis* effectively reduced the formation of TRAP-positive multinuclear osteoclasts without affecting cytotoxicity (Fig. 1).

To further confirm the inhibitory effects of GK-F on osteoclast differentiation, we analyzed the expression of osteoclastogenic markers by RT-PCR. Reflecting the reduced number of TRAP-positive cells, mRNA expression levels of NFAT2, Cathepsin K and c-Src were significantly reduced in the presence of GK-F.

We also found that GK-F suppressed osteoclast fusion mediated by receptor activator of nuclear factor kappa B (RANKL), TNF-α, and lipopolysaccharide (LPS). Reflecting its inhibitory effects on cell-cell fusion, GK-F suppressed the expression of fusion-mediated molecules such as the dendritic cell-specific transmembrane protein (DC-STAMP). Furthermore, GK-F disrupted cytoskeletal organization which is an essential event for osteoclast function, thereby resulting in the suppression of cell’s bone resorptive activity. Hence, we propose that GK-F might be useful as a therapeutic agent for the treatment of bone-related diseases such as osteoporosis.

DISCUSSION

Bone is continuously remodeled by a series of cellular actions of bone-resorbing osteoclasts and bone-forming osteoblasts. Excessive osteoclastic bone resorption plays a central role in the pathogenesis of most adult skeletal diseases, such as osteoporosis, periodontal disease, rheumatoid arthritis, multiple myeloma and metastatic cancers. In this study, we report Gymnasterkoreayne F (GK-F) as a novel inhibitor of osteoclast development and bone resorption.

Gymnasterkoreayne F, a compound identified from the medicinal plant, *Gymnaster koraiensis*, suppressed osteoclast differentiation and cytoskeletal organization, consequent to bone resorption. In addition, GK-F inhibited the fusion of osteoclast precursors during osteoclast differentiation. Therefore, GK-F might be a candidate for a drug development for the treatment of bone-related diseases such as osteoporosis and rheumatoid arthritis and periodontal disease.

REFERENCES


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Figure 1: GK-F inhibits osteoclast differentiation.

Figure 2: GK-F inhibits osteoclastic bone resorption.