A TANNIN SERIES NATURAL COMPOUND SUPPRESSES RECEPTOR ACTIVATOR OF NF-*K*B-INDUCED OSTEOCLAST DIFFERENTIATION BY INHIBITING P38MAP KINASE AND JNK

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Introduction

Bone remodeling is the continuing processes of bone formation and bone resorption mediated by osteoblasts and osteoclasts, respectively. Osteoclasts are differentiated from hematopoietic precursors of the monocyte/macrophage lineage to large multinucleated cells. In pathological conditions such as osteoporosis, resorption rate mediated by osteoclasts is higher than bone forming rate by osteoblasts, resulting in bone loss. Much effort has been made to develop drugs for controlling activities of osteoclasts and osteocblasts. We have screened natural compounds extracted from plants and found a candidate single compound related to tannin series (named as K16). In the present study, we have evaluated effect of the natural compound on the osteoclastogenesis and signal transduction pathways

Methods

The murine monocytic cell line RAW264.7 (ATCC, Rockville, MD) was maintained in DMEM containing 10% heat-inactivated FBS. For osteoclastogenesis experiments, cells was plated in 96 well cell culture plate at the number of 2000cells/well and cultured in α-MEM containing 10% FBS in the presence of 200ng/ml RANKL (Peprotech). Bone marrow cells (BMMs) were isolated from tibiae and femora of 6 to 8 week old ICR mice and cultured for 24h. After 24h in culture, the non-adherent cells were collected centrifuged on a Histopaque gradient, and the cells at the interface were collected and washed twice with PBS. For osteoclastogenesis experiments, cells was plated in 24 well cell culture plate at the density of 2×10^5cells/well and cultured in α-MEM containing 10% FBS in the presence of 100ng/ml RANKL and 30ng/ml M-CSF. Characterization of Osteoclast like cells was performed using the Tartrate resistant acid phosphatase assay kit (sigma) according to manufacturer's instruction. For resorption fit assay, cells was plated on the calcium phosphate apatite-coated 24well plate, OAAS (Oscotec, Korea). For Western blot analyses, 10-20? of cell lysates was resolved by 10% SDS-PAGE and transferred to PVDF membrane. The membrane was proved with anti-phospho p38, JNK and I?Ba. The same membrane was stripped and reproved with anti- p38 and JNK. For Electrophoretic mobility shift assays (EMSA), nuclear proteins were extracted and the consensus oligonucleotides were end labeled with polynucleotide kinase (Promega). The DNA protein complex was subjected to 5% polyacrylamide gel electrophoresis.

Results

The effects of K16 on differentiation of RAW264.7 and mouse bone marrow cells into osteoclastogenesis cells were investigated. K16 markedly inhibited osteoclastogenesis in the presence of RANKL. RAW264.7 cells could be differentiated into multinucleated TRAP-positive osteoclast like cells in the presence of RANKL. Treatment of K16 dramatically reduced the number of multinucleated TRAP-positive cells and TRAP activity. K16 also reduced osteoclastogenesis of mouse bone cells stimulated with RANKL and M-CSF. Analysis of signal transduction pathways reveals that K16 inhibits RANKL-induced activation of pJNK and p38 MAP Kinases. K16 also inhibits RANKL-mediated activation of AP-1 transcription factor.

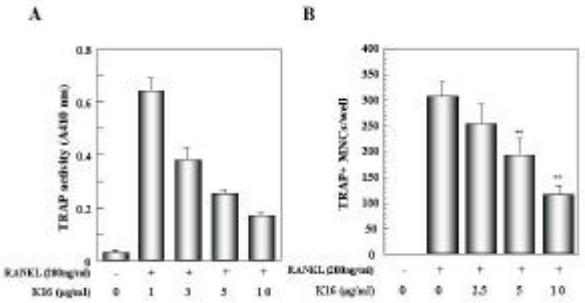

Fig. 1. Effect of K16 on RANKL-induced osteoclast formation from mouse bone marrow cells and RAW264.7 cells. (A) mouse bone marrow cells (B) RAW264.7 cells, The number of TRAP+ multinucleated cells containing three or more nuclei were scored.

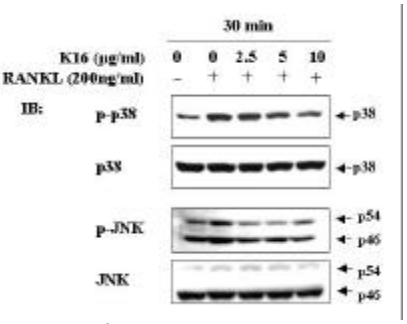

Fig. 2. Phosphorylation of p38 and JNK in RAW264.7 cells treated with RANKL and K16.

Discussion

We found that a tannin series natural compound has a potent inhibitory effect on osteoclast differentiation by inhibiting RANK-induced activation of p38MAP kinase, JNK and AP-1. These results suggest that K16 may be a candidate natural compound for osteoporotic treatment.

Acknowledgements

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