INHIBITORY EFFECTS OF HYDROLYSABLE TANNINS ON OSTEOCLAST DIFFERENTIATION AND FUNCTION THROUGH INHIBITION OF MAP KINASES AND AP-1/KF-KB ACTIVATIONS

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Introduction

Recently it has been shown that flavonoids of polyphenolic compounds such as genistein and daidzein isolated from soyfood exert a therapeutic effect on osteoporosis and that they inhibit osteoclast differentiation [1,2]. Moreover, some flavonoids such as quercetin, kaempferol, and condensed tannins such as (-)-epigallocatechin-gallate (EGCG) derived from vegetables, wine, and green tea induce apoptosis of mature osteoclasts in the same dose-range effective for inhibiting bone resorption [3]. Quercetin also has been shown to inhibit osteoclast differentiation of progenitors at low doses [4], and its glycoside, rutin (quercetin-3-*O*-*β*-D-rutinoside), has been shown to inhibit ovariectomy-induced osteopenia in rats. However, it was not clear whether hydrolysable tannins also exert similar inhibitory effects on osteoclast differentiation and function. Previously we have shown that furosin, a hydrolysable tannin, also have a potential to inhibit osteoclast differentiation and function by a mechanism involving inhibition of RANKL-induced MAP kinase activation and actin ring formation [5]. Therefore, we asked whether other members of hydrolysable tannins have similar inhibitory effects on osteoclast differentiation and function. In the present study, we investigated the effects of two hydrolysable tannins, named as K10 and K43, on osteoclast differentiation and function, and their influence on RANKL-induced early intracellular signaling pathways.

Materials and Methods

The murine monocytic cell line RAW264.7 (ATCC, Rockville, MD) was maintained in DMEM containing 10% heat-inactivated FBS. For osteoclastogenesis experiments, cells (2000cells/well) were 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 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 dentine disk. 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 IκBα. 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

K10 and K43 effectively reduced the formation of osteoclasts in mouse bone marrow cell cultures supported either by coculturing with osteoblasts. These results suggest that K10 and K43 may influence both osteoclast precursors and osteoblastic cells. As expected K10 and K43 significantly suppressed expression of RANKL in osteoblastic cells. We previously showed that furosin directly inhibit osteoclast precursors. Therefore, we tested the effect of K10 and K43 on osteoclast differentiation in bone marrow cells supported by adding macrophage colony stimulating factor (M-CSF) and receptor activator of nuclear factor-*k*B ligand (RANKL). K10 and K43 also strongly inhibited osteoclastic differentiation. These results demonstrate that K10 and K43 have the inhibitory effect on osteoclast differentiation.

On the other hand, we also asked whether K10 and K43 have inhibitory effects on bone resorption. Bone marrow cells cultured under M-CSF and RANKL resorbed dentine slice. However, in the presence of K10 and K43, bone resorption was dramatically reduced. In accordance, K10 and K43 inhibited expression of genes associated with bone resorption such as αvβ3 integrin, MMP, and Src. In addition, K10 and K43 disrupted integrity of actin ring, demonstration that K10 and K43 suppress bone resorption of osteoclasts.

To define the molecular mechanism(s) by which K10 and K43 inhibit osteoclast differentiation, we examined activities of MAP kinases using antibodies specific for phosphorylated form of p38MAPK and JNK. Significantly, the RANKL-induced phosphorylation of both p38MAPK and JNK was gradually inhibited by K10 and K43 in both Raw264.7 cells and mouse BMM cells, suggesting that K10 and K43 may exert its inhibitory function on the signaling pathways leading to activation of p38MAPK and JNK.

It is well known that RANKL activates JNK and IKK and subsequently activates AP-1 and NF-*k*B transcription factors. To determine the effects of K10 and K43 on AP-1 and NF-*k*B activation, RAW 264.7 cells were treated with RANKL alone or RANKL with K10 or K43, and EMSA was performed. As expected, the strong activation of AP-1 and NF-*k*B by RANKL stimulation was substantially inhibited by K10 and K43. Taken together, these data suggest that the inhibitory effects of K10 and K43 on osteoclast differentiation are in part mediated by down regulation of the RANKL-induced activation of p38MAPK, JNK, AP-1 and NF-*k*B pathways.

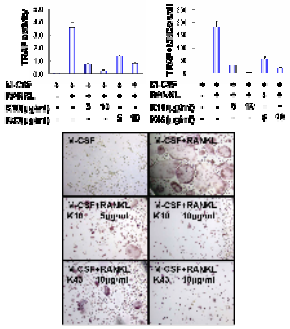

Figure 1. Inhibitory effect of K10 and K43 on osteoclastic differentiation of mouse bone marrow cells.

Discussion

We found that hydrolysable tannins purified from Euphorbiaceae plants have the potent inhibitory effects on osteoclast differentiation by inhibiting RANK-induced activation of p38MAP kinase, JNK and AP-1. These compounds also inhibited bone resorption by inhibiting expression of genes involved in bone resorption, and actin ring integrity. Therefore, hyfrolysable tannins may be candidate natural compounds for treatment of skeletal diseases such as osteoporosis and arthritis.

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