

## Inhibitory effects of R-518 on osteoclast differentiation and bone resorption

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### Objectives

Several plants and herbs have long been used for osteoporosis as folk medicine in oriental countries. By screening extracts and natural products from plants for the ability to reduce osteoclast differentiation, we identified R-518 to be potentially useful for bone lysis-associated diseases.

### Materials and Methods

#### Reagents

Natural compounds, ICR mouse, antibodies (phospho-ERK, ERK, phospho-JNK, JNK, phospho-p38, p38, phospho-IkB, IkB, phospho-CREB, CREB, NFATc1, actin, c-fos), and other chemicals were used.

#### Methods

Nonadherent bone marrow cells derived from 5-6 week-old ICR mice were plated on 100mm bacterial dishes, and cultured in alpha-MEM with 10% fetal bovine serum containing 30 ng/ml M-CSF. After 3 days, adherent cells were used as bone marrow macrophages (BMMs) after washing out the nonadherent cells including lymphocytes. mRNA expression levels were detected by RT-PCR analysis and protein levels were detected by Western blotting analysis.

### Results and Discussion

R-518 extract dramatically inhibited osteoclast differentiation. R-518 strongly reduced the induction of c-fos and NFATc1 after RANKL stimulation in a dose-dependent manner. R-518 had no effects on RANKL-induced activation of JNK, ERK, and p38. Although R-518 did not affect MAPK phosphorylation, it inhibited CREB and ATF1 phosphorylation in RANKL-stimulated BMMs. Consistently, R-518 reduced bone resorption *in vivo* in animal models of CIA and LPS-challenged mice.

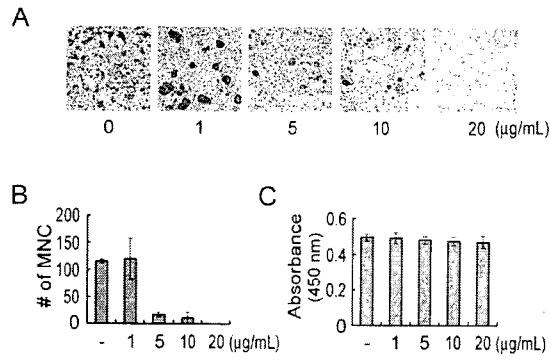


Figure 1. R-518 inhibits osteoclast differentiation.

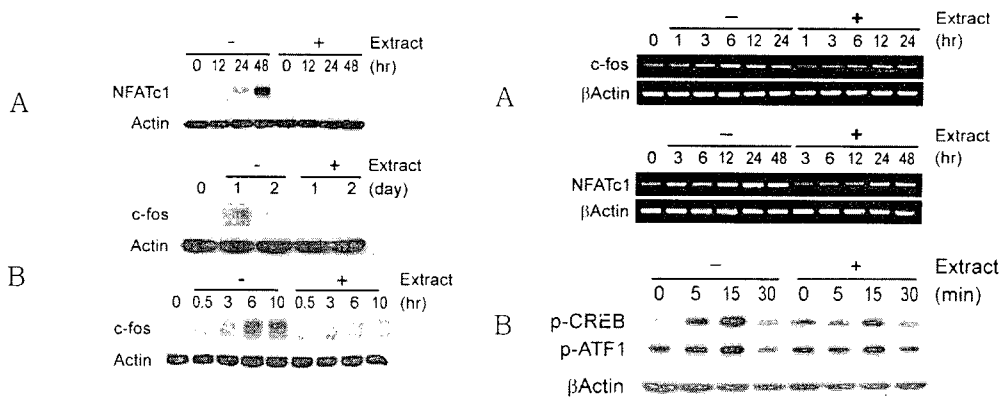
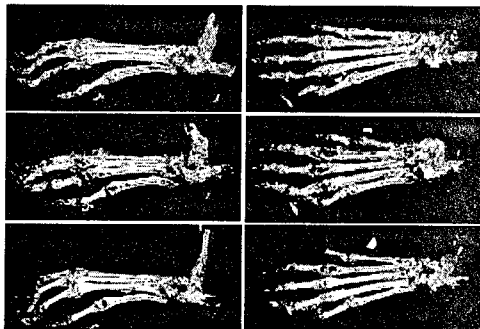


Figure 2. R-518 reduces c-fos (A) and NFATc1 (B) induction by RANKL.

Figure 3. (A) R-518 downregulates mRNA level of c-fos and NFATc1 in RANKL-stimulated BMMs. (B) R-518 reduces CREB and ATF1 phosphorylation by RANKL.



Control  
CIA+PBS  
CIA+R-518

Figure 4. In vivo suppression of bone resorbing activity by R-518 in CIA mice.