Ginsenoside Rh2(S) induces the differentiation and mineralization of osteoblastic MC3T3-E1 cells through activation of PKD and p38 MAPK pathways

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INTRODUCTION

Osteoporosis is a common disorder that is characterized by low bone mineral density and compromised bone strength, and predisposes the patient to increased risk of fracture (1). In normal bone remodeling or bone turnover, osteoblastic bone formation and osteoclastic bone resorption are coupled in a precise and orchestrated manner. The management of osteoporosis is among the greatest challenges faced by modern medicine. Traditional therapeutic agents for osteoporosis have been estrogen, calcitonin, and bisphosphonates, which inhibit bone resorption. These drugs seem to be the most effective method to reduce the rate of postmenopausal bone loss, but may be accompanied by severe side-effects such as breast cancer, venous thromboembolism, rhinitis, or esophageal ulcer (2, 3). Recently, attempts have been made to use a combination of anti-resorptive agents and bone formation-stimulating agents (4). However, the available bone-forming agents have serious adverse effects, may not improve bone quality, or may not reduce the susceptibility to fracture. Therefore, there is an increasing need for safer therapeutic agents with efficacy comparable to commercially available drugs for treating disorders of bone remodeling.

Ginseng, the root of Panax ginseng C.A. Meyer (Araliaceae), has been used as a traditional Chinese medicine for >2,000 years in Asia. In humans and animals, ginseng and its active ingredients show widely beneficial effects including improving immune function, preventing cancer, enhancing sexual function, and inhibiting adipocyte differentiation (5-8). However, few studies have been conducted to examine the anti-osteoporosis effect of ginseng (9, 10). Liu et al. speculated whether ginsenosides affect the differentiation of osteoclasts, and they found that ginsenosides Rh2(R) and Rh2(S) significantly depress osteoclast formation, and Rh2(R) shows a stronger inhibitory effect on osteoclast formation than Rh2(S) (10).

As part of the search for biologically active anti-osteoporotic agents which enhance differentiation and mineralization of osteoblastic MC3T3-E1 cells, we found ginsenoside Rh2(S) to be one of the most active ginsenosides. This effect was mediated by PKD and p38 mitogen-MAPK signaling pathways.

RESULTS

Effects of Rh2(S) on the differentiation and mineralization of MC3T3-E1 cells

To examine the effect of Rh2(S) on the differentiation and mineralization of osteoblasts, MC3T3-E1 osteoblast cells were incubated with Rh2(S) as indicated during osteoblast differen-
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Fig. 1. Effect of Rh2(S) on the differentiation and mineralization in MC3T3-E1 cells. Cells were cultured in osteogenic medium with or without Rh2(S) as indicated for 14 days. Differentiation and mineralization were evaluated by ALP activity and Alizarin Red/von Kossa staining, respectively (A-C). In panel A, vehicle denotes dimethyl sulfoxide in which Rh2(S) was dissolved. Quantification of Alizarin Red staining was performed by extraction with ethylpyridium chloride. The levels of gene expression were analyzed by real-time RT-PCR (D and E). Total RNA was collected on days 3, 7, and 14. Each value represents the mean ± SEM of the fold increase over the control. *P < 0.05, **P < 0.01, and ***P < 0.001 compared to the control group.

The ALP activity, an early-stage osteoblast differentiation marker, reached a maximum level at day 7 and then declined (Fig. 1A), and the activity increased in a concentration-dependent manner (Fig. 1B). Alizarin Red and von Kossa stainings showed that mineralized matrix in MC3T3-E1 cells was formed in a concentration-dependent manner by Rh2(S) on day 14 (Fig. 1C). Rh2(S) (40 μM) markedly increased ALP activity and mineralization by 4.8-fold and 2.5-fold, respectively, when compared to those activities in the control group. To examine the molecular mechanism underlying the promotion of mineralization, gene expression profiles of osteogenic markers, such as ALP, OCN, Osx, and Col-I were investigated after 3, 7 and 14 days of treatment with 40 μM of Rh2(S). As shown in Fig. 1D and 1E, Rh2(S) significantly increased the mRNA expression levels of ALP, OCN, Osx, and Col-I in concentration-dependent manners. The highest level of ALP mRNA expression was observed on day 3, and then the expression level abated, whereas OCN, Osx, and Col-I mRNA were gradually up-regulated until day 14. On day 7, Rh2(S) significantly increased the gene expression of OCN, Osx, and Col-I in concentration-dependent manners (Fig. 1E).

Rh2(S) induces differentiation and mineralization of MC3T3-E1 cells via p38 MAPK activation
We investigated whether p38 MAPK is associated with Rh2(S)-induced differentiation and mineralization of MC3T3-E1 cells. As shown in Fig. 2A and 2B, Rh2(S) markedly stimulated the phosphorylation of p38 MAPK in time- and concentration-dependent manners. Next, to confirm that Rh2(S)-induced differentiation and mineralization resulted from activation of p38 MAPK, a pharmacological approach using a p38 MAPK inhibitor (SB203580) was explored. As shown in Fig. 2C, phosphorylation of activating transcription factor 2 (ATF2), a specific target protein for p38 MAPK, was markedly decreased when MC3T3-E1 cells were pretreated with SB203580. In addition, Rh2(S)-induced stimulation of osteogenic gene expression, ALP activity, and mineralization were all attenuated in the presence of SB203580 (Fig. 2D, E and F). These results suggest...