ALENDRONATE ENHANCES OSTEOGENIC DIFFERENTIATION IN HUMAN MESENCHYMAL STEM CELLS DERIVED FROM BONE MARROW AND ADIPOSE TISSUE

Mei-Ling Ho1,2, Chain-Fu Chen1, Yu-Fang Chang2, Che-Yu Hsu2, Shih-Mao Chen1,2, Yun-Wei Lai1,2, Je-Ken Chang3,2, Gwo-Jaw Wang3,2
1Department of Physiology, Graduate Institute of Physiology, Kaohsiung Medical University, Kaohsiung, Taiwan; 2Orthopaedic Research Center, Kaohsiung Medical University, Kaohsiung, Taiwan; 3Department of Orthopaedics, Kaohsiung Medical University, Kaohsiung, Taiwan chencf@ntu.edu.tw

Introduction: Bisphosphonates are well known potent inhibitors of osteoclast activity and are widely used in the clinical treatment of various systemic metabolic bone diseases. Recently, there is increasing evidence that bisphosphonates also interact with osteoblasts [1, 2]. Bisphosphonates act as blockers of the mevalonate pathway through inhibition of farnesyl pyrophosphate (FPP) synthase [3]. According to the interference with mevalonate pathway, statins, a class of drugs clinically used to suppress cholesterol synthesis, are found to increase osteoblastic bone formation [4]. Some recent studies further indicated that bisphosphonates might have anabolic effects on osteoblasts; however, the findings were controversial [5, 6]. In this study, we investigated the effect of alendronate on osteogenesis of mesenchymal stem cells in vitro. The influence of alendronate on expressions of osteogenic genes and mineralization potential in human bone marrow mesenchymal stem cells (hBMSCs) and human adipose tissue-derived stem cells (hADSCs) were examined.

Materials and Methods: Human bone marrow was obtained from the iliac crest of young trauma patient who did not have other bone disorders. After percoll separation, hBMSCs were selected by K-NAC medium. In order to isolate the hADSCs, the adipose tissues were digested with collagenase and hADSCs were also selected by K-NAC medium. Cell cultures within 15 doublings were used for experiments. Cultures were treated with alendronate (1-5 μM) for 3, 5, and 7 days. The mRNA expressions of osteogenic-related genes, BMP2 (bone morphogenetic protein 2) was tested by quantitative RT-PCR. After alendronate treatment, cells were cultured in osteo-induction medium. Cultures were examined β-catenin translocation by confocal microscopy 5 min to 2 hours after alendronate (10μM) treatment. Alkaline phosphatase (ALP) activity was tested 5-7 days after osteo-induction by using the Phospha-LightTM system (Applied Biosystems). Mineralization was determined 7-21 days after osteo-induction by using Alizarin Red S staining and the quantitative analysis of Alizarin Red S staining was performed by osteogenesis quantification kit (CHEMICON).

Results: A 1-hr treatment of alendronate induced the traslocation of β-catenin into nucleus in ADSC (Fig. 1). Three to seven days treatments of alendronate (5μM) increased the mRNA expression of BMP2 in hBMSCs and hADSCs (Fig. 2A). Moreover, a 5-day treatment of alendronate significantly enhanced ALP activity 5-7 days after osteo-induction in ADSCs (Fig. 2B) and increased mineralization 7-21 days after osteo-induction in both hBMSCs and hADSCs (Fig. 2C, D).

Discussion: In this study, we found that a very short time treatment of alendronate induced β-catenin translocation into nucleus, and further demonstrated that alendronate increases the expressions of BMP2, the osteogenic gene, in cultured hBMSCs and hADSCs. Furthermore, the ALP activity and mineralization were consequently enhanced at both matrix maturation and mineralization stages. These results suggest that alendronate can enhance osteogenesis of human mesenchymal stem cells via inducing the expressions of osteogenic gene, at least BMP2. However, the molecular mechanism of alendronate effects on β-catenin pathway and BMP2 expression need to be further investigated.