Osthole Enhances Osteogenesis in Preosteoblasts via cAMP/PKA Pathway

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Disclosures: None.

Introduction: Bone healing takes place in consequence of bone fractures, bone defects and orthopedic surgery. Multiple factors including patients’ systemic diseases, severe soft tissue damage and inadequate medical treatment may lead to impairment of bone regeneration and ultimately, delayed union or non-union. Enhancement of fracture healing is therefore a worthwhile and challenging subject. Osthole (Ost) is a coumarin-like derivative isolated from herb Cnidium monnieri. It promoted osteogenesis via upregulating several key growth factors such as BMP-2, FGF-2 and IGF-1 [1-6]. On the other hand, osthole increased cyclic adenosine monophosphate (cAMP) level by inhibiting cAMP phosphodiesterases (PDE) in non-osseous tissue [7-10]. Thereby, we hypothesised that the cAMP-elevating effect of osthole may also contribute to enhanced osteogenesis in osteoblast.

Methods: To test this hypothesis, the effect of osthole on osteogenic differentiation was investigated on mouse preosteoblast MC3T3-E1. The osteogenic differentiation was assessed by alkaline phosphate (ALP) activity and mineralization status. Expression of growth factors and osteogenesis-related genes was examined by real-time RT-PCR and western blot analysis. A cAMP competitive enzyme immunoassay kit was utilized for intracellular cAMP quantification.

Results: Results showed that osthole promoted osteogenesis by enhancing ALP activity and mineralization dose dependently in the range of 1-100µM (Fig.1). The anabolic effect of osthole on MC3T3-E1 cell was ascribable to activation of BMP-2 (Fig.2A) followed by inducing Runx2, and further triggering the expression of other downstream osteogenic-related genes such as Osx, ALP, OCN and Col-1 (Fig. 2B). Application of BMP receptor blocker noggin confirmed that osthole-induced osteogenesis was mainly associated with BMP signaling pathway (Fig.3). On the other hand, cAMP-EIA test suggested that osthole application can significantly elevate MC3T3-E1 cellular cAMP level in a dose dependent manner (Fig.4A); and then increased CREB phosphorylation consequently (Fig.4B). Co-treatment with protein kinase A (PKA) inhibitor KT5720 was able to significantly down-regulate osteogenic-related genes and partially suppress osthole-mediated osteogenesis (Fig.4C).

Discussion: Together, these results demonstrate that osthole promotes osteogenesis in MC3T3-E1 via BMP pathway which is mediated, at least in part, by cAMP/PKA signaling. Based on previous literatures and current in vitro results, osthole is a potential agent to facilitate bone fracture healing.

Significance: The underlying mechanism of the anabolic effect of osthole still remains unclear. The finding of this study increase the knowledge and understanding of osthole on osteogenesis model and provide experimental evidence to apply osthole on treatment of bone injury or defect.

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**Figure 1:** ALP activity (A) and Alizarin Red S-stained photography (B) of MC3T3-E1 cell treated with osthole for 12 and 24 days respectively. (One-way ANOVA, **p<0.01, ***p<0.001;
Figure 2: RT-PCR analysis of mRNA level of growth factors (A) and osteogenic genes (B) in MC3T3-E1 cell treated with osthole for 12 hours and 6 days respectively.
Figure 3: ALP activity (A) and RT-PCR analysis of osteogenic genes mRNA level (B) of MC3T3-E1 cell treated by osthole with or without noggin. (T-test, *p<0.05, **p<0.01)
Figure 4: cAMP level (A) and western blot analysis of p-CREB and CREB protein level (B) in MC3T3-E1 cell treated with osthole. (C) RT-PCR analysis of osteogenic genes mRNA level of MC3T3-E1 cell treated by osthole with or without KT5720. (One-way ANOVA, *p<0.05; T-test, *p<0.05)

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