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Psoralidin, a prenylated coumestan, as a novel anti-osteoporosis candidate to enhance bone formation of osteoblasts and decrease bone resorption of osteoclasts

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ABSTRACT

Traditional Chinese medicines (TCM) have been proven to prevent osteoporosis, but their clinical applications are not widely recognized due to their complicated ingredients. Psoralidin, a prenylated coumestan, has been reported to prevent bone loss of ovariectomized rats, but detailed mechanisms are still not clear. In current study, we found that both psoralidin and coumestrol promoted osteoblast proliferation and differentiation, as evidenced by improvements in cell proliferation and alkaline phosphatase activity; increased formation of ALP colonies and calcified nodules; enhanced secretion of collagen-I, BMP-2, osteocalcin and osteopontin; and stimulation of the expression of IGF-1, β-catenin, Runx-2, Osterix, and OPG, as well as the mRNA ratio of OPG/RANKL, while significantly decreasing the expression of RANKL. In addition, both psoralidin and coumestrol inhibited osteoclast formation and osteoclastic bone resorption, as demonstrated by the lower tartrate-resistant acid phosphatase activity and smaller area, with fewer resorption pits formed. Interestingly, psoralidin showed much stronger effects than coumestrol at enhancing osteoblast proliferation/differentiation or inhibiting osteoclast differentiation and bone resorption. Moreover, we found that both psoralidin and coumestrol suppressed COX-2 and ROS production in rat osteoblastic calvarias cells, and psoralidin showed stronger effects than coumestrol. Furthermore, we detected that by blocking estrogen receptors with ICI 182.780 (an estrogen receptor antagonist), the osteoprotective effects of psoralidin and coumestrol were also blocked. Our findings demonstrated that psoralidin and coumestrol exert their bone-protective effects by enhancing bone formation of osteoblasts and inhibiting bone resorption of osteoclasts. These roles might be mediated by their antioxidant activity and transduced through estrogen receptor signaling.

1. Introduction

Osteoporosis is referred to as a ‘silent disease’ that caused by a metabolic disorder of bone that often leads to osteoporotic fractures, especially vertebral and hip fractures. Osteoporosis-related fragility fractures are a major source of morbidity and mortality in the aging population and result in a significant health and economic burden on society (Giangregorio et al., 2014; Larsson and Fazzalari, 2014). Although anti-osteoporotic agents have already obtained satisfactory effects, they still possess some side effects, such as that large doses of bisphosphonates increase the potential incidences of osteonecrosis of the jaw bone (Mouri et al., 2009), high doses of parathyroid hormone (PTH) may cause osteosarcomas (Khosla et al., 2008), and prolonged use of hormone replacement therapy (HRT) increases the risk for endometrial and breast cancers (Wei et al., 2012). Recently, many researchers have begun to focus on Chinese Herbal Medicines (CHM) because of their clear osteoprotective effects, lower costs, fewer side effects and thousands of years of usage in osteoporosis clinical practice (Huang et al., 2015; Wang et al., 2013).

Psoralea corylifolia L. is a classic herb for treating osteopenia and bone fractures (Zhai et al., 2012b) and has been used in many classic Chinese medicine formulas to strengthen bone, such as Xianling Gubao

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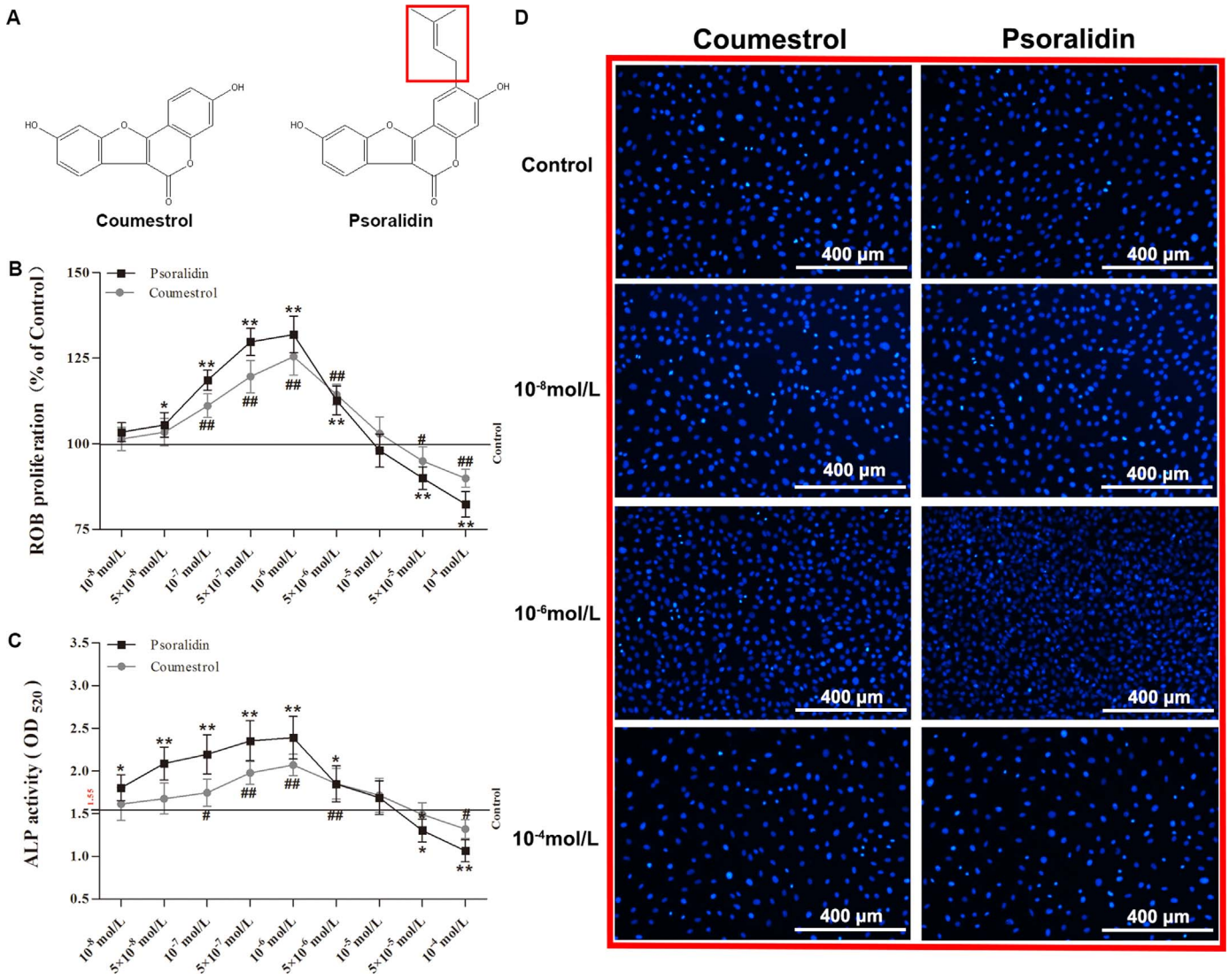

Fig. 1. The effects of coumestrol and psoralidin on cell proliferation and ALP activity in rat calvarial osteoblasts (ROB). The chemical structures of coumestrol and psoralidin are compared (A). Dose-dependent effects of coumestrol and psoralidin on cell proliferation (B) and ALP activity (C) in ROB cells. ROB cells were cultured in culturing medium and treated with different doses of coumestrol and psoralidin for 48 h; then, the cell proliferation rates were measured by the MTT assay (B); the same treated cells were stained using Hoechst 33342 nuclear dye and photographed by microscopy (D). ROB cells were cultured in osteogenic culture medium and supplemented with different doses of coumestrol and psoralidin for 9 days, and the alkaline phosphatase (ALP) activity was measured (C). The data represented three experiments and are shown as the mean ± *S.D.* $^{*}P < 0.05$, $^{**}P < 0.01$ represents psoralidin *vs.* control; $^{\#}P < 0.05$, $^{\#\#}P < 0.01$ represents coumestrol *vs.* control.

Capsule (Wu et al., 2009a), Er-Xian Decoction (Wong et al., 2014) and others. *Psoralea corylifolia* L. contains many ingredients, including psoralen, isopsoralen, bavachin, backuchiol, bavachalcone, psoralidin and corylifol A. Many previous studies have concentrated on the osteotropic activities of psoralen and isopsoralen (Tang et al., 2011; Yang et al., 2012; Zhai et al., 2012a), and have we also reported that another ingredient, bavachin, preserved osteoprotective effects (Kong et al., 2013). Psoralidin, a prenylated coumestan (the chemical structure is shown in Fig. 1A), used to be known for its strong anti-tumor effects (Bronikowska et al., 2012) and antidepressant-like effects (Yi et al., 2008). Recently, a study has reported that psoralidin can prevent bone loss in ovariectomized rats through up-regulation of the bone density of the lumbar vertebrae and femur, increasing the maximum bending strength of the femur and the serum levels of estrogen and calcitonin (Li et al., 2013), although the underlying molecular mechanism for these effects is unclear. In the present study, we demonstrated that psoralidin can enhance osteoblast proliferation and differentiation as well as inhibit osteoclast differentiation and bone resorption *in vitro*. Both of the osteoprotective activities of psoralidin were much stronger than those of coumestrol.

2. Materials and methods

2.1. Animals and reagents

New born *Sprague-Dawley* rats in the study were supplied from the Experimental Animal Center of Henan Province (Zhengzhou, China). All of the procedures for the handling of the rats were carried out according to the 'Guide for the Care and Use of Laboratory Animals' published by the US National Institutes of Health and approved by the Ethics Committee of Luoyang Orthopedic Hospital of Henan Province.

Coumestrol (purity: 98%) was obtained from Enzo Life Science Company (Farmingdale, NY, USA). Psoralidin (purity: 98%) was purchased from Winherb Medical Science Company (Shanghai, China). Alpha Minimal Essential Media (α-MEM) culture media and Penicillin-Streptomycin solution were purchased from HyClone (South Logan, Utah, USA). Fetal bovine serum (FBS) and Trypsin-EDTA solution were obtained from Gibco BRL (Gaithersburg, MD, USA). The majority of other chemicals/reagents, including dexamethasone, β-glycerophosphate, macrophage colony stimulating factor (M-CSF), recombinant of sRANK ligand (RANKL), dimethyl sulfoxide (DMSO),