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Extract of Magnoliae Flos inhibits ovariectomy-induced osteoporosis by blocking osteoclastogenesis and reducing osteoclast-mediated bone resorption

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ABSTRACT

Bone homeostasis is maintained by a balance between bone resorption by osteoclasts and bone formation by osteoblasts. Osteoporosis occurs when osteoclast activity surpasses osteoblast activity. Pro-inflammatory cytokines stimulate osteoclast differentiation and activity by increasing production of macrophage-colony stimulating factor and receptor activator of nuclear factor-κB ligand (RANKL). In this study, we investigated whether Magnoliae Flos (MF), one of the most commonly used Chinese medicinal herbs for managing rhinitis, sinusitis and headache, could effectively inhibit osteoporosis. In ovariectomized (OVX) mice compared to sham mice, the body weight increased and serum levels of alkaline phosphatase (ALP), tartrate resistant acid phosphatase 5b, calcium, and osteocalcin were significantly elevated. However, orally administrated MF extract substantially inhibited the increased body weight and serum levels of bone turnover markers, without any evidence of tissue toxicity. MF extract treatment significantly reversed the morphometric parameters of ovariectomy-induced bone loss, including trabecular bone volume, thickness, number, separation, and bone density, to almost the same levels of the sham mice. Furthermore, MF extract reduced the RANKL-mediated osteoclast differentiation and bone resorption by inhibiting the activities of matrix metalloproteinases (MMPs) and cathepsin K in mouse bone marrow macrophages. MF extract appeared to increase ALP activity in murine osteoblastic cells. Taken together, MF extract may be a beneficial supplement for the blockade of osteoporosis progression, particularly for the management of postmenopausal osteoporosis.

1. Introduction

Osteoporosis is a major worldwide public health problem in which patients suffer from a related fracture, and that reduces functional independence and poses a great economic burden to the patients' families and society. Osteoporosis is predominantly a disease of aging, affecting postmenopausal women in particular, with a 50% fracture risk in all women after the age of 50 years and a 25% risk in men [1]. In addition, osteoporosis and fragility fractures are prevalent in inflammatory diseases, including rheumatoid arthritis, systemic lupus erythematous, inflammatory bowel disease, and chronic obstructive pulmonary disease compared to a healthy population. Proinflammatory cytokines are closely associated with bone resorption in age- and estrogen

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deficiency-related bone loss, as well as in these inflammatory diseases [2].

Treatment modalities for osteoporosis focus on preventing further bone loss and reducing the risk of fracture. Currently available agents used to treat osteoporosis include estrogen, raloxifene, bisphosphonates, and calcitonin. The mechanisms of action of these agents are based on the inhibition of osteoclastic bone resorption [3]. However, these anti-resorptive agents have numerous side effects, causing many patients to discontinue their use [4,5]. Administration of IL-1 receptor antagonist and TNF-α antibody has also been shown to reduce joint destruction and secondary osteoporosis in rheumatoid arthritis and to completely abolish ovariectomy-induced bone loss, as has estrogen replacement [6]. However, these agents are associated with a substantial risk of toxicity and require parenteral administration [7]. Thus, it is necessary to develop novel agents with fewer undesirable side effects to prevent or reverse osteoporosis.

Medicinal plants with anti-inflammatory activity have been recognized as a potential source of promising agents for preventing and treating bone diseases. Magnoliae Flos (MF, Chinese name: Xin-yi) is one of the most commonly used herbs in traditional Chinese medicine to treat nasal congestion with headache, sinusitis and allergic rhinitis [8]. More than 20 different *Magnolia* species have been used clinically. Three of these species, *Magnolia biondii*, *M. denudate* and *M. sprengeri* are listed in the Chinese Pharmacopoeia [9]. Several studies have reported the inhibitory activity of MF extract and its active compound on inducible nitric oxide synthase in human respiratory epithelial cells [10] and on proliferation of synovial cells isolated from collagen-induced arthritis mice and patients [11]. Moreover, the lignans in MF are converted to active phytoestrogens, so-called “mammalian lignans” enterodiol and enterolactone, by microflora in the proximal colon [12]. In the present study, we determined the anti-osteoporotic potential of MF extract by investigating its effects on ovariectomy-induced osteoporosis in mice and osteoclastogenesis in mouse bone marrow macrophages.

2. Materials and methods

2.1. Plant material and extraction

The dried Magnoliae Flos (*M. biondii*, Magnoliaceae) was purchased from Yangnyeongsi herbal medicine market (Seoul, Korea) and authenticated by Professor Lee, J.-H. in Dongguk University (Gyeongju, Korea). A voucher specimen (LJH2007-8) was deposited in the herbarium of the College of Oriental Medicine, Dongguk University. Magnoliae Flos (10.45 kg) was successively extracted three times with methanol (20 l) for 5 h at 60 °C and the solvent was evaporated under reduced pressure to obtain the methanol extract (864.91 g). Lignan compounds, eudesmin, magnolin, and lirioresinol B dimethyl ether, were isolated as described previously [13].

2.2. Chemicals

MF extract and lignan compounds were dissolved with dimethyl sulfoxide (DMSO), respectively, and diluted with media just before use. The final concentration of DMSO in the cell culture media was maintained below 0.1%. Minimum essential medium-alpha (α-MEM), fetal bovine serum (FBS), Dulbecco's phosphate buffered saline (PBS), antibiotic–antimycotic (100×), penicillin–streptomycin and 25% trypsin–EDTA were purchased from Gibco BRL (Grand Island, NY). Histopaque-1083, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) and DMSO were purchased from Sigma-Aldrich (St. Louis, MO). Recombinant mouse soluble RANK Ligand (sRANKL) and macrophage-colony stimulating factor (M-CSF) were purchased from Koma Biotech (Seoul, Korea) and R&D Systems (Minneapolis, MN), respectively. All reagents used in this study were of analytical grade.

2.3. Animals

4-week-old male ICR mice (20±2 g) and 8-week-old female control, sham-operated, and surgically ovariectomized (OVX) ICR mice (29±1 g) were purchased from Central Lab Animal Inc. (Seoul, Korea). All mice were provided free access to a standard chow diet (Orient, Seongnam, Korea) and tap water. They were maintained at 22±2 °C, with a relative humidity of 50±5% and a 12 h light–dark cycle. The animal studies were conducted in accordance with the rules and regulations established by the Institutional Animal Ethics Committee of the Yonsei University College of Dentistry.

2.4. Induction of ovariectomy-induced osteoporosis in mice

ICR mice were randomly divided into 6 groups (10 mice per group): control, sham, OVX, 17β-estradiol (E2, 10 μg/kg body weight (BW))-treated OVX, and MF extract (0.1 and 1 mg/kg BW)-treated OVX groups. MF extract was orally administrated and E2 was subcutaneously injected into the mice 5 times per week for 12 weeks. Sham and OVX mice were treated with vehicle (PBS containing 0.1% DMSO) instead of MF extract or E2. The mice were weighed every 4 weeks. At the end of treatment, the mice were sacrificed after the blood was collected by cardiac puncture. The livers and kidneys of the mice were excised and then immediately fixed in 10% neutral buffered formalin. The femurs were removed and stored at −20 °C.

2.5. Determination of serum biochemical parameters

Blood samples were maintained at room temperature for 1 h, and then centrifuged at 1910 ×*g* for 20 min to obtain serum. Serum alkaline phosphatase (ALP) and osteocalcin levels, as the biomarkers of bone formation, were estimated with QuantiChrom ALP and Calcium Assay Kits (BioAssay Systems, Hayward, CA) and Osteocalcin EIA Kit (Biomedical Technologies, Stoughton, MA) according to the manufacturers' instructions, respectively. Serum levels of calcium and tartrate-resistant acid phosphatase 5b (TRAP5b), as a biomarker of bone resorption, were evaluated using a mouse TRAP Assay Kit (Immunodiagnostic Systems, Scottsdale, AZ). Serum alanine aminotransferase (ALT) and blood urea nitrogen (BUN) levels were measured as an indicator of kidney and liver function, using commercially available kits (YD Diagnostics, Yongin, Korea), respectively.