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Triterpenoids from Cimicifugae rhizoma, a novel class of inhibitors on bone resorption and ovariectomy-induced bone loss

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Abstract

Objective: Increasing research suggested that Cimicifugae rhizoma might be protective against osteoporosis. In this study, we investigated the effects of three cycloartane-type triterpenoids isolated from Cimicifugae rhizoma, cimicidol-3-*O*-β-D-xyloside (**1**), cimicidanol-3-*O*-β-D-xyloside (**2**) and acetylacteol-3-*O*-β-D-xyloside (**3**) on bone resorption in vitro and bone loss in ovariectomized (OVX) mice.
Methods: The activities of the tested compounds on bone resorption were evaluated using three assays, neonatal mouse parietal bone organ culture, osteoclast-like cells (OCLs) formation and pit formation. The effects on bone mineral density (BMD) and uterine weight were examined using OVX mice. Using LC–MS/MS method, the serum concentrations of the triterpenoids were measured in mice serum collected at 0.5, 1, 3, 6 and 12 h following its oral administration.
Results: All of the tested compounds exerted the inhibitory effects on bone resorption in bone organ culture, suppressed both of the formation and the resorbing activity of OCLs. Furthermore, a synergistic effect was observed among those compounds. In vivo studies revealed that compounds **1**–**3** and the mixture of compounds **1**–**3** prevented the bone loss in OVX mice without affecting uterine weight, and each compound was detected in the mice serum after single oral administration.
Conclusions: The triterpenoids exerted the inhibitory effects on osteoclastic bone resorption through the suppression of both OCLs formation and the resorbing activity of OCLs, and also showed a significant protective effect on BMD in OVX mice. The present results might provide a new pharmacological potential for the treatment of osteoporosis.

Keywords: Triterpenoids; Cimicifugae rhizoma; Bone resorption; Osteoclast-like cells; Pit formation; Ovariectomized (OVX) mice; Serum concentration; Bone mineral density (BMD)

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1. Introduction

Osteoporosis is a silent disease characterized by low bone mineral density and structural deterioration of bone tissue, which leads to bone fragility and increased susceptibility to fractures. The disease is widely recognized as a major public health problem, particularly among postmenopausal women [1]. Approximately half of postmenopausal women will sustain an osteoporosis-related fracture and 15% will sustain a hip fracture in their lifetime [2]. The cause of the disease stems from an imbalance of the bone remodeling process with bone resorption exceeding bone formation due to a dramatic deficiency of female hormone. Currently, estrogen replacement therapy (ERT) or hormone replacement therapy (HRT) showed an established benefit in the prevention of fractures and bone loss in postmenopausal women, however, ERT/HRT significantly increase the risks of breast cancer, endometrial cancer, heart attacks, strokes, and blood clots, which are generally called estrogen-like side effects [3]. Therefore, the use of alternatively preventive and treatment strategies for osteoporosis sourced from natural plants to avoid the increased significant risks might potentially be achieved [4].

Cimicifugae rhizoma, listed in Chinese pharmacopoeia [5], has long been used in traditional medicine systems for the treatment of various illnesses. The rhizoma sourced from a number of *Cimicifuga* species, but only four species, *C. dahurica*, *C. foetida*, *C. heracleifolia* and *C. racemosa* are available in market. Up to date, a lot of efforts have been dedicated to the studies on their chemical constituents, pharmacological effects and clinical uses. It has been clarified that main constituents of the rhizoma are highly oxidized cycloartane-type triterpenoids and phenol-type derivatives, and a variety of biological activities such as relief of hot flash, anti-human immunodeficiency virus (HIV), antiinflammatory, antidiabetes, antimalaria have also been discovered [6]. Especially, the root and rhizoma sourced from *C. racemosa*, common name black cohosh, is currently available for the treatment of climacteric complaints [7].

In a research project aimed at finding active compounds or principles from natural resources with potential of anti-osteoporosis, we screened a number of crude drug extracts for their inhibitory activity on bone resorption stimulated by parathyroid hormone (PTH) in a bone organ culture system and discovered that the methanol extracts of rhizomes sourced from *C. dahurica*, *C. heracleifolia* and *C. foetida* showed the inhibitory activity [8]. Further research revealed that ethyl acetate (EtOAc) soluble fractions of methanol extracts of *C. heracleifolia* and *C. foetida* possessed a stronger inhibitory activity on bone resorption and the EtOAc soluble fraction of the methanol extract prevented the loss of bone mineral density (BMD) in OVX rats [8,9]. It has also been reported that the extracts from black cohosh could significantly improve overall bone quality in orchidectomized rats [10], increase serum levels of bone-specific alkaline phosphatase, an indicative marker of activity of osteoblast that functions as bone constructor [11]. These data together with our results proved that Cimicifugae rhizoma might possess a great potential on the treatment of osteoporosis, but its active components are still not well understood.

In the present study, as a continuing research of our project, we report the inhibitory activities of three cycloartane-type triterpenoids isolated for *C. foetida*, cimicidol-3-*O*-β-D-xyloside (**1**), cimicidanol-3-*O*-β-D-xyloside (**2**), acetylacteol-3-*O*-β-D-xyloside (**3**) and their mixture on bone resorption, the formation and resorbing activity of osteoclast-like cells (OCLs). The effects of each compound and the mixture on uterine weight and the maintenance of bone mineral density (BMD) of lumber spine (L1-4) in ovariectomized (OVX) mice and the measurement of the serum concentrations of the compounds in mice are also incorporated.

2. Materials and methods

2.1. Chemicals and reagents

The tested triterpenoids, compounds **1**–**3** were isolated and purified from the rhizome of *C. foetida* by our group (Fig. 1) [12]. The contents of compounds **1**, **2** and **3** in EtOAc extract are about 1.8%, 1.4% and 1.6% (g/g), respectively.

The chemicals, reagents and cells were obtained from the following sources: Ham's F-12 medium from Nissui Pharmaceutical Co. Ltd. (Tokyo, Japan); RPMI-1640 medium from GIBCO Invitrogen Corporation (NY, USA); $^{45}CaCl_2$ from NEN Research