Biochimie 111 (2015) 107–118

Contents lists available at ScienceDirect

Biochimie

journal homepage: www.elsevier.com/locate/biochi

Research paper

Inhibitory effects of ursolic acid on osteoclastogenesis and titanium particle-induced osteolysis are mediated primarily via suppression of NF-κB signaling

Chuan Jiang [a], Fei Xiao [a], Xinfeng Gu [b], Zanjing Zhai [a], Xuqiang Liu [a], Wengang Wang [a], Tingting Tang [a], You Wang [a], Zhenan Zhu [a], Kerong Dai [a], An Qin [a, *], Jinwu Wang [a, *]

[a] Shanghai Key Laboratory of Orthopaedic Implant, Department of Orthopaedic Surgery, Shanghai Ninth People's Hospital Affiliated Shanghai Jiao Tong University School of Medicine, Shanghai, China
[b] School of Biomedical Engineering, Shanghai Jiao Tong University, Shanghai, China

ARTICLE INFO

Article history:
Received 27 October 2014
Accepted 1 February 2015
Available online 11 February 2015

Keywords:
Ursolic acid
Osteoclastogenesis
NF-κB
Titanium particle
Osteolysis

ABSTRACT

Ursolic acid (UA), a pentacyclic triterpenoid found in a variety of plants, has attracted considerable attention because of its important biological and pharmacological activities. However, its effect on osteoclasts and mechanism of action require further investigation. In this study, we evaluated the effects of UA on osteoclastogenesis and osteoclast-mediated osteolysis *in vitro and in vivo*, and explored its possible mechanism of action. The results indicated that UA could inhibit receptor activator of nuclear factor-κB ligand (RANKL)-mediated osteoclastogenesis and the bone resorptive function of osteoclasts in a concentration-dependent manner *in vitro*. Further, UA effectively inhibited the mRNA and protein expression of NFATc1, primarily via the suppression of nuclear factor-κB (NF-κB) signaling, and partly through the suppression of c-Jun N-terminal kinase (JNK) signaling. Additionally, UA treatment down-regulated the expression of NFATc1-regulated osteoclast marker genes. Likewise, UA induced dose-dependent attenuation of titanium (Ti) particle-induced mouse calvarial bone loss, and decreased the number of tartrate-resistant acid phosphatase (TRAP)-positive osteoclasts. In conclusion, these results demonstrate that UA protects against wear particle-induced osteolysis by suppressing osteoclast formation and function. These effects are associated with the inhibition of the NF-κB- and JNK-related signaling pathways.

1. Introduction

Bone metabolism mediated by osteoclasts and osteoblasts has a direct impact on bone mass [1,2]. Thus, osteoblast-osteoclast coordination is critical for the maintenance of skeletal integrity and normal physiological function. Imbalance between bone resorption by osteoclasts and bone formation by osteoblasts during skeletal turnover can give rise to various orthopedic disorders, particularly bone loss caused by excessive bone resorption [3]. Currently, therapeutics for the treatment of low bone mineral density include anti-resorptive and bone anabolic agents. However, the identification of bone-protecting agents that are able to mediate both anti-

Abbreviations: AP-1, activator protein-1; BV/TV, bone volume/tissue volume; BMD, bone mineral density; BMC, bone mineral content; BMMs, bone marrow monocytes; CK, cathepsin K; CT, computed tomography; CTR, calcitonin receptor; DC-STAMP, dendritic cell-specific transmembrane protein; ERK, extracellular signal-regulated kinase; GAPDH, glyceraldehyde-3-phosphate dehydrogenase; IC_{50}, half-maximal inhibitory concentration; IκB, inhibitor of NF-κB; IKK, IκB kinase; JNK, c-Jun N-terminal kinase; MAPKs, mitogen-activated protein kinases; M-CSF, macrophage-colony stimulating factor; MNCs, multinucleated cells; NFATc1, nuclear factor of activated T-cells, cytoplasmic, calcineurin-dependent 1; NF-κB, nuclear factor-κB; PCR, polymerase chain reaction; PMMA, polymethyl methacrylate; PPAR, peroxisome proliferator-activated receptor; RANKL, receptor activator of nuclear factor-κB ligand; ROI, region of interest; STAT3, signal transducer and activator of transcription 3; TGF-β, transforming growth factor-β; Ti, titanium; TRAP, tartrate-resistant acid phosphatase; UA, ursolic acid; UHMWPE, ultrahigh molecular weight polyethylene.

* Corresponding authors. Shanghai Key Laboratory of Orthopaedic Implant, Department of Orthopaedic Surgery, Shanghai Ninth People's Hospital Affiliated Shanghai Jiao Tong University School of Medicine, Room 703, No. 3 Building, 639 Zhizaoju Road, Shanghai 200011, China. Tel.: +86 13301773680; fax: +86 21 63139920.
E-mail addresses: dr_qinan@163.com (A. Qin), jinwu_wang@163.com (J. Wang).

http://dx.doi.org/10.1016/j.biochi.2015.02.002
0300-9084/

resorptive and anabolic effects is of growing interest, and has become a central focus for drug development.

Ursolic acid (UA), a natural pentacyclic triterpene compound (Fig. 1A), is distributed over more than 100 medicinal plants in nature, such as *Ligustrum lucidum* and *Eriobotrya japonica* [4]. Similar to other triterpenoids, UA possesses a wide variety of pharmacological properties, including anti-tumor, anti-inflammation, anti-microbial, and anti-oxidant activities [5,6]. Several studies evaluating the pharmacological mechanism of UA action have focused on its anticancer, hepatoprotective, anti-hyperlipidemic, and anti-depression effects. These studies have revealed that UA modulates signal transducer and activator of transcription 3 (STAT3) [7], mitogen-activated protein kinases (MAPKs) [8], peroxisome proliferator-activated receptors (PPARs) [9], transforming growth factor-β (TGF-β)/Smad signaling [10], and nuclear factor-κB (NF-κB) [11]. However, the effects of UA on bone metabolism remain unclear.

Interestingly, Lee et al. reported that UA promotes bone formation and induces bone-forming activity *in vivo*. Furthermore, they showed that the expression of osteoblast-specific genes was enhanced after UA treatment, and that UA could induce osteoblastogenesis and mineralization of osteoblasts *in vitro*, which was associated with the activation of MAPKs, activator protein-1 (AP-1), and NF-κB [12]. Further, Tan et al. observed that UA extracted from loquat leaves inhibited bone mineral density loss in ovariectomized mice [13]. These findings suggest that UA may be valuable for the treatment of metabolic osteopathy characterized by bone mass loss. Nevertheless, the mechanism of UA action remains unclear.

In this study, we investigated the effects of UA on receptor activator of nuclear factor κB ligand (RANKL)-mediated osteoclast differentiation and bone resorption in the presence of macrophage-colony stimulating factor (M-CSF), and elucidated its underlying molecular mechanisms. In addition, to further validate the effects of UA on osteoclasts *in vivo*, the titanium (Ti) particle-induced calvarial osteolysis model was chosen. This murine model was well recognized for its ability to lead to a series of biologic effects including osteoclast formation and bone resorption, and allowed to quantitatively assess the degree of bone loss [14,15]. Thus, by using this model, we attempted to explore the protective effect of UA on bone mass through the suppression of osteoclastogenesis *in vivo*.

2. Materials and methods

2.1. Reagents and titanium particles

UA (Sigma–Aldrich, St. Louis, MO, USA) was dissolved in dimethyl sulfoxide without exposure to light and stored at −20 °C. Cell culture medium (α-MEM) was obtained from Hyclone (Logan, UT, USA). Penicillin-streptomycin solution, trypsin-EDTA solution (0.25%), and fetal bovine serum (FBS) were obtained from Gibco (Gaithersburg, MD, USA). Recombinant mouse RANKL and M-CSF were provided by PeproTech (Rocky Hill, NJ, USA). The tartrate-

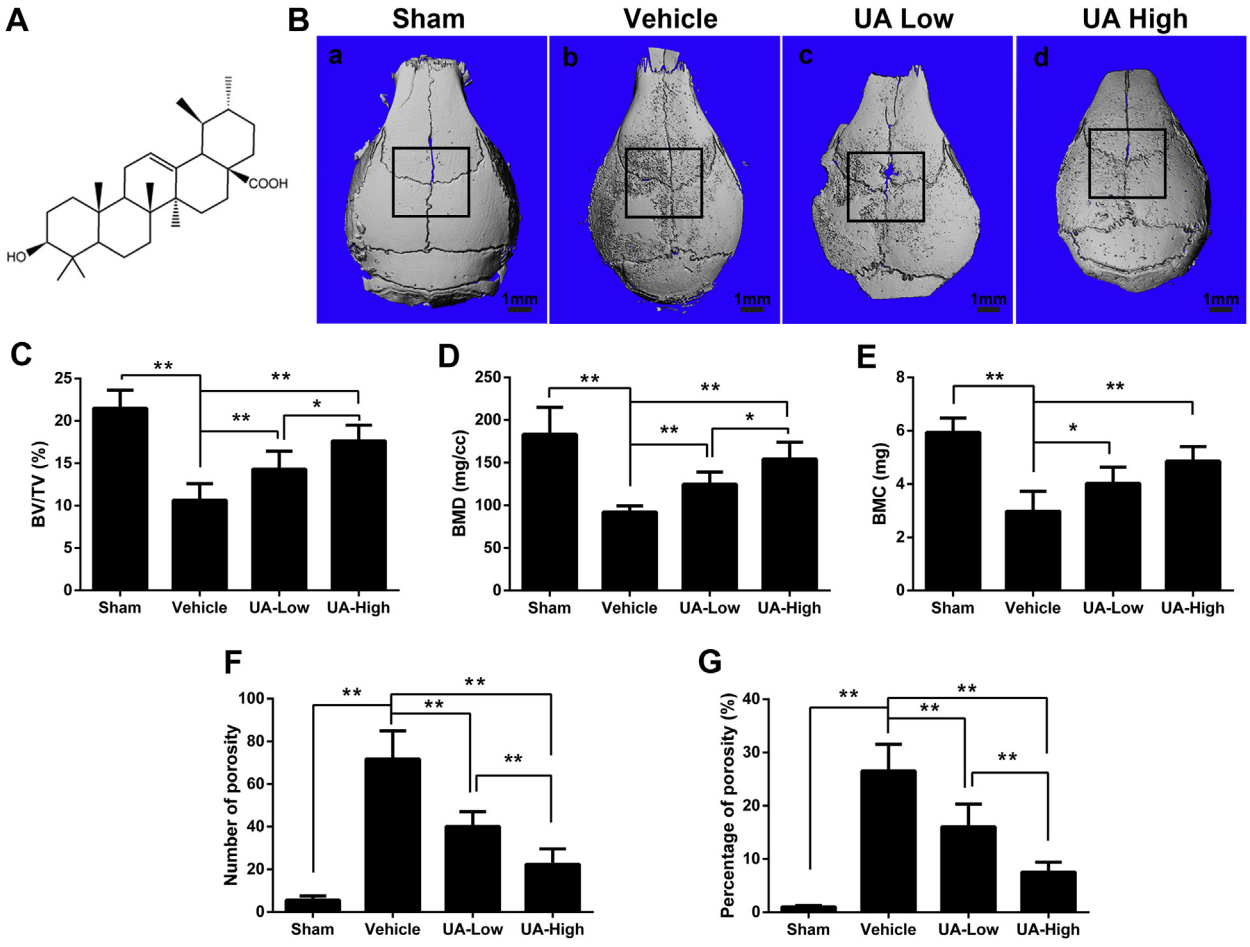

Fig. 1. UA inhibited titanium (Ti) particle-induced murine calvarial osteolysis in a dose-dependent manner. (A) The chemical structure of UA (B) Representative three-dimensional reconstructed micro-computed tomography images from each group. The square-shaped regions of interest (ROI) are shown in the boxes (C–G) BMD, BV/TV, BMC, porosity number, and porosity percentage in the ROI were measured as described in the methods. $^{*}p < 0.05$ and $^{**}p < 0.01$ versus the vehicle group or the indicated groups. Scale bar = 1 mm.