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Diarylheptanoid from *Curcuma comosa* Roxb. suppresses RANKL-induced osteoclast differentiation by decreasing NFATc1 and c-Fos expression via MAPK pathway

Supatta Chawalitpong [a], Nilubon Sornkaew [b], Apichart Suksamrarn [b], Tanapat Palaga [c,d,*]

[a] Graduate Program in Biotechnology, Faculty of Science, Chulalongkorn University, Phayathai Road, Pathumwan, Bangkok 10330, Thailand
[b] Department of Chemistry and Center of Excellence for Innovation in Chemistry, Faculty of Science, Ramkhamhaeng University, Ramkhamhaeng Rd, Bang Kapi, Bangkok 10240, Thailand
[c] Department of Microbiology, Faculty of Science, Chulalongkorn University, Phayathai Road, Pathumwan, Bangkok 10330, Thailand
[d] Center of Excellence in Immunology and Immune-mediated Diseases, Chulalongkorn University, Phayathai Road, Pathumwan, Bangkok 10330, Thailand

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Fast red violet LB salt (PubChem CID: 16219357)
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sulfanilamide (PubChem CID: 5333)
formaldehyde (PubChem CID: 712)
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ABSTRACT

Osteoporosis is caused by a functional imbalance between osteoblasts and osteoclasts. The increased activation of osteoclasts that is a hallmark of osteoporosis results in the progressive loss of bone mass and therefore in an increased susceptibility to bone fractures. Diarylheptanoids are a group of phytoestrogens that have been isolated from a number of plant species, including the rhizomes of *Curcuma comosa* Roxb. In this study, the effect of one of diarylheptanoids, (3*S*)-1-(3,4-dihydroxyphenyl)-3-hydroxy-7-phenyl-(6*E*)-6-heptene (DHPH), was investigated for anti-inflammatory and anti-osteoclastogenic activity. DHPH significantly inhibited nitric oxide production in RAW264.7 cell line following their activation by lipopolysaccharide and interferon-γ, with no cytotoxicity. In primary mouse bone-marrow-derived macrophage precursors, DHPH suppressed osteoclastogenesis induced by receptor activator of nuclear factor-κB (RANK) ligand at an inhibitory concentration 50 of 325 ± 1.37 nM. DHPH treatment delayed and reduced the expression of master regulators of osteoclast differentiation, NFATc1 and c-Fos. Consistent with this result, the mRNA level of *cathepsin K*, associated with osteoclast differentiation, was decreased whereas the reduction in the mRNA of *irf8*, a negative regulator of osteoclast differentiation, was similar to that measured in the vehicle-treated control cells. DHPH reduced the phosphorylation of p38 MAPK, ERK (p44/42). Furthermore, DHPH suppressed the bone absorption activity of osteoclasts and enhanced osteoblast differentiation. Taken together, DHPH interrupts the immediate downstream signaling cascade of RANK and interferes with osteoclast differentiation and its function while enhances osteoblast differentiation. These results demonstrate the potential of this diarylheptanoid as a new therapeutic agent in osteoporosis.

1. Introduction

Osteoporosis is caused in part by a functional imbalance between osteoblasts and osteoclasts. It is a major health concern in aging communities, as the progressive decrease in bone mass leads to an increased susceptibility to fractures. Osteoclasts are multinucleated giant cells that differentiate from hematopoietic stem cells in response to two essential cytokines, macrophage colony-stimulating factor (M-CSF) and RANKL (Edwards and Mundy, 2011). Interactions between RANKL and its receptor, RANK, on osteoclast precursors activate nuclear factor-κB (NF-κB) and mitogen-activated protein kinase (MAPK) pathways (Lee and Kim, 2003). In turn, these pathways trigger the activation of nuclear factor of activated T cells c1 (NFATc1), a master transcriptional regulator of osteoclast differentiation (Takayanagi et al., 2002). An activator protein (AP)-1 complex containing c-Fos is essential for the auto-amplification of NFATc1, led to the robust induction of NFATc1 (Asagiri and Takayanagi, 2007). Osteoclastogenesis is also responsive to the cytokines and reactive oxygen species produced during inflammation, as both ensure osteoclast activation and survival. Thus, bone destruction appears to be tightly linked to inflammation (Bartell et al., 2014; Komatsu and Takayanagi, 2012).

Phytoestrogens are groups of naturally occurring compounds

* Corresponding author at: Department of Microbiology, Faculty of Science, Chulalongkorn University, Phayathai Road, Pathumwan, Bangkok 10330, Thailand.
E-mail address: tanapat.p@chula.ac.th (T. Palaga).

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isolated from plants that mimic or modulate the biological activities of estrogen. The various biological activities of phytoestrogens account for their use as traditional medicines and the increasing interest in these compounds as therapeutic agents (Bolego et al., 2003). Studies in animals and in humans have demonstrated the potential ability of phytoestrogens to prevent bone resorption (Fu et al., 2014; Malaivijitnond, 2012; Poluzzi et al., 2014). Diarylheptanoids are mainly found in the roots and rhizomes of *Alpinia*, *Zingiber*, and *Curcuma* species. Their wide range of biological activities includes anti-cancer, anti-oxidant, and antimicrobial effects (Lv and She, 2010). Diarylheptanoids isolated from the rhizomes of *Curcuma comosa* Roxb. have estrogenic activities (Suksamrarn et al., 2008; Winuthayanon et al., 2009). They inhibit nitric oxide production in response to lipopolysaccharide (LPS) stimulation of RAW264.7 cells and inflammatory cytokine production in the THP-1 cell line (Sodsai et al., 2007; Sornkaew et al., 2015). A diarylheptanoid from *C. comosa* was shown to have bone-sparing effects, based on the decreased reduction of total bone calcium and the dose-dependent increase in bone density in an ovariectomized rat model. Both effects mimic those of estrogen treatment (Tantikanlayaporn et al., 2013b). However, the detailed molecular mechanism underlying the direct action of diarylheptanoids on osteoclasts has not been investigated. In the present study, we examined the effect of diarylheptanoids on osteoclast differentiation, their function in bone resorption, and the signaling pathways that are involved.

2. Materials and methods

2.1. Bioactive compound and reagents

The diarylheptanoid (3*S*)-1-(3,4-dihydroxyphenyl)-3-hydroxy-7-phenyl-(6*E*)-6-heptene (DHPH) (Fig. 1A) was isolated from *Curcuma comosa* as described previously (Suksamrarn et al., 2008). Rhizomes of *C. comosa* were purchased from the Kampaengsaen District, Nakorn Pathom Province, Thailand, and were identified taxonomically based on a previous report (Soontornchainaksaeng and Jenjittikul, 2010). The voucher herbarium specimen (SCMU no. 300) was deposited at the Department of Plant Science, Faculty of Science, Mahidol University (Thailand). Antibodies against p38, phospho-p38, SAPK-JNK, phospho- SAPK-JNK (pSAPK-JNK), ERK, phospho-ERK (pERK), NF-κB p65, phospho- NF-κB p65, IκB-α, NFATc1 and c-Fos were purchased from Cell Signaling Technology (Danvers, MA, USA) while antibody against β-actin was purchased from Merck Millipore (Darmstadt, Germany).

2.2. Cell cultures

Macrophage-like RAW264.7 (ATCC TIB-71) cells were cultured in DMEM medium (Thermo Fisher Scientific, Runcorn, UK) containing 10% (v/v) fetal bovine serum (GIBCO-Invitrogen, Carlsbad, CA, USA), 1% HEPES free acid, 1% sodium pyruvate, and 1% penicillin-streptomycin (Thermo Fisher Scientific) at 37 °C in an incubator with a 5% CO_2 atmosphere (Thermo Electron Corporation, Waltham, MA). Bone marrow cells were isolated from the femur and tibia of 6- to 8-week-old BALB/c female mice (National Laboratory Animal Center, Mahidol University, Thailand). All procedures involving laboratory animals were approved by the Institutional Animal Care and Use Committee of Chulalongkorn University.

2.3. Cell viability assay

RAW264.7 cells were treated with DHPH at concentrations of 100, 31.62, 10, 3.162, 1, and 0.316 μM for 24 h. Cell viability was determined using MTS solution (Promega, Madison, WI, USA), as described by the manufacturer, and expressed as a percentage

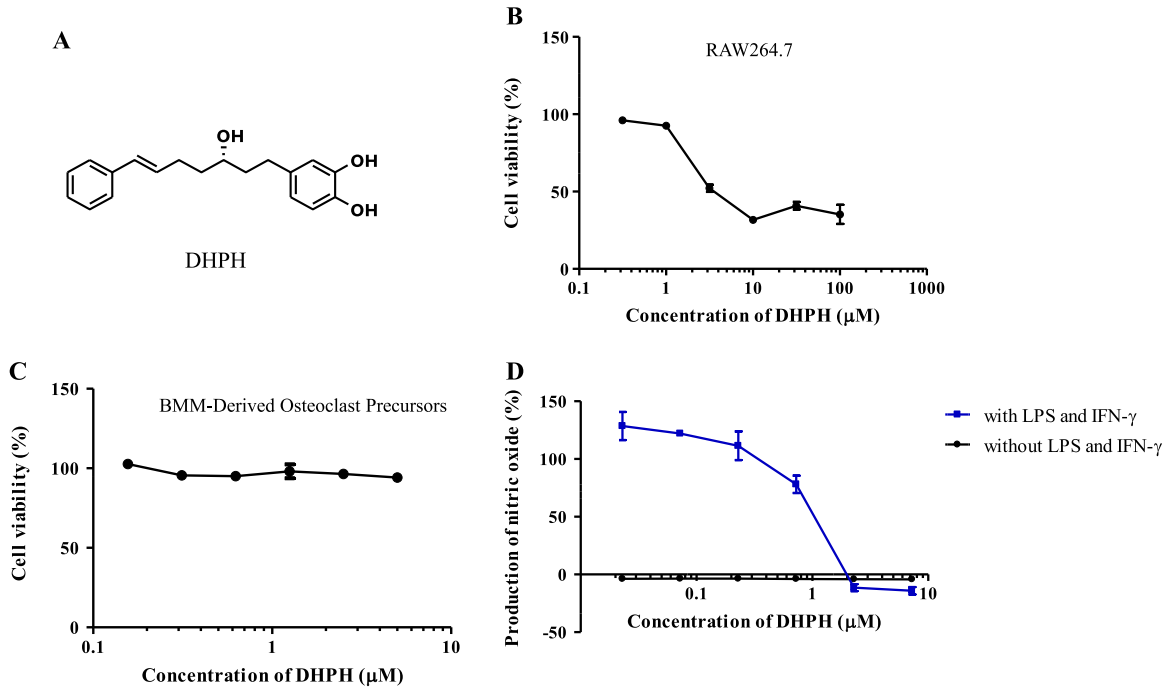

Fig. 1. Effect of DHPH on cell viability and nitric oxide production in macrophage-like RAW264 cells and osteoclast precursors. (A) The chemical structure of DHPH from *Curcuma comosa* Roxb. (B) Effect of DHPH on the viability of RAW264.7 cells treated with various concentrations of DHPH for 24 h. Cell viability was determined in an MTS assay. The IC50 was 7.055 ± 0.0195 μM. (C) Primary mouse bone-marrow-derived macrophage precursors (BMMs) were treated with various concentrations of DHPH for 24 h. Cell viability was determined in an MTS assay. (D) RAW264.7 cells were cultured with or without lipopolysaccharide (100 ng/ml) and interferon-γ (10 ng/ml) in the presence of DHPH at various concentrations. The amount of nitric oxide present in the culture supernatant was measured. The data are representative of two independent experiments and presented as the mean ± S.D.