Available online at www.sciencedirect.com

ScienceDirect

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

Original article

Effects of gallotannin on osteoclastogenesis and the p38 MAP kinase pathway

Yukiko Hosoyama D.D.S. [a], Eisuke Domae D.D.S., Ph.D. [b,*], Seiji Goda D.D.S., Ph.D. [c], Naoyuki Matsumoto D.D.S., Ph.D. [d]

[a] Graduate School of Dentistry, Department of Orthodontics, Osaka Dental University, 8-1 Kuzuhahanazonocho, Hirakata, Osaka 573-1121, Japan
[b] Department of Biochemistry, Osaka Dental University, 8-1 Kuzuhahanazonocho, Hirakata, Osaka 573-1121, Japan
[c] Department of Oral Science, Graduate School of Dentistry, Kanagawa Dental University, Yokosuka, Kanagawa 238-8580, Japan
[d] Department of Orthodontics, Osaka Dental University, 8-1 Kuzuhahanazonocho, Hirakata, Osaka 573-1121, Japan

ARTICLE INFO

Article history:
Received 26 September 2016
Received in revised form
14 October 2016
Accepted 18 October 2016
Available online 9 November 2016

Keywords:
Osteoclast
Gallotannin
Tannin
p38 MAP kinase
NFATc1

ABSTRACT

Purpose: Osteoclasts are multinucleated giant cells that specialize in bone resorption and work together with bone-forming osteoblasts to maintain bone homeostasis. However, excessive osteoclast activation accounts for bone diseases, such as osteoporosis and periodontitis. In previous studies, natural small-molecule compounds have been shown to regulate osteoclastogenesis and osteoclast functions. Here we demonstrate that gallotannin, a hydrolyzable plant tannin, suppresses osteoclast differentiation.

Methods: We first used an *ex vivo* bone marrow culture system containing both osteoclast precursors and surrounding cells, thereby resembling physiological conditions, to evaluate the suppressive effect of gallotannin. We also used a RANKL-induced osteoclastogenesis assay containing only osteoclast precursors to confirm the suppressive effect of gallotannin in the absence of effects from other cells.

Results: The suppressive effect of gallotannin was associated with the reduced RANKL-mediated induction of NFATc1, a critical transcription factor involved in osteoclast differentiation. We further confirmed that gallotannin reduced the p38 MAPK pathway activation, which is mediated by M-CSF and RANKL. This pathway suppression might underlie the suppression of NFATc1 production and subsequent reduction in osteoclast differentiation.

Conclusion: Our data indicate that the natural small-molecule compound gallotannin might be useful as a novel anti-bone resorptive agent.

© 2016 Elsevier Ltd and The Japanese Orthodontic Society. All rights reserved.

* *Corresponding author*. Fax: +81 72 864 3155.
E-mail address: domae-ei@cc.osaka-dent.ac.jp (E. Domae).

http://dx.doi.org/10.1016/j.odw.2016.10.007
1344-0241/© 2016 Elsevier Ltd and The Japanese Orthodontic Society. All rights reserved.

1. Introduction

The normal adult bone undergoes continuous formation and degradation to maintain skeletal homeostasis. Accordingly, an imbalance between osteoblast-mediated bone formation and osteoclast-mediated bone degradation results in bone diseases, such as osteoporosis, rheumatoid arthritis, and periodontitis [1]. The development and activation of osteoclasts, multinucleated giant cells that arise from monocyte-lineage hematopoietic cells, are tightly regulated by the surrounding cells, including osteoblasts, osteocytes, and immune cells [2].

The receptor activator of nuclear factor kappa-B ligand (RANKL) and monocyte colony-stimulating factor (M-CSF) are the key cytokines involved in osteoclast differentiation and function. A deficiency in signaling mediated by either cytokine results in severe osteopetrosis *in vivo* [3,4], and the presence of both cytokines is sufficient to induce the differentiation of osteoclast precursors into osteoclasts *in vitro* [5]. Although M-CSF is constitutively produced by osteoblasts, RANKL is produced by osteoblasts only in response to osteotropic factors [6]. The interaction of RANKL with its cognate receptor, RANK, activates downstream signaling pathways, such as the NF-κB, p38, ERK, JNK, and Akt pathways, thereby inducing the expression of osteoclastogenic transcription factors, such as c-fos, MITF, and NFATc1. These molecules are considered to be key targets in the regulation of osteoclast differentiation and activation [7].

A previous study has demonstrated that natural small-molecule compounds with unique pharmacological activities can provide beneficial effects in the context of human medicine [8]. Ellagitannins and gallotannins, the two subclasses of hydrolyzable tannins, are examples of such compounds that are widely distributed throughout the plant kingdom (*e.g.*, in beans, fruits, vegetables, and nuts) [9]. Ellagitannin and gallotannin are polyesters of glucose with organic acids which are ellagic acid and gallic acid, respectively [9]. Previous study has described the ellagitannin-mediated suppression of RANKL-induced osteoclastogenesis *via* the suppression of p38, JNK, and AP-1 activation [10]. Recently, ellagic acid, the acid component of ellagitannin, was also reported to suppress osteoclast differentiation and function [11]; in other words, both tannins and their acid components might regulate osteoclast differentiation. Gallotannin, the simplest hydrolyzable tannin, exhibits various biological effects, including anti-cancer [12,13] and anti-inflammatory effects [14,15], as well as protective effects against atherosclerosis [16], fatty diet-induced diabetes [17], and diabetic nephropathy [18]. However, the effects of gallotannin on osteoclast differentiation have not yet been characterized.

In the present study, we examined the effects of gallotannin on osteoclast differentiation *in vitro*. We found that gallotannin could suppress osteoclast differentiation in both bone marrow (BM) and bone marrow macrophage (BMM) culture systems. These suppressive effects of gallotannin were associated with decreased RANKL-induced NFATc1 expression in gallotannin-treated osteoclast precursors. Furthermore, we found that gallotannin treatment reduced the activation of p38 MAP kinase in RANKL- and M-CSF-treated osteoclast precursors.

2. Materials and methods

2.1. Ethics

This study was approved by the Institutional Animal Care and Use Committee of Osaka Dental University.

2.2. Cell culture

The ST-2 osteoblastic cell line was obtained from the RIKEN BioResource Center (Tsukuba, Japan) and cultured in α-Modified Eagle's Medium (MEM; Wako, Osaka, Japan) supplemented with 10% fetal bovine serum (FBS), 4 mM L-glutamine, 100 U/mL penicillin, and 100 μg/mL streptomycin (complete medium).

For BMM preparation, BM was isolated from the tibiae and femora of 6-week-old male ddY mice. Following red blood cell elimination with RBC lysis buffer (BioLegend, San Diego, CA, USA), the BM cells were cultured in complete medium supplemented with 25 ng/mL M-CSF (BioLegend) in a non-tissue culture dish (Sarstedt, Nümbrecht, Germany) for 5–7 days. For further experiments, BMMs were re-plated onto non-tissue culture plates (Sarstedt) prior to flow cytometry or onto tissue culture plates (TPP, Trasadingen, Switzerland) for all other experiments. Cells were counted by hemocytometer in the presence of trypan blue, then calculated the total cell number.

2.3. Osteoclast differentiation

Osteoclast differentiation was assessed by TRAP staining [19]. Briefly, the cells were fixed with 10% formalin and acetone–methanol (1:1), and subsequently incubated with TRAP staining buffer containing 0.1 mg/mL naphthol AS-MX phosphate (Sigma–Aldrich, St. Louis, MO, USA), 0.5% *N*,*N*-dimethylformamide (Wako), and 0.6 mg/mL fast red violet LB salt (Sigma–Aldrich) in 0.1 M sodium acetate buffer pH 5.0 with 50 mM sodium tartrate (Wako). TRAP-positive multi nucleated (more than three nuclei) cells were considered osteoclasts.

To induce osteoclast differentiation in a BM culture system, isolated red blood cell-free BM cells were cultured for 7 days in 96-well plates at a density of 6×10^3 cells/well in the presence of 50 nM 1,25 dihydroxyvitamin D_3 ($1,25(OH)_2D_3$; Santa Cruz Biotechnology, Dallas, TX USA). To induce RANKL-mediated osteoclast differentiation, BMMs were stimulated for 3 days in 96-well plates at a density of 1×10^4 cells/well in the presence of 25 ng/mL M-CSF and 50 ng/mL RANKL (Wako). Gallotannin (Santa Cruz Biotechnology) was added to both culture systems at concentrations of 0.1, 1, 10, and 100 nM.

2.4. Flow cytometry (FACS)

In preparation for FACS analysis, adherent cells were washed with PBS, exposed to accutase (Nacalai Tesque, Kyoto, Japan) for 5 min to induce detachment from the culture plate, and immediately washed with FACS buffer (phosphate-buffered saline [PBS] supplemented with 2% calf serum and 0.1% azide).

ST-2 cells were cultured for 3 days in a 6-well plate at a density of 1×10^5 cells/well in the presence of 50 nM 1,25(OH)$_2D_3$ and 50 nM dexamethasone (Wako). Subsequently, the

Download English Version:

https://daneshyari.com/en/article/8708041

Download Persian Version:

https://daneshyari.com/article/8708041

Daneshyari.com