Journal of Functional Foods 22 (2016) 243–256

Available online at www.sciencedirect.com

ScienceDirect

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

Curcumin ameliorates alveolar bone destruction of experimental periodontitis by modulating osteoclast differentiation, activation and function

Lian-Ping Mau [a,b], *Wan-Chien Cheng* [c], *Jen-Kun Chen* [c,d], *Yi-Shing Shieh* [e], *David L. Cochran* [f], *Ren-Yeong Huang* [c,*]

[a] *Division of Periodontics, Department of Dentistry, Chi Mei Medical Center, Liouying, Tainan 73659, Taiwan*
[b] *Department of Long Term Care, Chung Hwa University of Medical Technology, Tainan 71703, Taiwan*
[c] *Department of Periodontology, School of Dentistry, Tri-Service General Hospital and National Defense Medical Center, Taipei 11490, Taiwan*
[d] *Institute of Biomedical Engineering and Nanomedicine, National Health Research Institutes, Miaoli 35053, Taiwan*
[e] *Department of Oral Diagnosis and Pathology, School of Dentistry, Tri-Service General Hospital and National Defense Medical Center, Taipei 11490, Taiwan*
[f] *Department of Periodontics, The University of Texas Health Science Center at San Antonio, San Antonio, TX 78229, USA*

ARTICLE INFO

Article history:
Received 22 June 2015
Received in revised form 11 January 2016
Accepted 13 January 2016
Available online

Keywords:
Curcumin
Osteoclastogenesis
Bone resorption
Periodontal disease

ABSTRACT

This study evaluated as to whether curcumin (CCM) could ameliorate alveolar bone destruction *in vivo* and dissect its mechanism *in vitro*. The tartrate-resistant acid phosphatase (TRAP) activity, TRAP staining, quantitative RT-PCR, gelatin zymography, actin-ring formation, and pits formation assay were used to analyse the effect of CCM on receptor activator of nuclear factor κB ligand (RANKL)-induced osteoclast differentiation, activation, and function. *Porphyromonas gingivalis* LPS- and ligature-induced experimental periodontitis were established to evaluate the therapeutic benefits of CCM *in vivo*. The results demonstrated that CCM dose dependently diminishes RANKL-induced osteoclast differentiation, and osteoclastic specific genes expression accompanied by a significant attenuation of actin-ring and resorptive pits formation *in vitro*. Moreover, CCM reduces alveolar bone destruction, decreases TRAP-positive and polymorphonuclear cells infiltration, and suppresses myeloperoxidase activity *in vivo*. Thus, CCM reduces inflammatory bone loss *in vivo* by modulating RANKL-mediated osteoclast differentiation, activation, and function.

* *Corresponding author.* Department of Periodontology, School of Dentistry, Tri-Service General Hospital and National Defense Medical Center, Taipei 11490, Taiwan. Tel.: +886 2 87923311 12933; fax: +886 2 87927147.
E-mail address: ndmcandy@ndmctsgh.edu.tw (R.-Y. Huang).

Abbreviations: *A.a*, *Aggregatibacter actinomycetemcomitans*; ABC, alveolar bone crest; α-MEM, alpha-minimum essential medium; CCM, curcumin; CEJ-ABC, cementoenamel junction-alveolar bone crest; DC-STAMP, dendritic cell-specific transmembrane protein; DMEM, Dulbecco's Modified Eagle Medium; DMSO, dimethyl sulphoxide; EDTA, ethylenediaminetetraacetic acid; FBS, foetal bovine serum; GAPDH, glyceraldehyde-3-phosphate dehydrogenase; HGFs, human gingival fibroblasts; HTAB, hexadecyltrimethylammonium bromide; LPS, lipopolysaccharide; M-CSF, macrophage-colony stimulating factor; Micro-CT, micro-computerised tomography; MMP-9, matrix metalloproteinase-9; MNCs, multinucleated cells; MPO, myeloperoxidase; OPG, osteoprotegerin; PBS, phosphate-buffered saline; *P.g*, *Porphyromonas gingivalis*; *P. intermedia*, *Prevotella intermedia*; PMN, polymorphonuclear leukocyte; qRT-PCR, quantitative reverse transcription-polymerase chain reaction; RANKL, receptor activator of nuclear factor κB ligand; SDS-PAGE, sodium dodecyl sulphate-polyacrylamide gel; TRAP, tartrate-resistant acid phosphatase

http://dx.doi.org/10.1016/j.jff.2016.01.025
1756-4646/

1. Introduction

Pathological bone destruction occurs in diseases such as rheumatoid arthritis and periodontitis where osteoclasts are present in elevated numbers and/or exhibit enhanced activity, leading to excessive bone resorption (Cochran, 2008; Hajishengallis, 2014). Periodontitis, a chronic inflammatory disease, is initiated by the colonisation of complex subgingival bacterial plaque biofilms, including specific species of gram negative microorganisms such as *Porphyromonas gingivalis* (*P. gingivalis*) and *Aggregatibacter actinomycetemcomitans* (*A.a*), resulting in a host immune response and subsequent gingival tissue inflammation (Hajishengallis, 2014). Consequently, dysregulated immunoinflammatory processes may lead to osteoclast differentiation and function in the alveolar bone and be responsible for the excessive bone resorption (Cochran, 2008; Kayal, 2013). Therefore, substances targeting bacteria-induced inflammation or osteoclast activation and bone resorption can potentially be used for the treatment of periodontal diseases (Cheng et al., 2010; Lu, Huang, & Chou, 2013) Curcumin (diferuloylmethane), 1,6-heptadiene-3,5-dione-1,7-bis(4-hydroxy-3-methoxyphenyl)-(1E,6E), has been used in dietary and traditional medicine for centuries (Prasad, Gupta, Tyagi, & Aggarwal, 2014). Its wide range of biological activities and pivotal role in healthcare has been widely documented in recent years (Prasad et al., 2014). These studies have revealed that curcumin (CCM) has antioxidant, antimicrobial, anti-inflammatory, anti-carcinogenic and pro-apoptotic effects (Prasad et al., 2014; Zorofchian Moghadamtousi et al., 2014). It also has therapeutic effects against arthritis (Kuncha, Naidu, Sahu, Gadepalli, & Sistla, 2014) and various neurological (Han et al., 2014), pulmonary (Chong et al., 2014), metabolic (Jimenez-Flores, Lopez-Briones, Macias-Cervantes, Ramirez-Emiliano, & Perez-Vazquez, 2014), haematological (Yu et al., 2013), and cardiovascular ailments (Madaric et al., 2013). Moreover, there is sufficient evidence indicating that CCM exhibits pleiotropic characteristics and has the potential to modulate biological activities. Additionally, the most promising rationale is its good safety profile to date; no studies either in animals or in humans have discovered any toxicity associated with the use of CCM, and even at very high doses (Panahi et al., 2014; Prasad et al., 2014).

Although various biological and pharmacological activities of CCM have been reported, its therapeutic potential within the field of periodontology is limited (Chen, Nie, Fan, & Bian, 2008; Guimaraes et al., 2011, 2012; Hu, Huang, & Chen, 2013; Kim, 2011; Muglikar, Patil, Shivswami, & Hegde, 2013; Nagpal & Sood, 2013; Zhou et al., 2013). Previous studies have demonstrated that CCM can suppress COX-2 mRNA and protein synthesis in lipopolysaccharide (LPS)-stimulated human gingival fibroblasts (HGFs) through the inhibition of the NF-κB pathway (Hu et al., 2013). In addition, CCM reduces the host-destructive processes mediated by the production of inflammatory cytokines, such as TNF-α, IL-1β, or IL-6 in RAW264.7 cells stimulated by LPS from *P. gingivalis* or *Prevotella intermedia* (*P. intermedia*), through inhibition of the NF-κB and STAT1 signalling pathway (Chen et al., 2008; Kim, 2011). Therefore, CCM effectively reduces inflammation and modulates innate immune responses in ligature- and LPS-induced experimental periodontitis (Guimaraes et al., 2011, 2012; Zhou et al., 2013). However, the protective effect of CCM on alveolar bone destruction remains controversial, and comprehensive deciphering of the underlying mechanism is still lacking.

In the present study, we investigated the effects of CCM on RANKL-induced osteoclastogenesis *in vitro* and those on LPS- and ligature-induced experimental periodontitis *in vivo* to test the hypothesis that CCM attenuates the alveolar bone destruction by modulating osteoclast formation, activation, and function.

2. Material and methods

2.1. Osteoclast differentiation of RAW264.7 cells

The mouse macrophage cell line RAW264.7 cells were cultured in Dulbecco's Modified Eagle Medium (DMEM, Gibco BRL, Grand Island, NY, USA) supplemented with 10% fetal bovine serum (FBS; Biological Industries, Kibbutz Beit Haemek, Israel), and maintained at 37 °C in 5% CO_2 humidified air. The cells (1×10^5 per well) were seeded in 24-well plates and incubated to adhere overnight. After that, the cells were cultured for an additional 5 days in alpha-minimum essential medium (α-MEM, Sigma, St. Louis, MO, USA) containing 100 ng/mL recombinant murine receptor activator of nuclear factor κB ligand (RANKL, R&D Systems, Minneapolis, MN, USA), defined as differentiation medium, in the absence or presence of CCM (Sigma). Cells were treated with different concentration of CCM (0–20 μM) dissolved in dimethyl sulphoxide (DMSO, Sigma) and were added to their respective medium for a final DMSO concentration of 0.1%. After initial seeding, all media were replaced and additional factors (RANKL and/or CCM) were replenished at the third day. The culture wells were then incubated for further analysis.

2.2. TRAP activity measurement and TRAP-positive multinucleated cells (TRAP$^+$ MNCs) staining

The TRAP activity assay was performed as previously described (Lu et al., 2013). RAW264.7 cells (1×10^5 per well in a 24-well plate) were incubated with CCM (0–20 μM) in the presence or absence of 100 ng/mL RANKL. Cells were lysed with 0.1 Triton X-100 (Sigma), then the supernatants were collected and reacted with pNPP substrate solution (Sigma) at 37 °C for 15 min. The reaction was stopped with 0.1 N NaOH and was analysed spectrophotometrically at 405 nm.

For the TRAP staining assay, cells (1×10^5 per well in a 24-well plate) were fixed with 3.7% formalin for 10 min and 0.1% Triton X-100 for 1 min, and then stained by TRAP staining solution (Sigma) at 37 °C for 30 min. The images of TRAP-positive multinucleated cells (MNCs) ≥ three nuclei were captured and counted as performed as previously described (Lu et al., 2013).

2.3. Cell viability assay

The MTT (3-[4,5-dimethylthiazol-2-yl]-2,5-dipheyltetrazolium bromide) (Sigma) assay for cell viability was performed on Day 6 of culture (Lu et al., 2013). Briefly, RAW264.7 cells (1×10^5 per