Archives of Oral Biology 71 (2016) 155–161

Contents lists available at ScienceDirect

Archives of Oral Biology

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

Mangiferin inhibits lipopolysaccharide-induced production of interleukin-6 in human oral epithelial cells by suppressing toll-like receptor signaling

Hao Li[a,b,*], Qi Wang[b,c], Xinmin Chen[b], Yi Ding[b], Wei Li[b]

[a] Department of Prosthodontics, The Affiliated Hospital of Stomatology, Guangxi Medical University, 10 Shuangyong Road, Nanning 530021, PR China
[b] State Key Laboratory of Oral Diseases, West China Hospital of Stomatology, Sichuan University, 14, 3rd Section S Renmin Road, Chengdu 610041, PR China
[c] Loma Linda University School of Dentistry, 24876 Taylor Street, Loma Linda, CA 92354, USA

ARTICLE INFO

Article history:
Received 10 September 2015
Received in revised form 27 July 2016
Accepted 3 August 2016

Keywords:
Mangiferin
Oral epithelial cells
Lipopolysaccharide
Interleukin-6
Toll-like receptors

ABSTRACT

Objective: Oral epithelial cells have currently been found to play an important role in inflammatory modulation in periodontitis. Mangiferin is a natural glucosylxanthone with anti-inflammatory activity. The aim of this study was to investigate the regulatory effect of mangiferin on lipopolysaccharide (LPS)-induced production of proinflammatory cytokine interleukin-6 (IL-6) in oral epithelial cells and the underlying mechanisms.
Design: The levels of LPS-induced IL-6 production in OKF6/TERT-2 oral keratinocytes were detected using enzyme-linked immunosorbent assay (ELISA). The expression of Toll-like receptor (TLR) 2 and TLR4 was determined using western blot analysis. And the phosphorylation of TLR downstream nuclear factor-κB (NF-κB), p38 mitogen-activated protein kinase (p38 MAPK) and c-Jun N-terminal kinase (JNK) was examined using cell-based protein phosphorylation ELISA kits.
Results: We found that mangiferin reduced LPS-upregulated IL-6 production in OKF6/TERT-2 cells. Additionally, mangiferin inhibited LPS-induced TLR2 and TLR4 overexpression, and suppressed the phosphorylation of NF-κB, p38 MAPK and JNK. Moreover, mangiferin repressed IL-6 production and TLR signaling activation in a dose-dependent manner after 24 h treatment.
Conclusions: Mangiferin decreases LPS-induced production of IL-6 in human oral epithelial cells by suppressing TLR signaling, and this glucosylxanthone may have potential for the treatment of periodontitis.

1. Introduction

Periodontitis is a chronic inflammatory disease caused by periodontal microorganisms (Marques, Maor, de Andrade, Rodrigues, & Benatti, 2016). It often results in destruction of alveolar bone and periodontal connective tissues, and has become a major cause of tooth loss in adults (Baelum & López, 2013). The inflammatory response in periodontitis is the major contributor to the damage of structural components in the periodontium. Lipopolysaccharide (LPS) is one of the key virulent attributes of periodontal pathogens (Jain & Darveau, 2010). It can stimulate the host cells including oral epithelial cells to produce various proinflammatory cytokines including interleukin-6 (IL-6), and promote the progression of periodontitis (Imai et al., 2014; Souza et al., 2010). IL-6 is accepted as an important stimulator of alveolar bone resorption, and excessive production of IL-6 can contribute to periodontal tissue damage (Cho et al., 2013; Keles, Keles, Avci, Cetinkaya, & Emingil, 2014).

Oral epithelial cells are the initial barrier to oral microbial intrusion, and their modulatory function in inflammatory response in periodontal diseases has been highlighted currently. They can produce a variety of inflammation-related proteins, including Toll-like receptors (TLRs), and regulate the development of periodontal diseases (Huang et al., 2015; Shin & Choi, 2010). As a family of pattern recognition receptors that recognize microbial components, TLRs mediate the activation of host response (Akira & Takeda, 2004). LPS is considered to induce inflammatory responses in periodontal diseases via prolonged TLR signaling (Firth et al., 2013). The stimulation of TLR2 and TLR4 by LPS activates downstream proteins nuclear factor-κB (NF-κB), p38 mitogen-activated protein kinase (MAPK) and c-Jun N-terminal kinase

* Corresponding author at: Department of Prosthodontics, The Affiliated Hospital of Stomatology, Guangxi Medical University, 10 Shuangyong Road, Nanning 530021, PR China.
E-mail address: sherrylee2011@126.com (H. Li).

http://dx.doi.org/10.1016/j.archoralbio.2016.08.003
0003-9969/

(JNK), leading to the production of critical proinflammatory cytokines that are necessary to activate potent immune responses (Akira & Takeda, 2004). For this reason, inhibiting the activation of TLR signaling is thought to be an effective strategy for periodontitis therapy.

During recent years, there has been growing interest in therapeutic use of natural components of plants to attenuate tissue damage caused by dysregulated inflammatory response. More and more evidences have demonstrated the potent anti-inflammatory properties of natural plants, suggesting that these substances can be used as therapy for inflammation-related diseases (Austenaa, Carlsen, Hollung, Blomhoff, & Blomhoff, 2009; Rivera et al., 2011; Xie et al., 2012). Mangiferin (2-C-β-D-glucopyranosyl- 1,3,6,7-tetrahydroxyxanthone) is a natural glucosylxanthone, primarily found in the fruit, leaves and stem bark of the mango tree (Mangifera indica (Telang, Dhulap, Mandhare, & Hirwani, 2013). It has pleiotropic bioactivities including the inhibition of inflammatory mediators (Guo et al., 2014), induction of antioxidation (Prabhu, Jainu, Sabitha, & Devi, 2006), and improvement of glucose intolerance (Wang et al., 2014). Its reported oral LD50 value in mice was 400 mg/kg (Jagetia & Baliga, 2005), and high concentrations of this xanthone (up to 100 μM) showed no cytotoxicity in cell culture in vitro (Rao et al., 2012; Satish Rao, Sreedevi, & Nageshwar Rao, 2009). It possesses little adverse effects, and has become a promising candidate for developing natural medicine. It has been reported to exert anti-inflammatory effects and diminish alveolar bone resorption in animal periodontitis models (Carvalho et al., 2009; Li, Wang, Ding, Bao, & Li, 2016), while the potential mechanism remains unclear. Recent studies have shown its suppressive effect on LPS-induced IL-6 production in a mouse model of brain injury (Fu et al., 2014). In experimental periodontitis, it has been observed to ameliorate inflammatory response by inhibiting the phosphorylation of TLR downstream NF-κB signaling (Li et al., 2016). Additionally, in other inflammatory diseases, such as colitis, this xanthone can alleviate inflammatory damage by suppressing the activation of MAPK pathways, also TLR downstream signalings (Dou et al., 2014; Jeong, Jang, Hyam, Han, & Kim, 2014). These research imply that mangiferin might modulate inflammatory response in periodontitis through suppressing LPS-activated TLR signaling. In order to confirm the hypothesis, we incubated OKF6/TERT-2 oral keratinocytes with LPS and different concentrations of mangiferin, and investigated the changes of IL-6 production and TLR signaling activation.

2. Materials and methods

2.1. Cell culture

The immortalized human oral keratinocyte cell line OKF6/TERT-2 was kindly provided by Dr J. Rheinwald (Harvard University, Boston, MA), and cultured in keratinocyte serum-free medium supplemented with 25 μg/ml bovine pituitary extract (Invitrogen, Carlsbad, CA), 0.2 ng/ml epidermal growth factor (Invitrogen, Carlsbad, CA), 2 mM L-glutamine, 100 IU/ml penicillin, 100 mg/ml streptomycin and 0.4 μM calcium chloride (Sigma-Aldrich, St. Louis, MO). The cells (1×10^5 cells/well) were cultured in 96-well plates at 37 °C, and were incubated for 6, 12 or 24 h, in the presence of 1 μg/ml *Porphyromonas gingivalis* LPS (Sigma-Aldrich, St. Louis, MO) and different concentrations of mangiferin (0, 10, 20, or 40 μM) (Sigma-Aldrich, St. Louis, MO).

2.2. IL-6 enzyme-linked immunosorbent assay (ELISA)

Culture supernatants at each time point were harvested and concentrations of IL-6 were determined using ELISA kit (R&D Systems, Minneapolis, MN). Briefly, protein samples were pipetted into a microplate with anti-IL-6 antibody and incubated at 25 °C for 2 h. Then, enzyme-linked polyclonal anti-IL-6 antibody was added and incubated at 25 °C for 1 h. Subsequently, a substrate solution was added and incubated at 25 °C for 15 min. When blue color was developed in positive control wells, stopping reagent was added to each well to stop the reaction. The absorbance of the solution was detected at 450 nm using a microplate reader (Thermo Fisher Scientific, Waltham, MA).

2.3. Western blot analysis

At the indicated time points, the cultured cells were lysed, and the expression of TLR2 and TLR4 was detected using western blot analysis. Total protein was extracted using ReadyPre protein extraction kits (Biorad Laboratories, Hercules, CA). Protein concentration was measured using the Bradford protein assay (Biorad Laboratories, Hercules, CA) according to the manufacturer's protocol. Afterwards, protein samples were separated on 8% SDS-polyacrylamide gels by electrophoresis and transferred to polyvinylidene difluoride membranes. Subsequently, the membranes were incubated with monoclonal antibodies anti-TLR2 (1:300), anti-TLR4 (1:500) and anti-GAPDH (1:500) at 4 °C for 12 h. On the next day, the membranes were incubated with horseradish peroxidase (HRP)-conjugated secondary antibodies at 25 °C for 1 h. Bound antibody was detected using the SuperSignal West Pico Chemiluminescent Substrate System. The antibodies were all from Santa Cruz Biotechnology (Santa Cruz, CA).

2.4. Cell-based ELISA for NF-κB

Cell-based protein phosphorylation ELISA kits for detecting NF-κB p65 were purchased from R&D Systems (Minneapolis, MN), and the percentage of NF-κB p65 phosphorylation was calculated in accordance with the manufacturer's instructions. OKF6/TERT-2 cells in 96-well plates were incubated with 1 μg/ml LPS and different concentrations of mangiferin (Sigma-Aldrich, St. Louis, MO) for 6, 12 or 24 h. Then, supernatants were discarded and cells were fixed with 4% formaldehyde. Subsequently, the cells were permeabilised and quenched with 1% H_2O_2 and 0.1% azide. The cells were incubated with antibody-blocking buffer prior to the addition of anti-phosphorylated NF-κB p65 or anti-total NF-κB p65. Antibody dilution buffer only was added as negative control. After incubation at 4 °C for 12 h, the cells were incubated with HRP-conjugated secondary antibody and exposed to developing solution for 15 min. The absorbance of each well was measured at 570 nm using a microplate reader (Thermo Fisher Scientific, Waltham, MA), and the percentage of NF-κB p65 phosphorylation was analyzed. A crystal violet assay was performed to correct the data for cell numbers.

2.5. Cell-based ELISA for p38 MAPK and JNK

The effect of mangiferin on the activation of p38 MAPK and JNK was investigated using cell-based protein phosphorylation ELISA kits (R&D Systems, Minneapolis, MN). OKF6/TERT-2 cells in 96-well plates were treated with 1 μg/ml LPS and varying concentrations of mangiferin (Sigma-Aldrich, St. Louis, MO) for 6, 12 or 24 h. Supernatants were then discarded and cells were fixed with 4% formaldehyde. Subsequently, the cells were permeabilised and quenched with 1% H_2O_2 and 0.1% azide. After cells were incubated with antibody-blocking buffer, anti-phosphorylated p38 MAPK, anti-total p38 MAPK, anti-phosphorylated JNK, or anti-total JNK was added into wells. For negative control wells, antibody dilution buffer only was added. After incubation at 4 °C for 12 h, the cells were treated with HRP-conjugated secondary antibody and