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Resveratrol prevents alveolar bone loss in an experimental rat model of periodontitis

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ABSTRACT

Resveratrol is an antioxidant and anti-inflammatory polyphenol. Periodontitis is induced by oral pathogens, where a systemic inflammatory response accompanied by oxidative stress is the major event initiating disease. We investigated how resveratrol modulates cellular responses and the mechanisms related to this modulation in lipopolysaccharide (LPS)-stimulated human gingival fibroblasts (hGFs). We also explored whether resveratrol protects rats against alveolar bone loss in an experimental periodontitis model. Periodontitis was induced around the first upper molar of the rats by applying ligature infused with LPS. Stimulating hGFs with 5 μg/ml LPS augmented the expression of cyclooxygenase-2, matrix metalloproteinase (MMP)-2, MMP-9, and Toll-like receptor-4. LPS treatment also stimulated the production of reactive oxygen species (ROS) and the phosphorylation of several protein kinases in the cells. However, the expression of heme oxygenase-1 (HO-1) and nuclear factor-E2 related factor 2 (Nrf2) was inhibited by the addition of LPS. Resveratrol treatment almost completely inhibited all of these changes in LPS-stimulated cells. Specifically, resveratrol alone augmented HO-1 induction via Nrf2-mediated signaling. Histological and micro-CT analyses revealed that administration of resveratrol (5 mg/kg body weight) improved ligature/LPS-mediated alveolar bone loss in rats. Resveratrol also attenuated the production of inflammation-related proteins, the formation of osteoclasts, and the production of circulating ROS in periodontitis rats. Furthermore, resveratrol suppressed LPS-mediated decreases in HO-1 and Nrf2 levels in the inflamed periodontal tissues. Collectively, our findings suggest that resveratrol protects rats from periodontitic tissue damage by inhibiting inflammatory responses and by stimulating antioxidant defense systems.

Statement of significance

The aims of this study were to investigate how resveratrol modulates cellular responses and the mechanisms related to this modulation in lipopolysaccharide (LPS)-stimulated human gingival fibroblasts (hGFs) and protects rats against alveolar bone disruption in an experimental periodontitis model. Our findings suggest that resveratrol protects rats from periodontitic tissue damage by inhibiting inflammatory responses and by stimulating antioxidant defense systems. On the basis of our experiment studies, we proposed that resveratrol could be used as novel bioactive materials or therapeutic drug for the treatment of periodontitis or other inflammatory bone diseases like osteoporosis, arthritis etc. Furthermore, it could be also used for the modification or coating of implant materials as an antiinflammatory molecules which will help to accelerate bone formation.
There are a few of reports suggesting antioxidant and anti-inflammatory potentials of resveratrol. However, our results highlight the cellular mechanisms by which resveratrol inhibits LPS-mediated cellular damages using human-originated gingival fibroblasts and also support the potential of resveratrol to suppress periodontitis-mediated tissue damages. We believe that the present findings might improve a clinical approach of using of resveratrol on human, although further detailed experiments will be needed.

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1. Introduction

Periodontitis is an immune-inflammatory disorder that originates due to the formation of complex subgingival microbial biofilms. Periodontitis stimulates inflammatory cells to produce pro-inflammatory cytokines that leads to the destruction of connective tissues such as the subgingivae, periodontal ligament, and alveolar bone [1]. The induction of oxidative stress is a potential mechanism by which periodontitis manifests its systemic effects [2]. Considerable evidence also implicates the association of reactive oxygen species (ROS) with the pathogenesis of periodontitis [3]. Therefore, it is likely that natural compounds having antioxidant potential are capable of ameliorating periodontal damage by inhibiting inflammatory responses and ROS accumulation.

Human gingival fibroblasts (hGFs) play an important role in the local immune response that may initiate periodontitis or gingivitis [4]. The gram-negative bacterium *Porphyromonas gingivalis* is the main pathogen involved in the initiation and progression of periodontitis [5]. This pathogen produces several virulence factors that stimulate hGFs to produce inflammatory mediators, such as prostaglandin E2, matrix metalloproteinases (MMPs), and several pro-inflammatory cytokines. These mediators lead periodontal cells to mount an excessive host inflammatory response, resulting in periodontal destruction.

Various biological substances are known to have antibacterial activity that promotes the healing and regeneration of periodontal tissues [6,7]. The use of anti-inflammatory drugs or inhibitors specific to MMPs and pro-inflammatory cytokines are thought to exert beneficial effects on periodontal diseases [8]. However, prolonged exposure to and/or excessive use of antibiotics can contribute to the development of resistance to various antibiotics. The systemic use of several commercial drugs is also known to cause severe side effects leading to patient complications [9]. Accordingly, plant extracts or plant-derived active constituents have been considered as attractive materials to treat periodontitis or repair bone defects [10,11], as polyphenols isolated from various foods and herbs have been shown to prevent inflammatory diseases [12]. These findings suggest that naturally occurring bioactive substances can act as anti-inflammatory mediators in the process of periodontitis.

Resveratrol is a polyphenolic phytoalexin found in various plants and fruits and is known to exert various pharmacological activities, including antioxidation, anti-inflammation, anticancer, cardioprotection, and vasoprotection [13]. The administration of resveratrol was shown to inhibit the loss of alveolar bone and reduce interleukin (IL)-17 levels in gingival tissue [14]. The compound also activated the nuclear factor erythroid 2-related factor 2 (Nrf2) pathway and diminished oxidative stress and pro-inflammatory cytokine production in a rat periodontitis model [15]. In addition, resveratrol suppressed the inflammatory response of human gingival epithelial cells via the inhibition of nuclear factor (NF)-κB signaling [16]. Furthermore, no toxic effects of the compound were found, even with long-term use [17]. These findings indicate that resveratrol suppresses the inflammatory response in periodontal tissue and improves alveolar bone loss by elevating the expression of cellular redox-sensitive molecules without side effects.

In this study, we employed an experimental periodontitis model using lipopolysaccharide (LPS) and ligature to investigate the protective effect of resveratrol on periodontitic tissue damage. We also examined the mechanisms by which resveratrol prevents the LPS-induced production of inflammatory mediators and ROS in hGFs.

2. Materials and methods

2.1. Chemicals and laboratory wares

Resveratrol was obtained from Sigma–Aldrich Co. LLC (St. Louis, MO, USA) and dissolved in dimethylsulfoxide (DMSO) at a concentration of 35 mg/ml as the stock solution. Fetal bovine serum (FBS) and PIK-75, an inhibitor of Nrf2, were purchased from HyClone Laboratories, Inc. (Logan, UT, USA) and Selleck Chemicals (Houston, TX, USA), respectively. LPS produced from *P. gingivalis* was obtained from InvivoGen (San Diego, CA, USA). Primary antibodies specific for cyclooxygenase-2 (COX-2, BS1076), MMP-2 (BS1236), and MMP-9 (BS6893) were purchased from Bioworld Technology (St. Louis Park, MN, USA). Nrf2 (sc-722) and β-actin (sc-47778) were from Santa Cruz Biotechnology (Santa Cruz, CA, USA), while heme oxygenase-1 (HO-1) and Toll-like receptor 4 (TLR4) were from Enzo Life Sciences (Farmingdale, NY, USA) and Abcam (Cambridge, UK), respectively. Other protein kinase antibodies were obtained from Cell Signaling Technology (Danvers, MA, USA). Unless otherwise specified, all other chemicals were from Sigma–Aldrich Co. LLC (St. Louis, MO, USA) and laboratory items were Falcon Labware (Becton–Dickinson, Franklin Lakes, NJ, USA).

2.2. Cell culture

hGFs were obtained from healthy men aged 20–30 years who underwent molar extraction. Written informed consent for the use of periodontal tissue was obtained from all donors. The Institutional Review Board of Chonbuk National University Hospital approved this study. All culture procedures followed methods described previously [18]. hGFs were incubated in DMEM supplemented with 10% FBS and antibiotics (100 IU/ml penicillin G and 100 mg/ml streptomycin) at 37 °C in a humidified atmosphere of 5% CO_2. The cells cultured in 96-well plates and 60 mm culture dishes were used for viability and Western blot assays, respectively.

2.3. LPS treatment

The content of FBS in culture medium was reduced to 0.5% 16 h prior to LPS exposure. Cells were treated with resveratrol 1 h before stimulation with 5 μg/ml LPS. Cells treated with 0.02% DMSO alone were used as the vehicle control.

2.4. Measurement of cell viability

Cell viability was determined using a water-soluble tetrazolium salt (WST)-8 reagent (Dojindo Molecular Tech. Inc., Kumamoto, Japan). WST-8 is reduced by dehydrogenase activities in viable cells to give a yellow-color formazan dye, where the amounts of the dye are proportional to the number of living cells. In brief, hGFs were stimulated with various concentrations (0–200 μM) of resveratrol. After 24 and 48 h of incubation, the cells were treated with WST-8 reagent followed by incubation for an additional 2 h. WST-8-specific absorbance was measured at 450 nm using a microplate reader (Packard Instrument Co., Downers Grove, IL, USA).

2.5. Western blot analysis

Western blotting was carried out according to the methods described previously [19]. Briefly, whole protein lysates were prepared from hGFs or rat gingival tissues. Protein extracts (20 μg

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