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Inhibition of IL-6 and IL-8 production in LPS-stimulated human gingival fibroblasts by glycyrrhizin via activating LXRα

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ABSTRACT

The aim of this study was to clarify the anti-inflammatory effects and its molecular mechanism of glycyrrhizin on LPS-stimulated human gingival fibroblasts (HGFs), which will be of benefit for periodontitis treatment. An MTT assay was performed to assess the effects of glycyrrhizin on cellular viability. The levels of IL-6 and IL-8 were measured by ELISA. The expression of iNOS, COX-2, NF-κB, and LXRα were detected by western blot analysis. The results showed that glycyrrhizin significantly inhibited LPS-induced IL-6 and IL-8 production, as well as COX-2 and iNOS expression. LPS-induced NF-κB activation in HGFs was also inhibited by treatment of glycyrrhizin. Furthermore, glycyrrhizin increased the expression of LXRα in a concentration-dependent manner. In addition, the inhibition of glycyrrhizin on IL-6 and IL-8 production was reversed by LXRα inhibitor GGPP. In conclusion, these results indicated that glycyrrhizin exhibited its anti-inflammatory effects in HGFs by activating LXRα.

1. Introduction

Periodontitis is a common and often undiagnosed oral disease that characterized by inflammatory destruction of periodontal tissues [1]. Periodontal destruction is often caused by bacteria, which leads to the inflammation and bone resorption [2]. *Porphyromonas gingivalis* is the major pathogen that leads to periodontitis [3,4]. *Porphyromonas gingivalis* LPS could induce NF-κB activation and inflammatory mediators release in human gingival fibroblasts [5]. These inflammatory mediators, such as IL-6, IL-8, NO, and PGE2, have the ability to induce periodontal tissue injury [6]. Previous studies showed that inhibition of inflammatory response could attenuate the pathological process of periodontitis [7]. LXRα is a nuclear receptor that has previously been shown to regulate inflammatory response [8]. It has been reported that activating LXRα could inhibit LPS-induced NF-κB activation [9]. Therefore, LXRα may be used as a target for the treatment of periodontitis.

Glycyrrhizin, an active component of liquorice roots, has been reported to have anti-inflammatory effect. Previous study showed that glycyrrhizin inhibited LPS and D-galactosamine-induced liver injury in mice [10]. Glycyrrhizin also suppressed LPS-induced acute lung injury in mice by inhibiting inflammatory mediators production [11]. Glycyrrhizin was found to inhibit LPS-induced inflammatory cytokines production in mammary epithelial cells [12]. Moreover, glycyrrhizin has been known to inhibit HMGB1 secretion in LPS-stimulated RAW264.7 cells [13]. LPS-induced neuroinflammation and memory deficit were also inhibited by treatment of glycyrrhizin [14]. However, the anti-inflammatory effects of glycyrrhizin on LPS-induced inflammatory response in HGFs have not been reported. The aim of this study was to clarify the anti-inflammatory effects and mechanism of glycyrrhizin in LPS-stimulated HGFs.

2. Materials and methods

2.1. Chemicals and reagents

Glycyrrhizin (purity>98%) was purchased from the National Institute for the Control of Pharmaceutical and Biological Products (Beijing, China). GGPP was purchased from Sigma-Aldrich (CA, USA). LPS from *Porphyromonas gingivalis* was obtained from InvivoGen (San Diego, CA, USA). NF-κB p65, NF-κB p-p65, IκBα, p-IκBα, iNOS, COX-2, and β-actin were purchased from Cell Signaling Technology Inc (Beverly, MA). LXRα antibody was purchased from Santa Cruz Biotechnology. ELISA kits for IL-6 and IL-8 were purchased from R&D. All other reagents were of analytical grade.

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2.2. Cell culture

HGFs were isolated from explants of human normal gingival tissues as described previously [15]. This study was approved by the local ethic committee. The cells were suspended in DMEM containing 10% fetal bovine serum (FBS). Cells between passages 3 to 8 were used in this study. For LXRα inhibitory experiment, the cells were pretreated with or without 20 μM geranylgeranyl pyrophosphate (GGPP) for 2 h, then the cells were pretreated with glycyrrhizin 12 h before LPS treatment.

2.3. Cell viability

Cell viability was detected by MTT assay. In brief, HGFs were seeded at a 96-well plate at the density of 1×10^4 cells/well. Then, the cells were treated with LPS and glycyrrhizin for 24 h. MTT was added to each well. 4 h later, 150 μL of DMSO was added to each well. Optical density was measured at 450 nm using a Bio-Rad Microplate Reader (Model 680, Bio-Rad, USA).

2.4. Cytokines assay

The culture medium was collected and the production of cytokines IL-6 and IL-8 in the culture medium were measured using ELISA kits (R&D) according to the manufacturer's instructions.

2.5. Western blot analysis

HGFs were lysed using RIPA lysis buffer and the protein concentration was detected by BCA method. The protein (30 μg) were separated by 12% SDS-PAGE and transferred to nitrocellulose membrane (Bio-Rad, PA, USA). The membranes were incubated with antibodies against COX-2, iNOS, NF-κB p65, NF-κB p-p65, IκBα, p-IκBα, LXRα, and β-actin overnight at 4 °C. Then, the membranes were probed with HRP-conjugated secondary antibody for 1 h. The specific binding was detected using chemiluminescence detection system (Amersham, Berkshire, UK).

2.6. Statistical analysis

Results were presented as means ± SD. Differences between multiple treatments were analyzed with one-way analysis followed by the Newman-Keuls post hoc test. Statistically significance were considered at $P < 0.05$.

3. Results

3.1. Effects of glycyrrhizin on HGFs cell viability

Effects of glycyrrhizin on HGFs cell viability was assessed by the MTT assay. As shown in Fig. 1, glycyrrhizin at the doses of 40, 80, 160 μg/ml did not affect the cell viabilities of HGFs. Glycyrrhizin at the dose of 180 μg/ml decreased the cell viabilities of HGFs. Thus, in the subsequent studies, glycyrrhizin at the doses of 40, 80, 160 μg/ml were used.

3.2. Glycyrrhizin inhibits LPS-induced IL-6 and IL-8 production in HGFs

To evaluate the anti-inflammatory effects of glycyrrhizin on LPS-stimulated HGFs, the production of IL-6 and IL-8 were detected. As shown in Fig. 2, LPS treatment increased the production of IL-6 and IL-8 compared with the untreated group. However, pre-treatment of the cells with glycyrrhizin decreased the production of IL-6 and IL-8 compared with the LPS group.

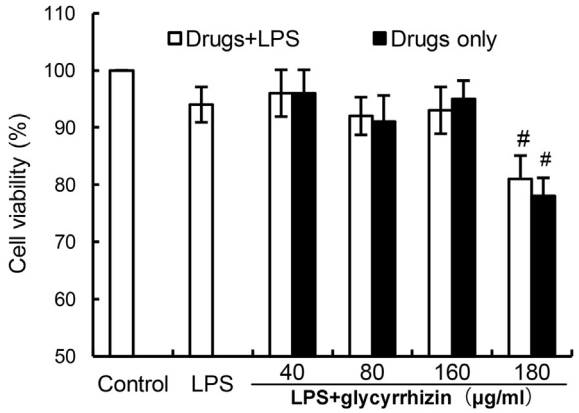

Fig. 1. Effects of glycyrrhizin on the cell viability of HGFs. Cells were cultured with different concentrations of glycyrrhizin (0–180 μg/ml) in the absence or presence of 1 μg/ml LPS for 24 h. The cell viability was determined by MTT assay. The values presented are the means ± SD of three independent experiments.

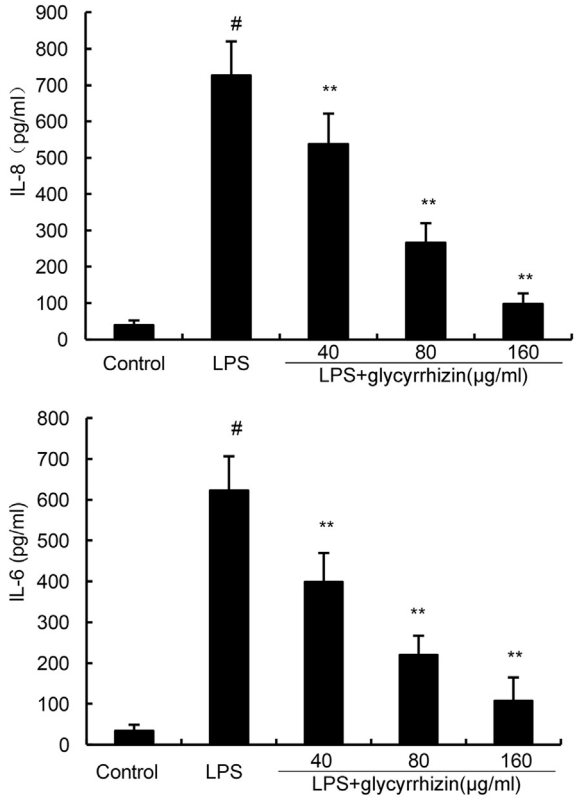

Fig. 2. Glycyrrhizin inhibits LPS-induced IL-8 and IL-6 production in HGFs. The data presented are the means ± SD of three independent experiments. $^{\#}p < 0.05$ *vs.* control group; $^{*}p < 0.05$, $^{**}p < 0.01$ *vs.* LPS group.

3.3. Glycyrrhizin inhibits LPS-induced COX-2 and iNOS expression in HGFs

To evaluate the anti-inflammatory effects of glycyrrhizin on LPS-stimulated HGFs, the production of IL-6 and IL-8 were detected. As shown in Fig. 3, LPS treatment increased the production of IL-6 and IL-8 compared with the untreated group. However, pre-treatment of the cells with glycyrrhizin decreased the production of IL-6 and IL-8 compared with the LPS group.