Ketoprofen Inhibits Expression of Inflammatory Mediators in Human Dental Pulp Cells

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Abstract

Introduction: Conventional root canal treatment is the treatment of choice for the irreversible pulpite caused by bacterial infection. More recently, vital pulp therapy has been proposed as an alternative for management of inflamed dental pulp. Ketoprofen is an anti-inflammatory agent commonly used as a component of mouth rinse for oral lesions. Here, we examined the effect and mechanisms of action of ketoprofen on the expression of inflammatory mediators induced by the lipopolysaccharide (LPS) in dental pulp cells. Methods: Human dental pulp cells were exposed to LPS or LPS + ketoprofen, and reverse-transcription polymerase chain reaction was used to detect interleukin-1β and tumor necrosis factor α. The effect of these treatments on mitogen-activated protein kinase pathways was assessed by Western blots for extracellular signal-regulated kinase and c-Jun N-terminal kinase. Results: LPS induced interleukin-1β and tumor necrosis factor α in dental pulp cells. Ketoprofen effectively inhibited interleukin-1β and tumor necrosis factor α production in LPS-stimulated dental pulp cells. Notably, ketoprofen inhibited phosphorylation of extracellular signal-regulated kinase and c-Jun N-terminal kinase. Conclusions: Ketoprofen inhibited expression inflammatory mediators in dental pulp cells stimulated with LPS. The inhibitory effect of ketoprofen on inflammatory cytokines is associated with inhibition of the mitogen-activated protein kinase pathway. (J Endod 2013;39:764–767)

Key Words
Interleukin-1β, ketoprofen, lipopolysaccharide, mitogen-activated protein kinase pathway, pulp inflammation, tumor necrosis factor α

Pulpitis is mainly caused by bacterial infections of dentin (1). The invasion of bacterial components and byproducts can cause irreversible pulpitis. Nonsurgical root canal treatment is conventionally the treatment of choice for irreversible pulpitis. However, vital pulp therapy has been proposed as an alternative for the management of inflamed dental pulp. The prevention of pulpite is very important for tooth viability. Inflammation is a multiple response mediated by the immune system. To avoid root canal treatment in teeth with pulpite, management of pulpal inflammation should be a critical factor.

Anti-inflammatory agents such as corticosteroids might be a candidate for reducing pulpal inflammation (2, 3). However, corticosteroids have many associated complications and cannot be considered for use for the treatment of pulp inflammation. Ketoprofen is a nonsteroidal anti-inflammatory agent that is used in the treatment of oral lesions in the form of a mouthwash. Ketoprofen is effective in reducing inflammatory symptoms in pharyngitis (4). Ketoprofen is also effective for pain relief and inflammation control in acute inflammation of the mouth in orthodontic therapy (5). However, the effect of ketoprofen on the expression of key inflammatory mediators by human pulp cells is unknown. In the present study, we examined the anti-inflammatory response of ketoprofen induced by lipopolysaccharides (LPSs) and determined the mechanism of the anti-inflammatory activity of ketoprofen in dental pulp cells.

Materials and Methods

Cell Culture

Human dental pulp cell (HDPC) lines immortalized by transfection with the telomerase catalytic subunit hTERT gene (6) were used. Cell cultures were maintained in Dulbecco modified Eagle medium (Invitrogen, Carlsbad, CA) supplemented with 10% fetal bovine serum (Invitrogen) and gentamicin (50 μg/mL) at 37°C in a humidified atmosphere containing 5% CO₂.

Cell Viability

Cell viability was determined by 3-[4,5-dimethylthiazol-2-yl]-2,5 diphenyltetrazolium bromide (MTT) assay. Cells were plated in 24-well plates at a density of 2.0 × 10⁴ cells per well and incubated with various amounts of ketoprofen (ie, 0.25, 0.5, 1, and 2 mg/mL). After incubation for 24 hours, MTT (0.5-mg/mL final concentration) was
added to each well and incubated for another 4 hours at 37°C under 5% CO2. The formazan crystals formed were dissolved in 150 μL dimethyl sulfoxide, and the optical density values were measured at 570 nm by using a MULTISKAN GO microplate reader (Thermo Scientific, Waltham, MA). Results are shown as a percentage of MTT reduction.

Cell Treatment

HDPCs were harvested from culture flasks and counted, and cells were seeded in a 3-cm dish at a density of 1.0 × 10⁵ cells per well for reverse-transcription polymerase chain reaction (RT-PCR) and Western blot in a final volume of 1 mL. The cells were treated with ketoprofen and Escherichia coli LPS or Porphyromonas gingivalis LPS for 4 hours.

RT-PCR

Total RNA was prepared with Trizol reagent (Invitrogen) as specified by the manufacturer and was quantified spectrophotometrically. First-strand complementary DNA was synthesized from 1 μg RNA using random primers (Promega, Madison, WI) and Moloney murine leukemia virus reverse transcriptase (Invitrogen). Two microliters of complementary DNA product was amplified in 25-μL volumes under a layer of mineral oil using a GeneAmp 2700 thermal cycler (Applied Biosystems, Foster City, CA). Each PCR reaction mixture contained 50 mM KCl, 10 mM Tris-HCl, 1.5 mM MgCl₂, 0.2 mM each deoxy-nucleotide-tri phosphate (dNTP), 1 U Taq DNA polymerase, and 0.5 μM each primer. Each reaction consisted of an initial denaturation at 94°C for 1 minute followed by 3-step cycling: denaturation at 94°C for 30 seconds, annealing at a temperature optimized for each primer pair for 30 seconds, and extension at 72°C for 60 seconds. After the requisite number of cycles (28–30 cycles), the reactions underwent a final extension at 72°C for 5 minutes. The primer sequences used for PCR amplification were as follows: interleukin (IL)-1β (548 base pairs [bp]): 5'-CAGTGAAAT-GATGGCTTTATAC-3', 5'-CTTTCAACAGGACAGGAGG-3'; tumor necrosis factor α (TNF-α) (261 bp): 5'-TGAAAGAGGCAGAACATCCA-3', 5'-AGCTTGAGGCCCAGTGAGT-3'; and β-actin (300 bp): 5'-AGCGGAATTCGTTGAGT-3', 5'-CAGGGTACATGGTGGTGCC-3'. Ten microliter volumes of the PCR products were fractionated on 1.2% (w/v) agarose gels containing ethidium bromide and were visualized by ultraviolet transillumination and photographed.

Western Blot

Total cell extracts were harvested in a lysis buffer (Cell Signaling Technology, Beverly, MA) and then centrifuged at 12,000 × g for 10 minutes at 4°C. Quantification of total protein was performed with a bicinchoninic acid protein assay reagent (Bio-Rad Laboratories, Hercules, CA). Proteins were resolved by 10% sodium dodecyl sulfate—polyacrylamide gel electrophoresis and transferred to a polyvinylidene difluoride membrane. The membranes were incubated with antiphospho extracellular signal–regulated kinase (ERK [1:1000]) (Cell Signaling Technology) and antiphospho c-Jun N-terminal kinase (JNK [1:1000]) (Cell Signaling Technology) antibodies. After washing, the blots were incubated with horseradish peroxidase–conjugated immunoglobulin G secondary antibody (1:1000) (Cell Signaling Technology) (Cell Signaling Technology) antibodies. Immunoreactive proteins were detected by enhanced chemiluminescence reagent (LumiGLO, Cell Signaling Technology) according to the manufacturer’s instructions. Densitometric analysis was conducted directly from the blotted membrane by using the LAS-4000 lumino image analyzer system (Fujifilm, Tokyo, Japan). The same membrane was successively stripped and reprobed with anti–β-actin (1:5000) (Sigma-Aldrich, St Louis, MO).

Statistical Analyses

Data were analyzed by using 1-way analysis of variance followed by a multiple-comparison Scheffe test with the use of SPSS 17.0 software program (SPSS Inc, Chicago, IL). Statistical significance was determined at \( P < .05 \).

Results

Cytotoxicity of Ketoprofen and LPS Induces IL-1β and TNF-α in HDPCs

Cell viability was not inhibited by the presence of ketoprofen. There was no statistically significant difference between untreated cells and cells treated with ketoprofen (Fig. 1A). To investigate whether E. coli and P. gingivalis LPS induced an inflammatory reaction in HDPCs, the influence of LPS on the expression of IL-1β and TNF-α was assessed. LPSs of both species significantly increased IL-1β and TNF-α expression (Fig. 1B). Increases in IL-1β and TNF-α expression were observed from 0.1 μg/mL E. coli and P. gingivalis LPS. Therefore, these concentrations were selected for subsequent experiments.

Effect of Ketoprofen on the Expression of IL-1β and TNF-α Production in LPS-treated HDPCs

To investigate the effect of ketoprofen on LPS-induced pulpal inflammation, IL-1β and TNF-α expression was assessed by RT-PCR. Ketoprofen treatment reduced the LPS-induced increase of IL-1β and TNF-α expression in a concentration-dependent manner (Fig. 2A).

Figure 1. The cytotoxicity of ketoprofen and LPS induce IL-1β and TNF-α in HDPCs. (A) HDPCs were incubated with 0, 0.25, 0.5, 1, and 2 mg/mL ketoprofen for 24 hours. Cell viability was assessed by using the MTT assay. The percentage of cell viability in the control group represented 100%. There was no statistically significant difference. Each value represents the mean of 3 replicates. (B) Cells were treated with 0.1, 1, and 10 μg/mL LPS. The IL-1β and TNF-α production were assessed using RT-PCR. β-actin was used as the loading control.
**Discussion**

Pulpal disease is associated with an immune response to bacteria. Both gram-negative bacteria and gram-positive bacteria have been detected in infected root canals and pulps (7, 8). Gram-negative bacteria are suspected to induce pulp inflammation. LPS is a component of the outer membrane of gram-negative bacteria and mediates bacterial activity that destroys host tissue (7).

IL-1β is considered to be a key mediator of inflammation (9). In pulp disease, an increase in the IL-1β in pulps might stimulate inflammation. Inflammatory activity could be attributed to IL-1β induced by LPS (10, 11). IL-1β is involved in the pulp inflammatory process via stimulation of vascular cell adhesion molecule-1 (VCAM-1) expression (12). TNF-α and IL-1β share many biological activities. Like IL-1β, TNF-α also induces inflammatory activity. TNF-α is related to clinical symptoms of endodontic disease (13). TNF-α increases matrix metalloproteinase-1 (MMP-1) expression in pulp fibroblasts, and it causes type I and type III collagen degradation (14). IL-1β and TNF-α are produced by human pulp fibroblasts exposed to LPS (15). In this study, E. coli LPS and P. gingivalis LPS increased the expression of IL-1β and TNF-α in HDPCs.

Studies related to the reduction of pulp inflammation have been performed with the aim of treating pulps without root canal therapy. Peroxisome proliferator–activated receptor γ (PPARγ), tea catechin, and terrein were introduced to reduce pulpal inflammation (16–18). Peroxisome proliferator–activated receptor gamma decreases the production of matrix metalloproteinases, intercellular adhesion molecule-1, and VCAM-1 (16). Epigallocatechin, which is an ingredient of tea, inhibits the production of IL-8 and prostaglandin E2 production in human dental pulp fibroblast (HPDF) (17). Terrein reduces intercellular adhesion molecule-1 and VCAM-1 induced by LPS (18).

In the present study, ketoprofen effectively reduced IL-1β and TNF-α expression induced by E. coli and P. gingivalis LPSs in a dose-dependent fashion. Ketoprofen is a highly potent and safe nonsteroidal anti-inflammatory agent of the propionic acid derivative group. It was synthesized in 1963 and subsequently introduced for anti-inflammatory use (19). It is also convenient to apply to dentin or pulp inflammation as part of mouthwash. It might be beneficial to use ketoprofen mouthwash for dentin or inflamed pulps to control inflammation and encourage pulp healing.

MAPKs have been implicated in many physiologic processes, including cell proliferation, differentiation, and death (20). MAPK pathways mediated by ERK, JNK, and p38 protein kinases also play a key role in regulating the inflammation response (21). The primary role of ERK 1/2–mediated signaling has been thought to be cell growth.
and proliferation. However, it has become clear that several inflammatory processes involve ERK 1/2 activation. The JNK signaling pathway has been implicated in a large variety of pathological conditions, including cancer, stroke, ischemic heart disease, and inflammatory disorders. p38 MAPK plays a central role in the regulation of a wide range of immunologic responses, as seen in inflammatory disorders. Therefore, the MAPK pathway is an attractive strategy for anti-inflammatory therapy (22).

In this study, the ketoprofen-mediated reduction of IL-1β and TNF-α production is thought to be the result of the inhibition of the MAPK pathway because ketoprofen suppressed phosphorylation of ERK and JNK, which was induced by LPS treatment. Ketoprofen likely suppressed the phosphorylation of ERK and JNK, inhibiting the MAPK pathway. Thus, inflammatory cytokines such as IL-1β and TNF-α production are reduced.

Collectively, our results suggest that the inhibition of MAPK activation might be a good therapeutic strategy for LPS-induced pulp inflammation. We propose that the inhibitory effect of ketoprofen on the inflammatory cytokine involves the blockade of the MAPK pathway. This study unveils the inhibitory effect of ketoprofen on the expression of inflammatory cytokines induced by LPS in HDPCs. These results suggest a therapeutic potential for ketoprofen in conservative approaches for the management of teeth with pulpitis.

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The authors deny any conflicts of interest related to this study.

References
