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IL-1β-induced MCP-1 expression and secretion of human dental pulp cells is related to TAK1, MEK/ERK, and PI3K/Akt signaling pathways

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ABSTRACT

Objective: Interleukin-1β (IL-1β) is an inflammatory molecule of the dental pulp. IL-1β stimulates cyclooxygenase-2 (COX-2) and prostaglandins production of pulp cells and affects the pulpal inflammation and repair. However, the effects of IL-1β on Monocyte Chemotactic Factor-1 (MCP-1) of dental pulp cells and its relation to transforming growth factor β-activated kinase-1 (TAK1), PI3K/Akt, and MEK/ERK signaling and COX activation are not fully clear.
Design: Human dental pulp cells were exposed to IL-1β with/without pretreatment and co-incubation by aspirin (a COX inhibitor), 5z-7-oxozeaenol (a TAK1 inhibitor), LY294002 (a PI3K/Akt inhibitor) or U0126 (a MEK/ERK inhibitor). Viable cell number was evaluated by MTT assay. MCP-1 mRNA expression was tested by reverse transcriptase-polymerase chain reaction (RT-PCR). MCP-1 and COX-2 protein expression was studied by western blot. MCP-1 in the culture medium was measure by ELISA.
Results: IL-1β showed little cytotoxicity to pulp cells. It stimulated MCP-1 mRNA and protein expression and MCP-1 secretion. Aspirin, U0126, LY294002 and 5z-7-oxozeaenol attenuated the IL-1β-induced MCP-1 expression. In addition, 5z-7-oxozeaenol, LY294002, U0126 and aspirin prevented the IL-1β-induced MCP-1 secretion of pulp cells.
Conclusion: These results indicate that IL-1β may be involved in the pulpal inflammatory and healing processes by inducing MCP-1 expression and secretion. These events are related to differential activation of TAK1, PI3K/Akt and MEK/ERK 1/2 signaling and COX activation. These results are important for future pharmacologic intervention of pulpal inflammatory diseases.

1. Introduction

Human dental pulp may suffer from the damage of outer physical, microbial and chemical injuries, leading to different extent of pulpal inflammation and even pulp necrosis and periapical infection (Seltzer & Boston, 1997; Scarano et al., 2003). While the mineralized dentin barrier has the capacity to protect dental pulp from exogenous insults, the damaged dental pulp has intrinsic activity to repair and regenerate the lost tissue. However, if the infection and injury to the dental pulp is not eradicated and well-controlled, tissue immune and inflammatory responses will prolong and result in pulpal damage and eventually necrosis. The pulpal inflammation and repair are very complex whereas a number of cells and molecules such as bacterial toxins, interleukin-1β (IL-1β), tumor necrosis factor-α (TNF-α), prostanoids, IL-6, IL-8, MCP-1 and many others are involved and contribute to hyperalgesia and pathogenesis of pulpitis (Hahn & Liewehr, 2007a, 2007b). These inflammatory mediators may bind to surface receptors and stimulate various signal transduction pathways to regulate downstream reactive molecules and play an important role in both dental pulp inflammation and repair (Cooper et al., 2010).

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IL-1β belongs to IL-1 family, which comprised of IL-1α, IL-1β, IL-1 receptor antagonist (IL-1ra), IL-18, IL-33, IL-36α, IL-36β and IL-36γ., IL-37 and IL-38 (Ballak, Stienstra, Tack, Dinarello, & van Diepen, 2015). IL-1β is an important and multi-functional pro-inflammatory cytokine, secreted by various cells, including monocytes, macrophages and fibroblasts. IL-1β is a proinflammatory cytokine that exhibits its biological effects via autocrine or paracrine manners by binding to surface IL-1 receptors (IL-1Rs) and stimulate signal transduction pathways such as transforming growth factor β-activated kinase-1 (TAK1), IL-1R-associated kinase (IRAK), MEK/ERK p38, PI3K/Akt, NF-κB and AP-1 to affect the expression of downstream repair and inflammatory molecules (Ballak et al., 2015; Debets et al., 2000; Dunne & O'Neill, 2003; Yamazaki et al., 2009). TAK1 is one of the kinase that may activate downstream NF-kB and mitogen-activated protein kinases (MAPKs) including ERK, p38 and JNK. TAK1 has been shown to play an important role in renal inflammation and fibrosis (Ma et al., 2011), suggesting its possible involvement in pulpal inflammation and repair.

Various chemotactic factors like IL-8, monocyte chemotactic factor-1 (MCP-1) and leukotriene B4 may regulate tissue inflammation, repair and regeneration (Artuc, Hermes, Stechelings, Grutzkau, & Henz, 1999; Dah & Rhee, 2009; Sampson, 2000), by inducing neutrophil and monocyte recruitment/chemotaxis and the prevention of neutrophils apoptosis. When binding of bacteria or immunoglobulin to TLR receptors of monocytes or macrophages, IL-1β production is stimulated. Recently we have found that IL-1β stimulates PGE_2, soluble VCAM-1 (sVCAM-1) and soluble ICAM-1 (sICAM-1) production of dental pulp cells (Chang et al., 2006, 2012, 2015a). However, the impact of IL-1β on MCP-1 expression and secretion of human dental pulp cells and their signaling via TAK1, PI3K/Akt and MEK/ERK pathways is not clear.

To know more about pulpal inflammation and repair, we hypothesize that IL-1β stimulates MCP-1 expression and production of pulp cells through activation of TAK1, MEK/ERK 1/2 and PI3K/Akt signaling and the activation of COX. We therefore design this study to further investigate the effects of IL-1β on MCP-1 expression/production of human dental pulp cells and its relation to TAK1, MEK/ERK1/2 and PI3K/Akt signaling and COX activation. These results are helpful to our understanding the molecular mechanisms of pulpal inflammation and repair.

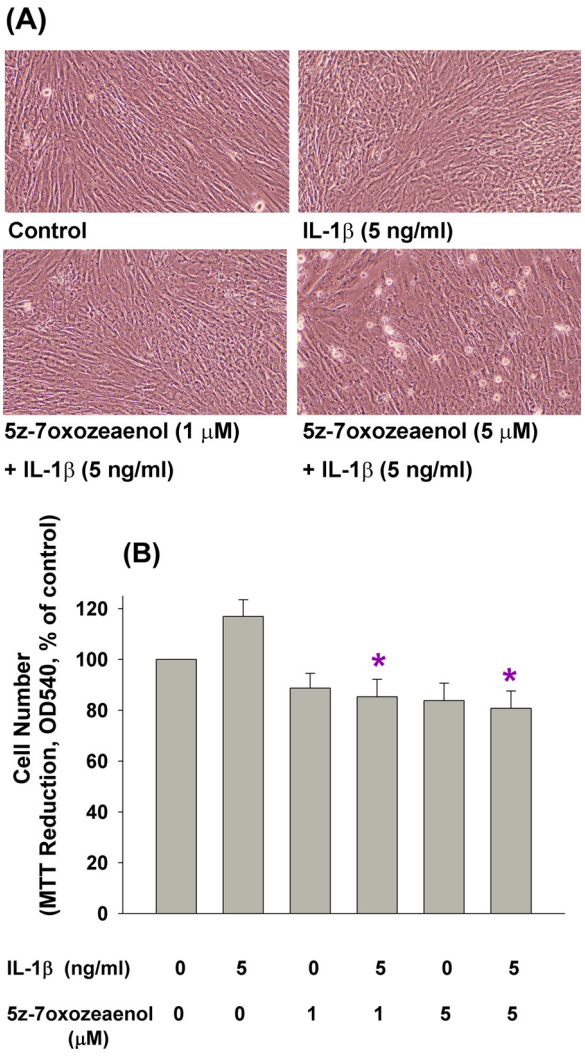

Fig. 1. (A) Morphology of control pulp cells and pulp cells exposed to IL-1β (5 ng/ml) alone, 5z-7oxozeaenol (1 μM) plus IL-1β (5 ng/ml) and 5z-7oxozeaenol (5 μM) plus IL-1β (5 ng/ml). (B) Number of pulp cells exposed to IL-1β with/without 5z-7oxozeaenol for 24 h, as analyzed by MTT assay. Results were expressed as % of control ($n = 6$). * Denotes statistically significant difference ($P < 0.05$) when compared with IL-1β solely group.

2. Materials and methods

2.1. Materials

Materials and reagents for pulp cell culture were purchased from Gibco Laboratories (Life technologies, Grand Island, NY, USA). Dimethyl sulfoxide (DMSO), 3-(4,5-dimethyldiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT), aspirin, U0126 (1,4-diamino-2,3-dicyano-1,4-bis(2-amino phenylthio) butadiene) and LY294002 were purchased from Sigma (Sigma Chemical Company, St. Louis, MO, USA). Total RNA isolation kits were obtained from Qiagen (Qiagen Company, Taiwan). PCR primers for β-actin (BAC) and MCP-1 were synthesized from Genemed Biotechnologies, Inc. (San Francisco, CA, USA). Enzyme-linked immunosorbant assay (ELISA) kits for MCP-1 were from R&D (R&D DuoSet, Minneapolis, MN, USA). IL-1β was obtained from Pepro-Tech (Pepro-Tech Asia, Israel). 5z-7-Oxozeaenol was purchased from Tocris (Tocris Cookson Ltd., Northpoint, Fourth Way, Avonmouth, UK). MCP-1, COX-2 and GAPDH antibodies were from Santa Cruz Biotechnology (LA, USA).

2.2. Culture of human dental pulp cells

By the approval of Ethics Committee, National Taiwan University Hospital, human dental pulp tissues were obtained from caries- and periodontitis-free premolars extracted from three young donors (12–20 years old) due to orthodontic reason. Each extracted teeth was split immediately, pulp tissues were taken and washed 3 times with Dulbecco modified Eagle medium (DMEM). They were cut into 1 mm^3 pieces by a surgical knife, placed onto culture dishes and cultured in DMEM containing 10% fetal bovine serum (FBS), 100 U/ml penicillin, and 100 μg/ml streptomycin (Life Technologies, Grand Island, NY) for 7–14 days. When the growth of pulp cells reached near confluence, they were subcultured at a ratio of 1:3. Three strains of dental pulp cells were established. The 3rd to 8th passages of dental pulp cells were used in this study with similar results (Chang et al., 2006, 2012, 2015a).

2.3. Effect of IL-1β with/without aspirin, LY294002, U0126, 5z-7-oxozeaenol on the viability of pulp cells

Briefly, 1×10^5 pulp cells/well were seeded onto 24-well cultures and treated with various concentrations of IL-1β (0.1, 0.5, 1, 5, 10 ng/ml) with/without 30 min of pretreatment and then co-incubation by aspirin (100 or 200 μM), LY294002 (10 and 20 μM), U0126 (10 and 20 μM) or 5z-7-oxozeaenol (1 and 5 μM) for 24-h. Morphology of pulp cells was observed under phase