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Original Article

IL-1β induced IL-8 and uPA expression/production of dental pulp cells: Role of TAK1 and MEK/ERK signaling

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Urokinase plasminogen activator

Background/purpose: Interleukin 1 beta (IL-1β) is a pro-inflammatory cytokine involved in the inflammatory processes of dental pulp. IL-8 and urokinase plasminogen activator (uPA) are two inflammatory mediators. However, the role of transforming growth factor beta-activated kinase-1 (TAK1) and mitogen-activated protein kinase kinase (MEK)/extracellular signal-regulated kinase (ERK) signaling pathways in responsible for the effects of IL-1β on IL-8 and uPA expression/secretion of dental pulp cells are not clear.
Methods: Human dental pulp cells were exposed to IL-1β with/without pretreatment with 5z-7-oxozeaneaeol (a TAK1 inhibitor) or U0126 (a MEK/ERK inhibitor). TAK1 activation was determined by immunofluorescent staining. The protein expression of IL-8 was tested by western blot. The expression of IL-8 and uPA mRNA was studied by reverse transcriptase-polymerase chain reaction (RT-PCR). The secretion of IL-8 and uPA was measured by enzyme-linked immunosorbent assay.

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Results: Exposure of dental pulp cells to IL-1β (0.1–10 ng/ml) stimulated IL-8 and uPA expression. IL-1β also induced IL-8 and uPA secretion of dental pulp cells. IL-1β stimulated p-TAK1 activation of pulp cells. Pretreatment and co-incubation of pulp cells by 5z-7oxozeaenol (1 and 2.5 μM) and U0126 (10 and 20 μM) prevented the IL-1β-induced IL-8 and uPA expression. 5z-7oxozeaenol and U0126 also attenuated the IL-1β-induced IL-8 and uPA secretion.
Conclusion: IL-1β is important in the pathogenesis of pulpal inflammatory diseases and repair via stimulation of IL-8 and uPA expression and secretion. These events are associated with TAK1 and MEK/ERK signaling. Blocking of TAK1 and MEK/ERK signaling has potential to control inflammation of dental pulp.

Introduction

The teeth often come into various challenges such as dental caries, trauma, dental restoration and many others. In order to eliminate the invading pathogens and allow tissue repair, dental pulp may suffer from different extent of inflammatory response.[1] Because of the unique feature of dental pulp, as a tissue confined in a hard chamber, the inflammation of dental pulp is more difficult to control. Thus, for the better management of the injured tissue, it is important to understand the inflammatory processes of the dental pulp.

During pulpal inflammation, a number of cellular mediators may be stimulated and released to control pulpal inflammation and induce repair. Interleukin-1β (IL-1β) is one of the pro-inflammatory cytokines involved in the acute and chronic inflammatory processes of the host and pulpal/periapical lesions.[2] An increased IL-1β and IL-8 expression in pulpitis tissues was observed by immunohistochemical staining.[3] IL-1β can be generated and released by different kind of cells residing in the dental pulp tissue and affects the biological activities of dental pulp.[4] The main function of IL-1 type cytokines is to control proinflammatory reactions in response to tissue injury, induced by pathogen-associated molecular patterns or damage-associated molecular patterns released from the damaged cells.[2,5]

Signal transduction of IL-1β begins with a ligand-induced conformational change in the first extracellular domain of the IL-1 receptor 1 (IL-1R1), which accelerates the recruitment of IL-1 receptor accessory protein (IL-1RacP).[6] This assembles a trimeric complex comprising myeloid differentiation primary response gene 88 (MYD88) and interleukin-1 receptor–activated protein kinase (IRAK) 4.[7] With the phosphorylation of IRAK1 and 4, tumor necrosis factor–associated factor 6 (TRAF6) is recruited and oligomerized.[8] In the signaling pathway, TRAF6 serves as an ubiquitin E3 ligase works with Ubiquitin E2 ligase as a complex,[9] attaches K63-linked polyubiquitin chains to several IL-1 signaling intermediates, such as IRAK1 and the adaptor proteins transforming growth factor–β (TGF-β)–activated protein kinase–binding protein 2 and 3 and Transforming growth factor β-activated kinase 1 (TAK1).[10–12] With the activation of these intermediates, the MEK/ERK, NF-κB, c-Jun N-terminal kinase (JNK), and p38 MAPK pathways are activated.[11,13] However, the signaling of TAK1 and MEK/ERK in IL-1β-induced events of dental pulp cells awaits further investigation.

IL-8 and urokinase plasminogen activator (uPA) are two inflammatory mediators. IL-8 as a potent chemokine, which recruits and activates neutrophils,[14] is associated with infectious and inflammatory diseases. It is proved that IL-8 can be produced at local site and is associated with pulpal inflammation.[15] Urokinase plasminogen activator promotes cell migration and proliferation, is involved in many physiological and pathological phenomena, such as inflammation and tissue remodeling.[16] It has been shown that IL-1β can stimulate expression of uPA mRNA and secretion of uPA by dental pulp cell.[17]

To know the pathogenesis of pulpal inflammation and repair, we proposed that during pulpal inflammation, IL-1β may stimulate IL-8 and uPA expression and secretion in dental pulp cells through the TAK1 or MEK/ERK signal transduction pathways.

Materials and methods

Materials

Reagents and materials for cell culture were obtained from Gibco Laboratories (Life technologies, Grand Island, NY, USA). Dimethyl sulfoxide (DMSO) and U0126 (1,4-diamino-2,3-dicyano-1,4-bis(2-amino phenylthio)butadiene) were purchased from Sigma (Sigma Chemical Company, St. Louis, MO, USA). 5z-7-Oxozeaenol was bought from Tocris (Tocris Cookson Ltd., Northpoint, Fourth Way, Avonmouth, UK). Total RNA isolation kits were from Qiagen (Qiagen Company, Taiwan). Antibody for western blotting analysis of IL-8 (GTX115959) was purchased from Gene Tex (GeneTex International Corporation, Hsin-Chu City, Taiwan). Glyceraldehyde 3-phosphate dehydrogenase (GAPDH) antibody was from Santa Cruz Biotechnology (Dallas, TX, USA) and p-TAK1 antibody was from AVIVA (San Diego, CA, USA). PCR primers for β–actin (BAC), IL-8, and uPA were synthesized from Genemed Biotechnologies, Inc. (San Francisco, CA, USA). Enzyme-linked immunosorbent assay (ELISA) kits for uPA were from R&D Systems (R&D DuoSet, Minneapolis, MN, USA). Recombinant IL-1β and IL-8 ELISA kits were obtained from PeproTech (PeproTech Asia, Israel).

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