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ORIGINAL ARTICLE

TGF-β1 stimulates cyclooxygenase-2 expression and PGE_2 production of human dental pulp cells: Role of ALK5/Smad2 and MEK/ERK signal transduction pathways

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Background/purposes: TGF-β1 is an important growth factor that may influence the odontoblast differentiation and matrix deposition in the reactionary/reparative dentinogenesis to dental caries or other tooth injuries. TGF-β1 exerts its effects through various signaling pathways, such as Smads and MAPKs. Cyclooxygenase-2 (COX-2) is a membrane-associated enzyme that produces prostaglandin E_2 (PGE_2) at sites of pulpal injury and inflammation, which leads to tissue swelling, redness and pain. The purposes of this study were to investigate the differential signal transduction pathways of TGF-β1 that mediate COX-2 stimulation and PGE_2 production in dental pulp cells.

Methods: Pulp cells were exposed to TGF-β1 with/without SB431542 (an ALK5/Smad2 inhibitor) and U0126 (a MEK/ERK inhibitor). MTT assay was used to estimate cell viability.

Conflicts of interest: The authors have no conflicts of interest relevant to this article.

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Enzyme-linked immunosorbent assay (ELISA) was used for measurement of PGE_2 levels. RT-PCR and western blot were used to determined COX-2 mRNA and protein, respectively.
Results: Exposure to TGF-β1 (1—10 ng/ml) increased the COX-2 mRNA and protein level of cultured pulp cells. Exposure to TGF-β1 (0.1—10 ng/mL) significantly stimulated PGE_2 production of dental pulp cells. Under the pretreatment of SB431542, the stimulatory effect of TGF-β1 on COX-2 level of pulp cells was inhibited. Similarly, U0126 also partly inhibited the TGF-β1-induced COX-2 expression.
Conclusion: TGF-β1 increased the COX-2 and PGE_2 level of cultured pulp cells. The effect of TGF-β1 on COX-2 protein expression was associated with ALK5/Smad2/3 and MEK/ERK pathways. These events are important in the early inflammation, repair and regeneration of dental pulp in response to injury.

Introduction

Prostaglandins are the principle mediators of tissue inflammation and play important roles in regulating cell function in physiological and pathological aspects.[1] Under physiological conditions, prostaglandin E_2 (PGE_2) regulates many important biological functions, such as immune responses, blood pressure, pain, body temperature, gastrointestinal integrity, and fertility.[2] Under pathological conditions, PGE_2 is a mediator of inflammation in many diseases such as rheumatoid arthritis and osteoarthritis. PGE_2 as a proinflammatory mediator regulates many processes, which lead to important signs of inflammation such as tissue swelling, redness and pain.[3]

Cyclooxygenase (COX) is a membrane-associated enzyme in the endoplasmic reticulum or nuclear membrane. COX can be further classified into two isoforms, cyclooxygenase-1 (COX-1) and COX-2. COX-1 maintains the baseline levels of prostaglandins. COX-2 produces prostaglandins through stimulation at the site of inflammation.[4] During tissue growth and inflammation, the level of prostaglandins is generated by COX-2. Inflammatory mediators such as IL-1, TNF-α, growth factors, lipopolysaccharide (LPS), and tumor cells are stimulators of the COX-2 expression.[4,5] Selective use of COX-2 inhibitors may provide a valuable tool in the control of inflammation and pain.[5]

Many pathological cytokines, such as IL-1 and TNF-α, stimulate COX-2 mRNA expression and PGE_2 production, which lead to tissue destruction and bony resorption. PGE_2 has been suggested as an indicator of pulpal inflammation and implicated in many inflammation processes, such as vasodilation, increased vascular permeability, bone resorption, chemotaxis, and pain.[6—8] COX-2 participates in the regulation of prostanoid formation in the pathogenesis of pulpal inflammation.[5] In inflamed pulp, fibroblasts and macrophages expressed COX-2, leading to PGE_2 production.[6] PGE_2 and other arachidonic acid metabolites increased vascular permeability in inflamed dental pulp.[7,9] PGE_2 enhances the bradykinin-induced release of calcitonin gene related peptide (CGRP) in bovine dental pulp.[10] During pulpal inflammation, Interleukin-1β (IL-1β) and TNF-α may stimulate PGE_2 production and activate prostaglandin EP receptors thereby induce Ca^{2+} signaling.[11] In the specimen of radicular cyst, COX-2 expression is significantly higher.[12] In periradicular lesions, PGE_2 level was increased, which may partly explain its bone resorbing activity.[13]

Small amount of PGE_2 can be detected in normal human dental pulp cells. However, in inflamed dental pulp, the production of PGE_2 and other arachidonic acid metabolites is significantly increased.[7,14] The level of PGE_2 is also correlated with the treatment outcome of vital pulp therapy in carious primary tooth.[15] However, PGE_2 also participate in tissue regeneration, which may stimulate osteoblast regeneration.[16] Dental pulp cells express EP1, EP2 and EP3, but little EP4 prostaglandin E_2 receptors and PGE_2 activates the downstream adenylate cyclase/cAMP signaling.[11,17] Interestingly, EP2/EP4 receptors can mediate bone and mineralized tissue regeneration, whereas EP1/EP3 receptor negatively regulates the differentiation of mesenchymal cells and rat calvarial cells,[18,19] suggesting the possible involvement of PGE_2 in regulation of pulp cells' behavior via stimulation of different EP receptors.

Human dental pulp tissue is situated inside the tooth, which is easily attacked by external stimuli such as bacteria, trauma and restorative materials. However, pulp tissue has a potential to repair and regeneration when suffering from injuries. Repair of human dental pulp is a complicated cellular and molecular process.[20] Transforming growth factor beta (TGF-βs) is one of the important growth factor that takes part in pulpal regeneration. TGF-β regulates a wide range of biological activities including cell proliferation, differentiation, chemotaxis and apoptosis in various cell types. TGF-β1 is a growth factor being shown to influence odontoblast differentiation and secretory behavior especially in the reactionary dentinogenesis to dental caries.[21] During tooth development and the reparative processes to carious injury, odontoblasts may respond to TGF-β isoforms released from demineralized dentin matrix.[22] TGF-β1 acts through the TGF-β type I and type II receptors to activate intracellular mediators, such as Smad proteins, the mitogen-activated protein kinase (MAPK), and the extracellular signal-regulated kinase (ERK) pathway. TGF-β2 may affect the dental pulp cells through autocrine or paracrine activation of the ALK/Smad2/3-signal transduction pathways.[23]

During tissue repair and regeneration by capping with TGF-β1, tissue inflammation and vessel dilatation are early