Induction of Cyclooxygenase-2 Expression in Human Tracheal Smooth Muscle Cells by Interleukin-1β: Involvement of p42/p44 and p38 Mitogen-Activated Protein Kinases and Nuclear Factor-κB

Chih-Chung Lin a Chi-Chin Sun b Shu-Fen Luo c An-Chi Tsai d Chin-Sung Chien d Li-Der Hsiao d Chiang-Wen Lee d Jen-Tsung Hsieh d Chuen-Mao Yang d,e

Departments of aAnesthetics and bOphthalmology, Chang Gung Memorial Hospital, Departments of cInternal Medicine and dPhysiology and Pharmacology, eGraduate Institute of Natural Products, College of Medicine, Chang Gung University, Taoyuan, Taiwan, ROC

Key Words
Cyclooxygenase-2 • Interleukin-1β • Mitogen-activated protein kinase • Nuclear factor-κB • Prostaglandin E2 • Tracheal smooth muscle cells

Abstract
Interleukin-1β (IL-1β) has been recognized as a potent stimulus for the synthesis of prostaglandin (PG), which has been implicated in inflammatory responses of the airways. However, the mechanisms underlying IL-1β-induced cyclooxygenase (COX) expression and PGE2 synthesis via activation of p42/p44 and p38 mitogen-activated protein kinases (MAPKs) in human tracheal smooth muscle cells (HTSMCs) are not completely understood. We found that IL-1β increased COX-2 expression and PGE2 synthesis in time- and concentration-dependent manners. Both specific phosphatidylincholine-phospholipase C inhibitor (D609) and protein kinase C inhibitor (GF109203X) attenuated IL-1β-induced responses in HTSMCs. IL-1β-induced COX-2 expression and PGE2 synthesis were also inhibited by an inhibitor of MEK1/2 (PD98059) and inhibitors of p38 MAPK (SB203580 and SB202190), respectively, suggesting the involvement of p42/p44 and p38 MAPKs in these responses. This hypothesis was further supported by the transient activation of p42/p44 and p38 MAPKs induced by IL-1β. Furthermore, IL-1β-induced activation of nuclear factor-κB (NF-κB) was inversely correlated with the degradation of IκB-α in HTSMCs. IL-1β-induced COX-2 expression and PGE2 synthesis were inhibited by the NF-κB inhibitor pyrrolidinedithiocarbamate. These findings suggest that the expression of COX-2 is correlated with the release of PGE2 from IL-1β-challenged HTSMCs, which is mediated, at least in part, through p42/p44 and p38 MAPKs and NF-κB signaling pathways in HTSMCs.

Introduction
Cytokines are potent immunoregulatory and proinflammatory mediators which are secreted by a variety of cells in response to infection and activated by lymphocyte...
products, microbial toxins and other stimuli [30]. Elevated levels of proinflammatory cytokines, including interleukin-1β (IL-1β), in bronchoalveolar lavage fluid, have been detected in allergic asthmatic patients [7, 23]. IL-1β is a potent stimulus for prostaglandin (PG) and thromboxane synthesis and has been implicated in inflammatory responses in the airways. Cyclooxygenase (COX), an enzyme responsible for the conversion of arachidonic acid to PGH₂, can be further metabolized to prostanoids that modulate various airway functions [25, 31, 36]. It has been demonstrated that COX exists in at least two isoforms. COX-1, expressed constitutively in most tissues, appears to support the levels of prostanoid biosynthesis required for maintaining normal physiological homeostasis [36]. In contrast, COX-2 is recognized to mediate inflammatory responses and is highly restricted under basal conditions, but is rapidly induced by proinflammatory cytokines [25, 36]. The expression of COX-2 appears to be highly regulated by a number of mitogen-activated protein kinases (MAPKs) and transcription factors, in particular nuclear factor (NF)-κB [4, 17, 28, 33, 37]. It has been shown that prostanoids are generated under physiological and pathophysiological conditions by a variety of cells in the airways [1, 16, 25, 31, 38]. However, the mechanisms of IL-1β-induced COX-2 expression in human tracheal smooth muscle cells (HTSMCs) have not been completely determined.

Several extracellular stimuli elicit a broad spectrum of biological responses through activation of MAPK cascades [2, 13]. There are 3 major superfamilies of MAPKs in several cell types, including p42/p44 MAPK, p38 MAPK and stress-activated protein kinase, all of which are activated by phosphorylation of a tyrosine and a threonine residue catalyzed by a dual-specificity MAPK kinase. Activation of MAPKs induces distinct cellular responses mediated by phosphorylation of specific target proteins [2]. Although cytokines, including IL-1β, are reported to activate all of these MAPKs [17, 37], the relationship between the activation of these pathways and COX-2 expression is not completely understood. In addition, we recently showed that IL-1β induced p42/p44 MAPK phosphorylation and enhanced bradykinin-stimulated signal transduction in canine TSMCs [41, 42]. However, whether activation of these MAPK pathways by IL-1β is linked to COX-2 expression has not been determined in HTSMCs.

In addition to activation of MAPKs, IL-1β has been shown to activate NF-κB in several cell types [28, 32, 40]. NF-κB transcription factors play key roles in regulating the expression of a variety of genes involved in immune and inflammatory responses, cell proliferation and apoptosis [2, 17, 36]. In unstimulated cells, NF-κB is sequestered in the cytoplasm with a specific inhibitory protein termed IκB-α [2, 39]. One MAPK kinase kinase, NF-κB-induced kinase, has been implicated in the activation of NF-κB/Rel following cytokine stimulation [21]. NF-κB-induced kinase, recruited to the receptors through an interaction with TRAF adaptor molecules, activates the IκB kinase (IKK) complex, which consists of IKK-α, IKK-β and IKK-γ [24]. Activation of the IKK complex is mediated through phosphorylation of either IKK-α or IKK-β [14, 33, 40], leading to the phosphorylation of the N-terminal Ser-32 and Ser-36 of IκB-α [40, 43]. This phosphorylation is followed by ubiquitination and rapid degradation through the proteasome pathway [8, 9, 14], allowing for translocation of the released NF-κB into the nucleus. NF-κB acts as a positive regulator of the expression of several genes involved in chronic inflammatory diseases [3]. In epithelial cells, proinflammatory cytokines such as IL-1β, which plays a major role in inflammation, rapidly induce NF-κB activation and cause upregulation of NF-κB-dependent genes, including COX-2 [28]. However, in HTSMCs, the molecular mechanisms of upregulation of COX-2 by IL-1β associated with NF-κB activation are not completely understood.

In addressing these questions, experiments were undertaken to investigate the effect of IL-1β on COX-2 expression and PGE₂ synthesis in cultured HTSMCs. Our findings suggest that the increased expression of COX-2 is correlated with the exaggerated release of PGE₂ from IL-1β-challenged cells, which is mediated, at least in part, through MAPK and NF-κB signaling pathways in HTSMCs. These results provide new insights into the mechanisms of action of IL-1β and suggest that MAPKs and NF-κB may be critical components controlling COX-2 expression and PGE₂ synthesis in HTSMCs.

Materials and Methods

Materials
DMEM/F-12 medium and FBS were purchased from Invitrogen (Carlsbad, Calif., USA). Hybond C membrane, the enhanced chemiluminescence (ECL) Western blotting detection system, poly(dl-dC) and [γ-³²P]ATP (>3,000 Ci/mmol) were from Amersham Pharmacia Biotech (Buckinghamshire, UK). Polyclonal antibodies for COX-1, COX-2 and p42 MAPK were from Santa Cruz (Santa Cruz, Calif., USA). PhosphoPlus p42/p44 MAPK and phosphoPlus MEK1/2 antibody kits were from New England Biolabs (Beverly, Mass., USA). PD98059, SB203580, SB202190, SB202474, genistein, D609, GF109203X and U73122 were from Biomol (Plymouth Meeting, Pa., USA). The PGE₂ enzyme immunoassay kit was from Cayman.