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Original article

The IL-1β signalling pathway and its role in regulating pro-inflammatory and pro-labour mediators in human primary myometrial cells

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ABSTRACT

Interleukin (IL)-1β plays a central role in the processes of human labour and delivery. The adaptor proteins involved in the IL-1β signalling pathway in human myometrium are not known. This study sought to determine the role of the adaptor proteins myeloid differentiation primary response 88 (MyD88), tumour necrosis factor receptor-associated factor 6 (TRAF6), IL-1 receptor-associated kinase 4 (IRAK4) and transforming growth factor beta-activated kinase 1 (TAK1) in IL-1β-induced formation of pro-inflammatory and pro-labour mediators in human myometrium. Human primary myometrial cells were transfected with siRNA against MyD88 (siMYD88), TRAF6 (siTRAF6), IRAK4 (siIRAK4) or TAK1 (siTAK1), treated with IL-1β, and assayed for the mRNA expression and or secretion of pro-inflammatory and pro-labour mediators. Transfection of primary myometrial cells with siMYD88, siTRAF6, siIRAK4 and siTAK1 significantly decreased IL-1β-induced IL-1α, IL-6, growth-regulated alpha protein (GRO-α), IL-8, monocyte chemoattractant protein (MCP)-1, intercellular adhesion molecule (ICAM)-1 and cyclooxygenase (COX)-2 mRNA expression and release of IL-6, GRO-α, IL-8, MCP-1, ICAM-1 and prostaglandin $PGF_{2\alpha}$. The expression and secretion of the extracellular matrix remodelling enzyme matrix metalloproteinase (MMP)-9 was significantly lower with siMYD88 and siTRAF6. Finally, IL-1β-induced nuclear factor κB (NF-κB) transcriptional activity was significantly attenuated by transfection with siMyD88, siTRAF6 and siIRAK4; there was no effect of siTAK1 transfection on NF-κB transcriptional activity. Collectively, these findings suggest that MyD88, TRAF6, IRAK4 and TAK1 are involved in IL-1β signalling in human myometrium. Further studies are required to determine if inhibition of these proteins can prevent preterm birth.

1. Introduction

Preterm birth has a global incidence of approximately 10% and is the leading cause of perinatal morbidity and mortality [1]. The life-long neurological and developmental problems suffered by survivors of preterm birth [2] impacts families emotionally and financially, while also being a fiscal burden on the health care system [3,4]. There are limited treatments available to prevent active labour [5]; however, prophylactic treatments in high-risk women have been shown to reduce the risk of preterm birth [6,7]. This is, in part, due to our lack of understanding of the mechanisms that regulate human labour and delivery [5].

Preterm birth is a heterogeneous syndrome with inflammation, however, firmly linked to preterm birth [8,9]. There is evidence by increased infiltration of leukocytes in the cervix, fetal membranes and myometrium during parturition [10,11]. Interleukin (IL)-1β, an acute phase pro-inflammatory cytokine, released from these invading leukocytes, has been implicated in the pathophysiology of spontaneous preterm birth [8,9,12]. Concentrations of IL-1β in gestational tissues and biological fluids are increased during human labour; while intraamniotic and/or systemic administration of IL-1β to mice and monkeys induces preterm labour [13–16]. Notably, a non-competitive IL-1 receptor–biased ligand (termed rytvela) prevents infection- and inflammation-induced preterm birth [16,17]. In myometrium, IL-1β has been shown to further increase cytokine production; promote the synthesis of cyclooxygenase (COX)-2, prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$), and the $PGF_{2\alpha}$ receptor (FP) [18–22] which are important regulators of myometrial contractility; activate the extracellular matrix (ECM) remodelling enzyme matrix metalloproteinase (MMP)-9 [23]; and induce the expression of adhesion molecules [21] that can further recruit leukocytes into the uterus.

IL-1β contributes to the onset of labour by stimulating the production of genes involved in myometrial contractions, cervical remodelling and rupture of fetal membranes via the pro-inflammatory transcription

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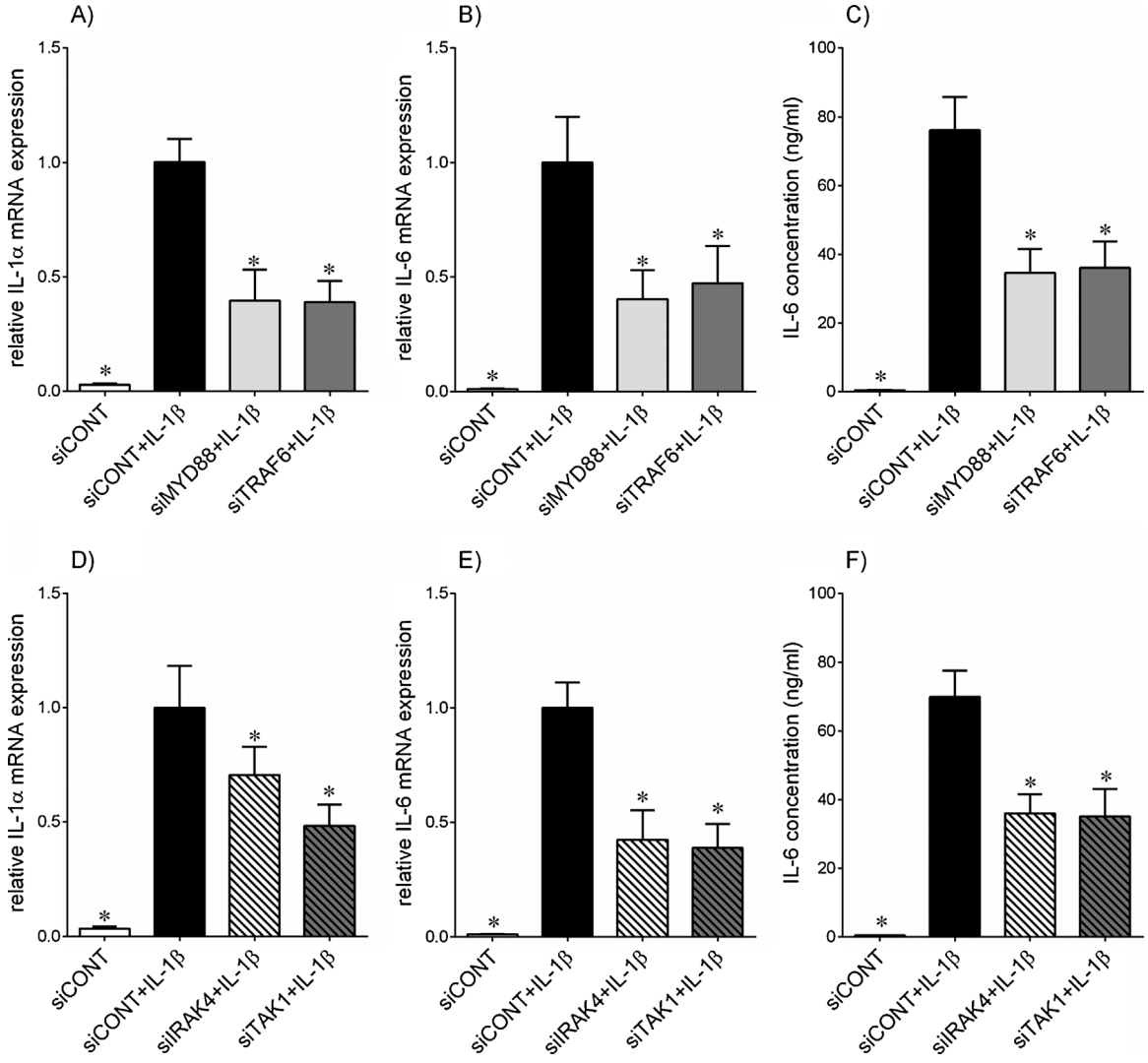

Fig. 1. Effect of siMYD88, siTRAF6, siIRAK4 and siTAK1 on IL-1β-induced expression of pro-inflammatory cytokines.
Human primary myometrial cells were transfected with (A–C) 100 nM siMYD88, siTRAF6 or siCONT, (D–F) 50 nM siIRAK4, siTAK1 or siCONT for 48 h and then treated with 1 ng/ml IL-1β for an additional 24 h (n = 5 patients). (A,B,D,E) IL-1α and IL-6 mRNA expression was analysed by qRT-PCR. (C,F) IL-6 concentration in the incubation medium was assayed by ELISA. For all data, the fold change was calculated relative to IL-1β-stimulated siCONT transfected cells, and displayed as mean ± SEM. *$P < 0.05$ vs. IL-1β-stimulated siCONT transfected cells (one-way repeated measures ANOVA).

factor nuclear factor-κB (NF-κB) [24,25]. In non-gestational tissues, the adaptor proteins myeloid differentiation primary response 88 (MyD88), tumour necrosis factor receptor-associated factor 6 (TRAF6), IL-1 receptor-associated kinase 4 (IRAK4) and transforming growth factor beta-activated kinase 1 (TAK1) have emerged as critical intermediates of IL-1β signalling through NF-κB [26–32]. The signalling cascade is initiated by the adaptor MyD88 binding to the toll/IL-1 receptor (TIR) domain. MyD88 oligomerizes and recruits IRAK4 to the complex via the N-terminal death domain (DD). IRAK4 is autophosphorylated, dissociates from the receptor complex, and interacts with TRAF6. TRAF6, through K63-linked polyubiquitination, is ubiquitinated and recruited to a complex containing TAK1. From TAK1, two signalling pathways diverge; one ultimately leads to NF-κB activation and the other to via mitogen-activated protein kinase (MAPK) activation.

There are no studies on the role of MyD88, TRAF6, IRAK4 and TAK1 in regulating IL-1β-induced inflammation in human myometrium. Thus, the aim of this study was to use siRNA to determine if MyD88, TRAF6, IRAK4 and TAK1 are involved in IL-1β-induced expression and secretion of pro-inflammatory cytokines; COX-2 expression and subsequent prostaglandin release; expression and secretion of the adhesion molecule ICAM-1; and expression of the ECM remodelling enzyme MMP-9 in primary human myometrial cells.

2. Materials and methods

2.1. Tissue collection

The Research Ethics Committee of Mercy Hospital for Women approved this study. Written, informed consent was obtained from all participating women. Myometrium was obtained from the upper margin of the lower uterine segment from women at term (37–41 weeks gestation) elective Caesarean section. None of the women were in labour and all women delivered a healthy singleton infant. All tissues were brought to the research laboratory and processed within 15 mins of the Caesarean delivery. Women with any underlying medical conditions such as diabetes, asthma, polycystic ovary syndrome, pre-eclampsia and macrovascular complications were excluded. Additionally, women with multiple pregnancies, obese women (BMI > 30 kg/m^2), and fetuses with chromosomal abnormalities were excluded.