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The effect of progesterone on genes involved in preterm labor

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ABSTRACT

The decidua is known to be a major source of intrauterine PGF2α during late gestation and labor, and inflammatory cytokines, including IL-1β, IL-6, and IL-8, are elevated in spontaneous preterm deliveries. In the present study, to elucidate how progesterone blocks the pathways associated with preterm birth, we determined the effects of P4 on the expression of PTGS-2 and PTGFR mRNA in human decidua fibroblast cells, as well as the genes, using microarray analysis. Senescence was induced in primary cultured human decidual cells treated with IL-1β. The IL-1β treatment implicated by microarray analysis increased gene expression levels of PTGS-2, PTGFR, NFκ-B p65, IL-17, and IL-8. In contrast, P4 + IL-1β decreased the expression levels of all of these genes in comparison to treatment with IL-1β alone ($p < 0.05$). IL-1β also increased the proportion of SA-β-gal-positive cells. Treatment with IL-1β also increased the p21 protein level in comparison to cells treated either with the vehicle or P4. Neither the p21 protein level nor the number of SA-β-gal-positive cells was increased in normal endometrial glandular cells by IL-1β ($p < 0.05$). Our studies demonstrated that P4 changes the level of gene expression in a manner that favors an anti-inflammatory milieu. Because IL-8 appears to be the cytokine whose expression is most significantly modulated by P4, further studies evaluating IL-8 as a therapeutic target are needed.

1. Introduction

Spontaneous preterm labor and delivery occur after premature labor with intact membranes, prelabor rupture of membranes, and cervical insufficiency (Romero et al., 2006a,b). Intra-amniotic infection accounts for 25%, and it activates all components of the common pathway of parturition, not only cervical ripening, but also evidence of myometrial activation and membrane/decidual activation (Tromp et al., 2004; Belt et al., 1999; Romero et al., 2006a,b).

The prostaglandin F2α receptor (PTGFR) and prostaglandin-endoperoxide synthase-2 (PTGS-2) are related to the induction of labor. Fuchs et al. (1982) reported that the decidua is the major source of intrauterine PGF2α during late gestation and labor. In general,

Abbreviations: CX-43, connexin43; FBS, fetal bovine serum; FDA, Food and Drug Administration; HEPES, *N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid; IGFBP-1, insulin-like growth factor binding protein-1; IL, interleukin (*e.g.*, IL-1); NFκ-B, nuclear factor-kappa B; OTR, oxytocin receptor; P4, progesterone; PGF2α, prostaglandin F2α; PRL, prolactin receptor; PROM, premature rupture of membranes; PTGFR, prostaglandin F2α receptor; PTGS-2, prostaglandin-endoperoxide synthase2/also known as COX-2 = cyclooxygenase-2; 17OHP-C, 17 alpha-hydroxyprogesterone caproate; RT-PCR, reverse transcriptase polymerase chain reaction; SA-β-gal, senescence associated-beta-galactosidase; VP, vaginal progesterone.

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the expression of myometrial PTGFR mRNA decreases progressively throughout gestation in the absence of labor. Moreover, the decidua produces large concentrations of prostaglandin F2α (Willman and Collins, 1976) and oxytocin (Chibbar et al., 1993). We have demonstrated that the decidua expresses PTGS-2 mRNA and protein, the oxytocin receptor (OTR), connexin43 (CX-43), and PTGFR; however, PTGFR was the only gene/protein in which there was an additional increase in expression at late gestation within this tissue (Makino et al., 2007).

Progesterone (P4) was discovered as a hormone produced by the corpus luteum, essential for pregnancy maintenance (Csapo et al., 1971, 1972; Lucovnik et al., 2011). In the first trimester, P4 produced by the corpus luteum is critical to the maintenance of early pregnancy until the placenta assumes this function at 7–9 weeks' gestation. In 2011, the US Food and Drug Administration (FDA) approved the use of 17 alpha-hydroxyprogesterone caproate (17OHP-C) supplementation during pregnancy to reduce the risk of recurrent preterm birth in women with a history of at least one prior spontaneous preterm delivery (Meis et al., 2003). Exogenous administration of 17P is now widely used for the prevention for premature delivery (Meis et al., 2003, 2005; Meis and Connors, 2004).

Vaginal progesterone (VP), natural progesterone (not the synthetic progestin), and 17OHP-C have been shown to prevent preterm birth (Hassan et al., 2000; Romero and Stanczyk, 2013; Kuon et al., 2010). These findings suggest that neither VP nor 17OHP-C has a significant effect on pathways involved in uterine activity or cervical remodeling in abnormal murine pregnancies (Koumans et al., 2012).

Thus, contrary to the expectation that progesterone blocks pathways associated with structural remodeling of the ante partum cervix, administration of VP has only been noted to increase the expression of the antimicrobial protein, defensin1 (Nold et al., 2013).

For women with a short cervix, vaginal progesterone reduced the rate of preterm labor (Romero et al., 2013). In women with a short cervix and a prior history of preterm birth, two strategies have been shown to be equally as effective: vaginal progesterone and cervical cerclage (Conde-Agudelo et al., 2013). However, the mechanism underlying the effect of P4 on the prevention of preterm labor is still unknown.p53 may also play a role in maintaining uterine quiescence in mice. Observations of senescence-associated restriction of uterine growth and preterm birth following the conditional deletion of uterine p53 in mice have revealed the critical role of p53 in uterine biology and parturition involving the p21/Akt/PTGS-2 pathway (Hirota et al., 2010). Senescence-associated growth restriction with increased levels of p21 in decidual cells is also likely involved in the pathophysiology of preterm labor (Hirota et al., 2010).

The purpose of the studies reported herein is to examine the mechanisms whereby progesterone may prevent preterm delivery. We studied the effect of P4 on the expression of PTGS-2 and PTGFR mRNA in decidual cells using microarray experiments as well as studying senescence in primary cultured human decidual fibroblast cells.

2. Materials and methods

2.1. Tissue collection and processing

The primary cell cultures derived from decidual fibroblast cells (decidual cells) were obtained following elective cesarean section from 15 patients at 37–38 weeks' gestation; the cells were isolated and cultured as previously described (Keelan et al., 1997). All patients provided informed consent at the Department of Obstetrics and Gynecology, Juntendo University Faculty of Medicine and Saitama Medical Center and this protocol was approved by the institutional ethics committee.

Samples were obtained from pregnant women who met the following inclusion criteria: median age (range) of 34.5 (20–40) years and single pregnancy. Exclusion criteria included: (1) use of tobacco or alcohol during the pregnancy; (2) pre-existing clinical conditions such as diabetes, hypertension or autoimmune disease; and (3) pregnancy-related complications such as induced hypertension, intrauterine growth restriction, and bleeding (Hanna et al., 2006) (Table 1).

Briefly, fetal membranes were washed three times with RPMI 1640 (Sigma–Aldrich, St. Louis, MO, USA) to remove adherent blood vessels/clots. The decidua was scraped off the underlying chorioamnion with a glass slide, washed in RPMI 1640 medium, and minced with a scalpel. After centrifugation at $1200 \times g$ for 5 min at 24 °C, the pellet was resuspended in RPMI 1640 and incubated at 37 °C for 10 min. After standing for 5 min to settle, the supernatant portion was collected and centrifuged at $1200 \times g$ for 5 min. This step resulted in the purification of decidual cells. The attached cells were cultured in the flask in RPMI 1640 (supplemented with 10% FBS, penicillin, and streptomycin) until the cells reached confluency for 14 days at a density between 5×10^5 and 1×10^6 cells/well in plastic plates (25 mm in diameter). The cultures were maintained at 37 °C in a humidified atmosphere consisting of 95% air and 5% CO_2.

The medium was then replaced with serum-free RPMI 1640 medium containing IL-1β (5 ng/ml; R&D Systems, Minneapolis, MN, USA (Friebe-Hoffmann et al., 2007; Keelan et al., 1997) and crystalline P4 dissolved in ethanol (P4, 1×10^{-6} M; Sigma–Aldrich) (Chanrachakul et al., 2005; Ishihara et al., 1995).

To confirm that decidual cells were present in the cultures, the levels of prolactin receptor (PRL; Santa Cruz Biotechnology, Santa Cruz, CA, USA) and insulin-like growth factor binding protein-1 IGFBP-1; (R&D Systems) were investigated in the cell lysate using Western blot analysis (Richards et al., 1995; data not shown). The levels of PRL and IGFBP-1 were increased in decidual cells compared with endometrial cells. Endometrial cells were used as the negative control and endometrial cells treated with cAMP + P4 served as the positive control (Enzo Life Sciences, NY, USA) (Gellersen and Brosens, 2003) (Fig. 1).

2.2. Endometrial epithelial cell culture

Suspensions of endometrial cells were obtained by enzymatic digestion and mechanical means, as described