BASIC SCIENCE: OBSTETRICS

Alteration of secretory leukocyte protease inhibitor in human myometrium during labor

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OBJECTIVE: Secretory leukocyte protease inhibitor (SLPI) has been shown to have antimicrobial and antiinflammatory properties. The aim of this study was to verify its expression in human myometrium.

STUDY DESIGN: Myometrium was obtained at time of cesarean delivery with (n = 9) or without (n = 11) labor. Expression of SLPI was detected using real-time polymerase chain reaction, enzyme-linked immunosorbent assay, and immunohistochemistry. SLPI expression relative to nuclear factor-κB p65 subunit was compared between subjects. SLPI response to inflammatory mediators was studied in myometrial explants.

RESULTS: SLPI was predominantly localized in the nuclei of myocytes and colocalized with $CD68^+$ macrophages. The nuclear immunoreactivity of SLPI was increased after the onset of labor and was associated with increased nuclear translocation of nuclear factor-κB p65 subunit. Treatment with lipopolysaccharide, interleukin-1β, or tumor necrosis factor-α increased SLPI messenger RNA and protein concentrations slightly in myometrium explants.

CONCLUSION: SLPI was expressed in human myometrium and increased after the onset of labor.

Key words: inflammation, myometrium, nuclear factor-κB, parturition, secretory leukocyte protease inhibitor

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Preterm birth is a leading cause of neonatal morbidity and mortality in developed countries.[1-3] Approximately 30-40% of preterm births are associated with an underlying infectious process.[4] Increasing evidence suggests that parturition is an inflammatory process[5] and inflammatory cell infiltration in the myometrium is 1 physiologic mechanism leading to parturition.[6] Many of the proinflammatory factors responsible for parturition are regulated by nuclear factor (NF)-κB, a transcription factor generally existing as a heterodimer of the p50 and p65 polypeptides.[7,8] After activation, NF-κB translocates to the nucleus, where it regulates transcription of its targeted genes responsible for inflammation, including interleukin (IL)-1β and tumor necrosis factor (TNF)-α.[9] Further understanding of the significance of the myometrium in generating labor-promoting signals and inflammatory reactions may aid in the development of interventions to prevent preterm birth.

Secretory leukocyte protease inhibitor (SLPI) is an 11.7-kDa cationic nonglycosylated protein containing 107 amino acids and found in a variety of mucosal fluids.[10,11] It is constitutively expressed by many epithelial cells (respiratory, intestinal, genital tract, and amnion epithelia) and by monocytes, macrophages, and neutrophils.[11] Evidence suggests that SLPI acts to inhibit various proinflammatory systems.[12,13] For example, SLPI decreases the production of matrix metalloproteinases in human monocytes[12] and inhibits activation of the NF-κB signal transduction pathway in mice macrophage cell lines.[13] It is thought that SLPI serves as an innate antimicrobial and as a buffer to the extracellular protease-mediated effects of inflammatory leukocytes.

In the reproductive context, SLPI was first demonstrated as a pregnancy-associated gene, because SLPI messenger RNA (mRNA) expression is the highest in the pig uterus during mid to late pregnancy.[14] In human endometrium and first-trimester decidua, SLPI was constitutively expressed and found only in glandular epithelium.[15] We previously demonstrated that increased endometrial SLPI expression was associated with improved endometrial bleeding.[16] In amniotic fluid, SLPI was found to be increased in the second trimester with a significant increase with the onset of labor.[17] Since then, a great deal of attention has been given to the use of SLPI concentrations in amniotic fluid for the prediction of preterm birth.[18-21] It has been proposed that within the gravid uterus SLPI was released by decidua, fetal membranes, and the fetal lung.[17,21] SLPI has also been identified in human

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cervical tissues with an increase during pregnancy and the postpartum period[22] and in vernix caseosa.[23]

In this study, we investigated SLPI expression in human nonpregnant myometrium and in term myometrium before and after the onset of labor. Furthermore, we studied the possible effects of lipopolysaccharide (LPS) and proinflammatory cytokines IL-1β and TNF-α on the expression of SLPI in human term myometrium in vitro.

MATERIALS AND METHODS

Tissue collection

This study was approved by our institutional review board. Informed consent was obtained from all subjects before tissue collection.

Tissues samples were obtained from nonpregnant women and pregnant women at term (> 37 completed weeks of gestation, either in labor or not in labor) who were undergoing hysterectomy or cesarean delivery, respectively. Nonpregnant subjects (n = 9) were all premenopausal and were undergoing hysterectomy for dysfunctional uterine bleeding due to leiomyomata. Gestational age was confirmed by the earliest ultrasound performed during the subject's prenatal care. Uterine contractions were measured by tocodynamometry. Labor was defined as regular uterine contractions occurring at least every 5 minutes or shorter in frequency that caused cervical dilation (n = 9). Subjects not in labor (n = 11) lacked regular uterine contractions and had their cervix examined before surgery to ensure that the cervix did not dilate. The indications for cesarean section for subjects not in labor included repeated cesarean birth and fetal malpresentation. The indications for cesarean section for subjects in labor included arrest disorders, fetal distress, and prior cesarean birth in labor electing for repeated cesarean birth. Pregnant subjects' demographics and clinical characteristics are listed in Table 1. In nonpregnant subjects, myometrium was trimmed from the lower uterine segment away from any leiomyomata. In pregnant subjects, after delivery of the fetus, myometrium (2 × 2 cm) was trimmed from the upper aspect of the lower uterine segment incision, and fetal membrane (2 × 2 cm) was trimmed from the closest area of rupture. The myometrium was carefully dissected and trimmed of any decidua to avoid heterogenous tissue sampling. A portion of each tissue specimen was immediately frozen on dry ice and stored at -70°C for total RNA isolation. Another portion was fixed in 10% buffered formalin for paraffin block and routine histologic evaluation and immunohistochemistry.

Explant cultures

Explant cultures were prepared from 11 not-in-labor term myometrial biopsy specimens. Myometrial tissues were immediately transferred to the laboratory after delivery. Portions of each sample were frozen and fixed in 10% buffered formalin for subsequent baseline measurements. Remaining specimens were rinsed with Dulbecco modified Eagle medium (Invitrogen, Carlsbad, CA) complemented with 1% antibiotic and antimycotic solution (containing 100 U/mL penicillin, 100 μg/mL streptomycin, and 250 ng/mL amphotericin B; Invitrogen), placed in a 100-mm plastic tissue culture dish (uncoated) (BD Biosciences, San Jose, CA) and cut with sterile scalpels into 10-15 mg per piece. In all, 8-10 pieces were combined for 1 incubation and placed into 1 well of a 12-well plate containing 1.5 mL of culture medium. After preincubation for 60 minutes in a humidified atmosphere of 95% O_2–5% carbon dioxide at 37°C, in addition to the control vehicle, explants were incubated with LPS (1 μg/mL) (Sigma-Aldrich, St Louis, MO), IL-1β (10 ng/mL) (R&D Systems, Minneapolis, MN), or TNF-α (10 ng/mL) (R&D Systems) in serum-free media for 6 or 24 hours. At the completion of the incubation interval, cultured supernatants were harvested, centrifuged, and stored at -70°C. In all, 3-4 pieces of explants were quick frozen and stored at -70°C for total RNA isolation and the remaining explants were fixed in 10% buffered formalin for paraffin block and immunohistochemistry study of SLPI and NF-κB activity.

Tissue viability and responsiveness were checked at the end of the incubation period. Based on results from our previous study and other studies,[24,25] LPS at 1 μg/mL was used as a positive control. A marked increase in the level of IL-1β and TNF-α was observed in LPS-treated explants after 6 and 24 hours, which indicated the ability of our system to respond to inflammatory mediators properly (data not shown). The dosage of IL-lβ and TNF-α used in this study was based on our previous study with smooth muscle cells and other studies of myometrial cells.[26,27]

Real-time polymerase chain reaction

Relative amounts of SLPI mRNA in human myometrium were assessed by real-time polymerase chain reaction (PCR) as described previously.[24,28-30] Briefly, total RNA from baseline or cultured myometrial samples was extracted using TRIzol Reagent (Invitrogen). Two μg of total RNA were reverse transcribed with SuperScript II First-Strand Synthesis System for real-time PCR (Invitrogen), according to the manufacturer's instructions. The quantification of SLPI mRNA was performed using a LightCycler (Roche, Mannheim, Germany). Oligonucleotide primers were designed using LightCycler Probe Design software (Roche). The nucleotide sequences of the primers used and resultant PCR product sizes were: (1) human SLPI: forward: 5′-GGTTATCCCAAAATGATAGCAC-3′ and reverse: 5′-TTCTCTTCGTTTTAGGATCAAAT-3′ (132 base pair); and (2) glyceraldehyde-3-phosphate dehydrogenase (GAPDH): forward: 5′-CTTCCCCTCCATCGTGGG-3′ and reverse: 5′-GTGGTACGGCCAGAGGCG-3′ (225 base pair). The assay for each sample was performed in duplicate. In addition, the amplicons were checked by agarose gel electrophoresis for a single band of the expected size.

Relative quantification analysis was carried out with LightCycler software, Version 4 (Roche). The results were expressed as normalized ratios. Two ratios were compared: the ratio of SLPI gene to a reference gene, (GAPDH) in samples of myometrium, to the ratio of the same 2

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