OBSTETRICS

Uterine overdistention induces preterm labor mediated by inflammation: observations in pregnant women and nonhuman primates

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OBJECTIVE: Uterine overdistention is thought to induce preterm labor in women with twin and multiple pregnancies, but the pathophysiology remains unclear. We investigated for the first time the pathogenesis of preterm birth associated with rapid uterine distention in a pregnant nonhuman primate model.

STUDY DESIGN: A nonhuman primate model of uterine overdistention was created using preterm chronically catheterized pregnant pigtail macaques (*Macaca nemestrina*) by inflation of intraamniotic balloons (N = 6), which were compared to saline controls (N = 5). Cesarean delivery was performed due to preterm labor or at experimental end. Microarray, quantitative reverse transcriptase polymerase chain reaction, Luminex (Austin, TX), and enzyme-linked immunosorbent assay were used to measure messenger RNA (mRNA) and/or protein levels from monkey (amniotic fluid, myometrium, maternal plasma) and human (amniocytes, amnion, myometrium) tissues. Statistical analysis employed analysis of covariance and Wilcoxon rank sum. Biomechanical forces were calculated using the law of Laplace.

RESULTS: Preterm labor occurred in 3 of 6 animals after balloon inflation and correlated with greater balloon volume and uterine wall stress. Significant elevations of inflammatory cytokines and prostaglandins occurred following uterine overdistention in an "inflammatory pulse" that correlated with preterm labor (interleukin [IL]-1β, tumor necrosis factor [TNF]-α, IL-6, IL-8, CCL2, prostaglandin E2, prostaglandin F2α, all $P < .05$). A similar inflammatory response was observed in amniocytes in vitro following mechanical stretch (IL1β, IL6, and IL8 mRNA multiple time points, $P < .05$), in amnion of women with polyhydramnios (IL6 and TNF mRNA, $P < .05$) and in amnion (TNF-α) and myometrium of women with twins in early labor (IL6, IL8, CCL2, all $P < .05$). Genes differentially expressed in the nonhuman primate after balloon inflation and in women with polyhydramnios and twins are involved in tissue remodeling and muscle growth.

CONCLUSION: Uterine overdistention by inflation of an intraamniotic balloon is associated with an inflammatory pulse that precedes and correlates with preterm labor. Our results indicate that inflammation is an early event after a mechanical stress on the uterus and leads to preterm labor when the stress is sufficiently great. Further, we find evidence of uterine tissue remodeling and muscle growth as a common, perhaps compensatory, response to uterine distension.

Key words: amniocyte, amnion, chemokine (C-C motif) ligand 2, choriodecidua, cytokines, interleukin-1, interleukin-8, *Macaca nemestrina*, monocyte chemotactic protein 1, myometrium, pregnancy, preterm labor, prostaglandin E2, prostaglandin F2α, tumor necrosis factor, uterine stress, uterine stretch

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Preterm delivery represents a significant public health and economic burden to our society. Preterm birth (PTB) occurs in 11.4% of births and is associated with neonatal mortality and long-term morbidity.[1] Over the course of gestation, uterine myocytes normally undergo hypertrophy and hyperplasia to accommodate the relatively slow-growing fetus, placenta, and amniotic fluid.[2,3] However, women tend to deliver preterm if they carry twins (54% rate),[4] have polyhydramnios, or have uterine anomalies, such as a unicornuate uterus.[5-7] Hence, there appears to be a limit to the degree the uterus can increase in size, and we propose that one of the factors predisposing women to PTB is an inability of the uterus to adapt to a continued increase in volume. A lack of appropriate animal models and the complex nature of hormonal and inflammatory factors coordinating the onset of labor have made it difficult to investigate the pathogenesis of preterm labor associated with increased uterine volume, sometimes referred to as excessive stretch. Although the idea of uterine stretch is intuitive for many obstetricians to describe the force of increasing uterine volume on the myometrium, the correct biomechanical term is "stress" (force per unit area).[8] Therefore, we have avoided the term uterine "stretch" and instead refer to myometrial stress, overdistention, or increasing uterine volume.

The use of extraamniotic balloons in human beings to increase uterine wall stress is well known to trigger abortion, labor at term, or uterine contractions postpartum. Extraamniotic balloon inflation induces abortion in the late second trimester with nearly half of subjects aborting within 24 hours.[9] At term, labor is often rapidly initiated in healthy women with extraamniotic balloon inflation and the volume of inflation

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correlates with a more rapid delivery.[10] Postpartum, balloon inflation also induces regular uterine activity, which was suppressed by indomethacin, supporting a central role for prostaglandins in this pathway of myometrial activity.[11] Ethical considerations in human studies prevent serial sampling of myometrium or amniotic fluid in the same patient; therefore, human studies are generally cross-sectional in design. This limits the ability to establish temporal relationships among increases in uterine volume, production of inflammatory mediators, and the evolution of preterm labor. As a result, mechanisms of preterm labor in women with polyhydramnios and multiple gestation are understudied, despite the fact that multiple gestations have a significant risk of PTB.[12]

Both rat and marsupial models of increasing uterine volume have been described, providing a greater opportunity to discover the underlying mechanisms involved. In the rat model, animals first undergo unilateral tubal ligation to ensure that they only become pregnant in a single uterine horn.[13] After becoming pregnant, a second procedure places a tube into the nongravid uterine horn to simulate increasing uterine volume. In the tammar wallaby, only a single uterine horn becomes pregnant naturally and one may compare samples between the nongravid horn with the gravid horn undergoing gradual expansion of the uterine volume during the pregnancy.[14] These studies and experiments in cultured human myometrial cells identified important roles for CCL2 (chemokine [C-C motif] ligand 2, also known as monocyte chemotactic protein 1), oxytocin receptor, and connexin-43.[14-17] However, biological differences in parturition between lower mammalian models and human beings/nonhuman primates present difficulty in translating these results to human pregnancy. For example, in women and primates, progesterone levels do not decline in maternal or fetal blood just prior to labor onset, as they do in almost all other animal species.[18-20] Progesterone modulates the expression of many genes associated with labor onset making it difficult to translate results of other animal studies to human parturition. These limitations have been overcome in our nonhuman primate model, where we have previously described infection-induced hormonal and immunologic events culminating in PTB.[21]

Our objective was to identify early biological events occurring in the amniotic fluid and myometrium after an increase in uterine volume induced by inflation of an intraamniotic balloon in a nonhuman primate (Video). We hypothesized that inflation of an intraamniotic balloon would increase uterine wall stress and trigger preterm labor with a unique pattern of mediators in the amniotic fluid and myometrium. A unique gene activation pattern due to stretch in uterine smooth muscle has yet to be elucidated, but such patterns have been described in vitro using primary cultures or muscle strips from cardiomyocytes, bladder, and airway smooth muscle.[22-24] Further, we used human amniocytes that were stretched in vitro and amnion and myometrial samples from women with twins or polyhydramnios to validate and extend our results.

Materials and Methods

Animals and study groups

Eleven chronically catheterized pregnant pigtail macaques (*Macaca nemestrina*) at 118-125 days' gestation (term = 172 days) received 1 of 2 experimental treatments: choriodecidual and intraamniotic saline infusions (n = 5), or

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