OBSTETRICS

In an in vitro model using human fetal membranes, 17-alpha hydroxyprogesterone caproate is not an optimal progestogen for inhibition of fetal membrane weakening

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BACKGROUND: The progestogen 17-alpha hydroxyprogesterone caproate is 1 of only 2 agents recommended for clinical use in the prevention of spontaneous preterm delivery, and studies of its efficacy have been conflicting. We have developed an in vitro model to study the fetal membrane weakening process that leads to rupture in preterm premature rupture of the fetal membranes. Inflammation/infection associated with tumor necrosis factor-alpha induction and decidual bleeding/abruption associated thrombin release are leading causes of preterm premature rupture of the fetal membranes. Both agents (tumor necrosis factor-alpha and thrombin) cause fetal membrane weakening in the model system. Furthermore, granulocyte-macrophage colony-stimulating factor is a critical intermediate for both tumor necrosis factor-alpha and thrombin-induced fetal membrane weakening. In a previous report, we demonstrated that 3 progestogens, progesterone, 17-alpha hydroxyprogesterone caproate, and medroxyprogesterone acetate, each inhibit both tumor necrosis factor-alpha— and thrombin-induced fetal membrane weakening at 2 distinct points of the fetal membrane weakening pathway. Each block both the production of and the downstream action of the critical intermediate granulocyte-macrophage colony-stimulating factor.

OBJECTIVE: The objective of the study was to characterize the inhibitory effects of 17-alpha hydroxyprogesterone caproate on tumor necrosis factor-alpha— and thrombin-induced fetal membrane weakening in vitro.

STUDY DESIGN: Full-thickness human fetal membrane fragments from uncomplicated term repeat cesarean deliveries were mounted in 2.5 cm Transwell inserts and cultured with/without 17-alpha hydroxyprogesterone caproate (10^{-9} to 10^{-7} M). After 24 hours, medium (supernatant) was removed and replaced with/without the addition of tumor necrosis factor-alpha (20 ng/mL) or thrombin (10 U/mL) or granulocyte-macrophage colony-stimulating factor (200 ng/mL). After 48 hours of culture, medium from the maternal side compartment of the model was assayed for granulocyte-macrophage colony-stimulating factor and the fetal membrane fragments were rupture strength tested.

RESULTS: Tumor necrosis factor-alpha and thrombin both weakened fetal membranes (43% and 62%, respectively) and increased granulocyte-macrophage colony-stimulating factor levels (3.7- and 5.9-fold, respectively). Pretreatment with 17-alpha hydroxyprogesterone caproate inhibited both tumor necrosis factor-alpha— and thrombin-induced fetal membrane weakening and concomitantly inhibited the induced increase in granulocyte-macrophage colony-stimulating factor in a concentration-dependent manner. However, contrary to our prior reports regarding progesterone and other progestogens, 17-alpha hydroxyprogesterone caproate did not also inhibit granulocyte-macrophage colony-stimulating factor—induced fetal membrane weakening.

CONCLUSION: 17-Alpha hydroxyprogesterone caproate blocks tumor necrosis factor-alpha— and thrombin-induced fetal membrane weakening by inhibiting the production of granulocyte-macrophage colony-stimulating factor. However, 17-alpha hydroxyprogesterone caproate did not also inhibit granulocyte-macrophage colony-stimulating factor—induced weakening. We speculate that progestogens other than 17-alpha hydroxyprogesterone caproate may be more efficacious in preventing preterm premature rupture of the fetal membranes—related spontaneous preterm birth.

Key words: 17-alpha hydroxyprogesterone caproate, biomechanical weakening, fetal membranes, granulocyte-macrophage colony—stimulating factor, medroxyprogesterone acetate, preterm premature rupture of the fetal membranes, progesterone, progestogens, thrombin, tumor necrosis factor-alpha

Progestogens are the only agents recommended for the prevention of preterm delivery.[1-4] Currently used procedures include weekly intramuscular administration of 17-alpha hydroxyprogesterone caproate (17OHP-C) for the prevention of recurrent spontaneous preterm birth and daily vaginal administration of progesterone for short cervical length.[1-4] However, controversy exists regarding the efficacy of these progestogens in preventing spontaneous preterm births. In a recent large study, 17OHP-C was reported to be ineffective in preventing recurrent preterm birth and was also associated with an increased rate of gestational diabetes.[5]

Within weeks after this study was published, 17OHP-C was separately reported to delay recurrent preterm births and improve neonatal outcomes when administration was initiated between 14 and $17^{1/7}$ weeks of gestation.[6] Similarly, studies of the use of vaginal progesterone in women at risk for spontaneous preterm birth have also produced inconsistent results, although a recent meta-analysis suggested that vaginal progesterone decreased preterm birth in women with singleton pregnancy and a short cervix.[7-11]

It is uncertain whether either of the 2 recommended progestogens, progesterone or 17OHP-C, is superior in preventing recurrent spontaneous preterm

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birth in women at risk because there have been few studies comparing them directly.[12-14] A recent meta-analysis of 3 trials comparing the efficacy of vaginal progesterone vs intramuscular 17OHP-C suggested that vaginal progesterone may be similar to or better than 17OHP-C in preventing recurrent preterm births in at-risk women carrying singleton pregnancies.[15] However, the authors cautioned that the quality of summary estimates was low or very low, rendering their estimate of noted effect to be questionable.

Differences in clinical effectiveness might be anticipated because 17OHP-C (a synthetic progestogen) and progesterone (a natural progestogen) are different in their biological activities in the myometrium and uterine cervix.[16] Progesterone has been reported to decrease myometrial contractility, but 17OHP-C has no such effect in vitro or in vivo.[17-19] Progesterone prevents cervical ripening, but 17OHP-C has no such known effect.[20]

17OHP-C has been shown to prevent preterm birth in a uterine inflammatory mouse model, suggesting an effect at the maternal-fetal interface.[21] However, another study in mice demonstrated that 17OHP-C had no effect, but vaginal progesterone demonstrated antiinflammatory effects at the cervical and maternal-fetal interface for the prevention of endotoxin-induced preterm birth.[22] These agents have not been previously evaluated for their effect on fetal membranes.

Preterm premature rupture of the fetal membranes (pPROM) causes about one third of preventable premature births and thus is a major cause of infant morbidity and mortality.[23,24] Infection-inflammation—associated excessive inflammatory cytokines (interleukin [IL]-1β, IL-6, IL-8, tumor necrosis factor-alpha [TNF-α), granulocyte-macrophage colony-stimulating factor (GM-CSF)], and thrombin production in amniotic fluid and fetal membranes complicate most pPROM.[24-37]

Animal models for the study of the process of fetal membrane weakening and rupture are lacking, in part because of interspecies differences in placentation. Animal models of premature birth typically involve evaluation of premature uterine contractions and delivery in response to stimuli, rather than pPROM. Progress in understanding the physiology of pPROM has suffered because these animal models of spontaneous preterm birth fail to mimic the human condition. Also, because most cell or tissue culture studies are not done in the context of extracellular matrix changes associated with fetal membrane weakening and rupture, any correlation with potential membrane weakening or pPROM would be speculative at best.

We have developed an in vitro explant model system of human fetal membrane weakening (Figure 1) that permits the correlation of human fetal membrane rupture strength, the major parameter of clinical interest, with concomitant biochemical changes within the membrane.[38-42]

In this in vitro model, TNF-α (used as a marker for infection/inflammation) and thrombin (as a marker for decidual bleeding/abruption) both individually cause marked weakening of full-thickness fetal membranes in a concentration-dependent manner.[26,39,41,42] This induced weakening is concomitantly associated with biochemical and histological tissue changes in the fetal membrane explant that mimic those reported in the zone of high morphological change, also called the physiological weak zone (fetal membrane rupture initiation site), in the fetal membrane region overlying the cervix.[39,41-49]

Based on clinical and experimental data from nonhuman primates and from laboratory studies, it has been hypothesized that pPROM or preterm labor may result secondary to infection that originates vaginally in an ascending manner, transplacentally, or through direct iatrogenic seeding.[50-53]

There are some data suggesting that in the context of chorioamnionitis, the inflammatory chemokines may be initially generated in the amniotic fluid followed by recruitment of inflammatory cells into the choriodecidua.[50] Infection leads to inflammation and an associated generation of inflammatory cytokines and proteases in amniotic fluid and placental and fetal membranes tissue. Amnion is the main strength-bearing component of the fetal membranes and must be weakened before the fetal membranes can rupture causing pPROM.[54]

Using our model system investigating the pathways of TNF-α/IL-1β- and thrombin-induced fetal membrane weakening, we have demonstrated that the cytokines TNF-α/IL-1β are unable to weaken isolated amnion (without any adherent choriodecidua) but easily weaken the intact (amnion adherent to the choriodecidua) fetal membranes.[26] Thus, the initial tissue and cellular targets of these agents are located in the choriodecidua sublayer of the fetal membranes rather than the [F1] amnion.[26,55-57]

This is consistent with in vivo inflammation or bleeding mainly originating from the maternal side of the fetal membranes. With enhancements to the model system allowing the study of directional signaling in the fetal membrane-weakening pathways, we have validated that TNF-α or thrombin applied solely to the choriodecidua (maternal) side of fetal membrane explants causes weakening in the same manner as when both sides of the fetal membranes explant were exposed.[56,57]

Using this enhanced experimental system (Figure 1), GM-CSF has been identified as a critical intermediate in the fetal membrane-weakening pathways induced by both TNF-α and thrombin individually.[56] The following 3 criteria were used to confirm that GM-CSF is a critical intermediate in this process: (1) GM-CSF is induced in the choriodecidua by both TNF-α and thrombin in a concentration-dependent manner concomitant with fetal membrane weakening; (2) fetal membrane weakening by both TNF-α and thrombin is inhibited by preincubation with specific neutralizing antibody to GM-CSF[56]; and (3) GM-CSF alone also causes concentration-dependent fetal membrane weakening.[56]

Consistent with this finding, fetal membranes from pregnancies complicated with chorioamnionitis stain more densely for GM-CSF localized to decidual cells than those from

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