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Mifepristone-induced cervical ripening: Structural, biomechanical, and molecular events

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Objective: The purpose of this study was to assess the structural, biomechanical, and biochemical effects of mifepristone-induced progesterone withdrawal on the rat cervix to identify possible mechanisms by which mifepristone incites cervical ripening.

Study design: After the administration of mifepristone, cervical tensile strength was determined by the cervical creep method. With polarized light microscopy and transmission electron microscopy, collagen organization and microstructure were quantified. Matrix metalloproteinase expression was assessed by Western Blot and Real-time reverse transcriptase–polymerase chain reaction.

Results: Mifepristone induced a decrease in cervical tensile strength at mid gestation that was associated with a decrease in collagen organization. Additionally, mifepristone led to collagen fragmentation with a significant decrease in fibril length and diameter, although fibril bundling remained unaffected. Matrix metalloproteinase-2 expression increased after the administration of mifepristone.

Conclusion: Mifepristone-induced cervical ripening is associated with collagen degradation, and the collagenase activity of matrix metalloproteinase-2 may play a role in this process.

Preterm delivery continues to be one of the leading causes of neonatal morbidity and death, second only to congenital anomalies.[1] To date, the study of preterm labor has been largely "myocentric." Although uterine activity is the most common sign of labor, cervical change is the defining characteristic of both term and preterm labor. A significant part of the assessment of premature labor relates to cervical change, and it is clear that the cervix plays an important role in both pregnancy maintenance and labor initiation. Clinical evidence of cervical "ripening" or remodeling often precedes an increase in uterine activity, and a shortened cervical length is considered an important risk factor for preterm birth.[2]

Progesterone is a key hormone that is responsible for pregnancy maintenance,[3] and its withdrawal through

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either decreased production[4] or through changes in receptor expression[5] is thought to play a significant role in labor initiation. Progesterone supplementation has shown promise in decreasing the incidence of preterm delivery in singleton pregnancies at increased risk.[6] In contrast, a number of animal studies have demonstrated that progesterone withdrawal with a progesterone receptor antagonist results in cervical ripening with subsequent preterm delivery.[7-9]

Mifepristone is a progesterone receptor antagonist that is used as a cervical ripening agent in the first and early second trimester. The molecular mechanisms that are responsible for cervical remodeling are poorly understood, and mifepristone's effect on cervical microstructure has not been quantified previously.[10]

The remodeling of collagen is regulated by multiple enzyme pathways, including a family of enzymes known as the matrix metalloproteinases (MMPs).[11] A number of MMPs have collagenase activity, and it is believed that these extracellular proteases are a major regulator of cervical collagen degradation.[12] Although increased collagenase activity has been described in association with cervical ripening, it is still unclear which members of the MMP family are responsible.

Mifepristone was administered to pregnant rats in an attempt to identify possible mechanisms by which progesterone withdrawal induces cervical ripening. We hypothesized that the cervical ripening that is induced by progesterone withdrawal with mifepristone results in decreased cervical tensile strength and collagen fragmentation mediated by MMPs.

Material and methods

Mifepristone-induced cervical ripening

Timed pregnant Sprague-Dawley rats were purchased from Charles Rivers Laboratories (Montreal, Canada). Animals were delivered 1 week before use and handled daily until killed. The day after mating was considered day 1 of gestation in the presence of a vaginal plug, and animals typically delivered on the evening of day 22 or morning of day 23.

Agents were administered on either day 15 (mid gestation) or day 20 (late gestation). Treatment animals were given subcutaneous injections of mifepristone 3 mg suspended in 0.1 mL of sesame oil.[13] Control animals received only the sesame oil vehicle. Before initiating this study, all procedures were approved by the Institutional Animal Care and Utilization Committee at the University of Vermont.

Tissue harvesting

All cervical tissue was harvested under pentobarbital sodium anesthesia. Tissue was obtained 16 to 18 hours after the administration of either mifepristone or vehicle. The cervix was separated from the uterus below the bifurcation of the uterine horns; care was taken to exclude the more muscular portion above the mainly fibrous cervix.

Cervical tensile strength by cervical creep method

Cervical tensile strength was determined with the cervical creep method, as previously described.[14,15] Immediately after tissue harvest, a cylindric segment of cervix, 5 mm long, was suspended in a heated 37°C water bath that contained Dulbecco's modified Eagle's media (Mediatech, Herndon, VA) bubbled with 95% oxygen and 5% carbon dioxide. The cervical segment was suspended between 2 horizontal hooks; 1 hook was fixed to the base of the water bath, and the other hook was connected to a linear variable differential transformer (TransTek Inc, Ellington, CT). Under a constant 50-g load, the rate of cervical distension was then determined.

The analog signal was digitized by a CB-68LP connector block (National Instruments, Austin, TX) wired to a PCI-6024E Multifunction I/O board (National Instruments, Dell Inc, Round Rock, TX). Data were collected and stored on a Pentium personal computer and with Labview software (version 6.1; National Instruments). The cervical creep was calculated from the plotted slope of distance versus time. Cervical creep measurements were obtained on 23 animals (day 16, 9 mifepristone-treated animals and 6 control animals; day 21, 4 mifepristone-treated animals and 4 control animals).

Extracellular matrix collagen with polarized light microscopy

Freshly excised tissue from treatment and control animals (4 per group) was rinsed in normal saline solution, embedded in OCT (Sakura Finotek, Tokyo, Japan), frozen in liquid nitrogen–cooled isopentane, and stored at −70°C until sectioning. Tissue blocks were cryostat sectioned at a thickness of 8 microns onto microscope slides (Superfrost Plus; Fisher Scientific, Philadelphia, PA). Slides were stained with 0.1% Sirius red F3BA (color index 35780; Pfaltz and Bauer, Waterbury, CT) in saturated picric acid, as previously described.[15]

Collagen birefringence on examination with polarized light allows quantification of collagen fibril organization. Organized collagen (fibrils oriented in parallel) appears brighter than disorganized collagen (fragmented and nonparallel fibrils). Digital images were obtained with a digital camera (Olympus MagnaFire; Optronics, Goleta, CA) at ×40 magnification with a 1280 × 1024 pixel image capture. All slides were prescreened to determine maximal birefringence by adjustment of the analyzer rotation, light intensity, and exposure time.