BASIC SCIENCE: OBSTETRICS

Progesterone protects fetal chorion and maternal decidua cells from calcium-induced death

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OBJECTIVE: The purpose of this study was to determine whether progesterone exerts a protective effect in chorion and decidua cells when exposed to calcimycin.

STUDY DESIGN: Fetal membrane samples were collected from term elective repeat cesarean deliveries and chorion and decidua cells that are separated and cultured. Cells were pretreated with progesterone and exposed to calcimycin. Cell viability was determined, and percent cell viability was calculated.

RESULTS: Exposure to calcimycin resulted in a reduction of cell viability in both chorion and decidua cells in a dose-dependent fashion. In chorion and decidua cells, progesterone pretreatment followed by calcimycin increased cell viability compared with calcimycin treatment alone (chorion, 67%, vs controls, 24%; $P < .001$; decidua, 58%, vs controls, 35%; $P < .001$). The progesterone receptor antagonist, RTI 6413-49a, blocked the protective effect of progesterone in both chorion and decidua cells.

CONCLUSION: These preliminary results suggest that progesterone may provide a protective effect in fetal membrane cells and that this effect may be mediated through the progesterone receptor.

Key words: progesterone, calcimycin, cytotoxicity, fetal membrane, membrane progesterone receptor

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Rupture of fetal membranes before 37 weeks of gestation without evidence of labor, termed PPROM, occurs in approximately 2%-3% of all pregnancies but is associated with 20% of all perinatal deaths. We have demonstrated previously, in patients at term and patients with PPROM, accelerated apoptosis in the chorion cells of fetal membranes in the presence of chorioamnionitis. Using immunohistochemistry, we were able to demonstrate that subjects with histologic chorioamnionitis exhibited twice as many apoptotic nuclei in the chorion cell layer as did subjects without chorioamnionitis.[1] When we studied subjects with PPROM separately, we confirmed our previous findings and additionally found that nearly 37% of subjects with PPROM completely lacked the chorion layer of fetal membranes. The cause of the cellular mechanisms that are involved in the destruction of the membranes is not understood. It is known that exposure to lipopolysaccharide and ischemia induces cell death and that elevated intracellular calcium concentrations are an important step in the process at the cellular level.[2]

Progesterone governs a wide range of biologic processes that are related to the maintenance of pregnancy, which includes an antiapoptotic action at the cellular level in various cell types and tissues.[2,3] Functions that are attributed to progesterone in pregnancy include the stimulation of growth and differentiation of the endometrium to allow for implantation, the inhibition of myometrial contractions, and the induction of immune tolerance to the fetus.[4,5]

Progesterone also has been the focus of intense clinical research in the area of preterm birth prevention.[6,7] Although continued evidence supports the use of progesterone for the prevention of recurrent preterm birth, little is understood about the exact mechanisms by which progesterone functions in these patients. The classic physiologic effects of progesterone are mediated by the interaction of the hormone with specific intracellular progesterone receptors that are members of the nuclear receptor family of transcription factors.[8-10] The nuclear progesterone receptors consist of 2 protein isoforms that are termed A and B that are expressed from a single gene in rodents and humans.[11,12] Preliminary data from our laboratory demonstrated the expression of progesterone receptor A and B messenger RNA and protein in human fetal membranes and determined that these levels do not change in cell culture.[13]

Recently published studies indicate that progesterone may also act through a nonclassic mechanism that involves G-protein– and/or protein kinase G–coupled signaling.[14,15] The relationship of the various possible progesterone signaling mechanisms to the initiation of cell death is unclear.

As previously noted, we have demonstrated that apoptosis is common in the

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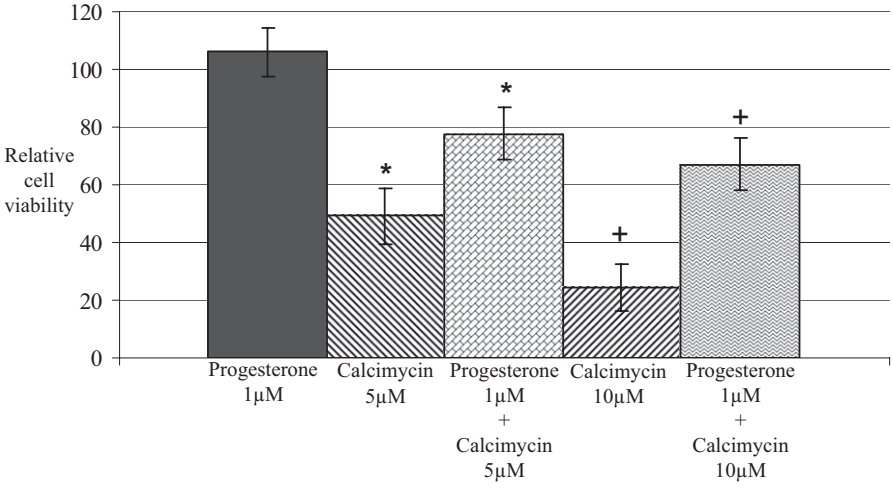
chorion layer of fetal membranes in the presence of chorioamnionitis and that this specific cell layer is destroyed in patients with PPROM.[1] Based on preliminary evidence, we believe that cell death in the fetal membranes is an important contributor to the risk for preterm delivery, specifically PPROM. We hypothesize that altered calcium homeostasis may contribute to cell death in the chorion and decidua cells of fetal membranes. Further, we hypothesize that progesterone offers a protective effect from calcium-mediated premature cell death in fetal membranes. Our objective was to determine whether progesterone protects cultured chorion and decidua cells from calcium-induced cell death.

FIGURE 1
Calcimycin vs progesterone + calcimycin in fetal chorion cells



Cells were pretreated for 24 hours with 1.0 μmol/L progesterone in DMEM and then exposed to calcimycin. Experimental conditions were repeated, and cell viability was assessed after an additional 48 hours in culture. Progesterone exposure was 1.0 μmol/L for all experimental arms that are shown. Data are shown relative to cell viability of untreated controls. Data represent mean ± SE. The *asterisk* indicates a probability value of .07 for calcimycin at 5.0 μmol/L; the *plus symbol* indicates a *P* value of <.003 for calcimycin at 10.0 μmol/L.

MATERIALS AND METHODS

Placentas were collected from women who underwent planned cesarean delivery at term, before labor, and without rupture of membranes (n = 5). Institutional review board approval was obtained for a waiver of consent to obtain de-identified tissue that was not to be used for clinical purposes. Tissue was transported to the laboratory in Dulbecco's modified Eagle medium (DMEM)–Hams F12 media. Fetal membrane tissue was cut into 2 × 2–inch squares with forceps and scalpel. The smooth layer of amnion was pulled off manually. Separation of the decidua and chorion involved blunt dissection with forceps and scalpel. Each layer was minced by cross-cutting with scalpel blades. Tissues were processed in digestion buffer I (0.125% trypsin and 0.02% DNase I) at 37°C for 30 minutes, followed by centrifugation at 2000 rpm for 10 minutes. The cell pellet was resuspended in digestion buffer II (0.125% trypsin, 0.02% DNase, 0.2% collagenase [Sigma Aldrich, St. Louis, MO]), and incubated for 60 minutes at 37°C. Cells were filtered through 4 layers of sterile gauze and centrifuged at 2000 rpm for 10 minutes.[16] A cell-separation gradient was prepared with an Optiprep column (Sigma Aldrich), with steps that ranged from 4% to 40% of 4 mL each (4%, 6%, 8%, 10%, 20%, 30%, and 40%). Processed chorion or decidua cells were added to the top of the gradient and then centrifuged (1000*g*) at room temperature for 30 minutes. Cells between densities of 1.049 and 1.062 g/mL represented the chorion layer. Cells between densities of 1.027-1.038 g/mL represented the decidual layer. Harvested cells were washed with DMEM, centrifuged, and resuspended in DMEM. Cell viability routinely was >90%, which was verified by trypan blue staining; cells types were plated at a density of 10^6 cells/mL in DMEM/Hams F-12/10% serum plus antibiotic/antimycotics (penicillin G 100 U/mL, streptomycin sulfate 100 mg/mL, amphotericin B 1.0 mg/mL) and cultured for 24 hours. Immunohistochemistry was performed to confirm the purity of cell culture, as previously described.[13]

The cells were analyzed with the CellTiter 96 AQueous Non-Radioactive Cell Proliferation Assay (Promega, Madison, WI) to assess cell viability. The assay is composed of solutions of a novel tetrazolium compound (3-[4,5-dimethylthiazol-2-yl]-5-[3-carboxymethoxyphenyl]-2-[4-sulfophenyl]-2H-tetrazolium, inner salt; MTS) and an electron coupling reagent (phenazine methosulfate). MTS is bioreduced by cells into a formazan product that is soluble in tissue culture medium. The absorbance of the formazan at 490 nm can be measured directly from 96-well assay plates without additional processing. The conversion of MTS into aqueous, soluble formazan is accomplished by dehydrogenase enzymes that are found in metabolically active cells. The quantity of formazan product, as measured by the amount of 490 nm absorbance, is directly proportional to the number of living cells in culture, which is determined every 30 minutes to a total of 4 hours by a spectrophotometric plate reader (Spectra MAX 190; Molecular Devices, Sunnyvale, CA) at 37°C. All samples, including blank wells that contained media only (no cells), were run in quadruplicate The quadruplicate data points from each experimental condition were pooled to create an average after the background reading from blank wells was subtracted. The percent of viable cells relative to untreated cells was determined by dividing the average optical density for each experimental condition by the average optical density for the untreated controls.

After 24 hours, cultured cells were pretreated with progesterone for 24 hours