OBSTETRICS

Progesterone blunts vascular endothelial cell secretion of endothelin-1 in response to placental ischemia

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OBJECTIVE: Preeclampsia (PE) is associated with hypertension and elevated endothelin (ET-1), an indicator of endothelial cell activation and dysfunction. Reduction of uteroplacental perfusion (RUPP) in the pregnant rat model of PE is characterized by elevated mean arterial pressure, inflammatory cytokines, and activation of the ET-1 system. We aim to determine whether 17-alpha-hydroxyprogesterone caproate (17-OHPC) or progesterone suppresses these pathways.

STUDY DESIGN: Plasma progesterone was purified from normal pregnant (NP) and PE patients and measured via enzyme-linked immunosorbent assay. Human umbilical vein endothelial cells were exposed to the sera with or without progesterone added and ET-1 was measured. Pregnant rats underwent the RUPP procedure with or without intraperitoneal 17-OHPC. Mean arterial pressure was compared in RUPP vs NP rats. Human umbilical vein endothelial cells were exposed to NP or RUPP sera, with and without progesterone and ET-1 measured.

RESULTS: Progesterone was significantly decreased in PE women compared with NP women. In response to human sera, ET-1 was elevated in PE women compared to NP women, and decreased with addition of progesterone. Mean arterial pressure was significantly elevated in RUPP vs NP rats but was attenuated by 17-OHPC. ET-1 secretion was stimulated significantly by RUPP compared to NP rat sera, but attenuated by progesterone.

CONCLUSION: Circulating progesterone is significantly lower in PE women compared to controls. 17-OHPC attenuates hypertension in response to placental ischemia in RUPP rats. Progesterone blunts vascular ET-1 stimulated at cellular level by sera from PE women or RUPP rats. Decreased circulating progesterone is associated with stimulation of ET-1. 17-OHPC supplementation blunts hypertension and progesterone blunts endothelial cell ET-1 secretion in response to placental ischemia.

Key words: endothelin, placental ischemia, preeclampsia, progesterone

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Preeclampsia (PE), defined as new-onset hypertension with proteinuria after the 20th week of gestation, affects 4-6% of all pregnancies in the United States.[1,2] Vascular complications from PE are a leading cause of maternal morbidity and death, and increase the need for premature delivery with resultant neonatal morbidity and mortality. PE is a multisystem syndromic disorder with origins thought to be subsequent to the shallow trophoblastic invasion of the uterine spiral arteries. Thus, a reduction of uteroplacental perfusion (RUPP) results in persistent placental ischemia.[3,4]

This diffuse dysfunction of the maternal vascular endothelium is associated with the release of inflammatory cytokines such as tumor necrosis factor (TNF)-alpha, interleukin (IL)-1, and IL-6, also known as "the early insult." These inflammatory cytokines have been shown to be elevated approximately 2-fold in women with PE when compared with normal pregnant (NP) controls.[5] Furthermore, the release of TNF-alpha has been shown to mediate endothelial dysfunction characterized by activation of the endothelin (ET-1) system, with blood pressure increases during pregnancy being mediated through the activation of ET-1 type A (ET_A) receptors.[6-8] Plasma concentrations of ET-1 are increased approximately 2- to 3-fold in patients with PE when compared with NP controls especially late in the disease process, hence a role in disease progression instead of initiation—"the late insult."[5,6]

An agent that has been used effectively for the prevention of recurrent preterm birth in singleton pregnancies is 17-alpha-hydroxyprogesterone caproate (17-OHPC).[9-12] The mechanism of action pathway is thought to be based on its antiinflammatory properties, with some studies showing inhibition of basal and TNF-alpha-induced apoptosis in fetal membranes.[13] A role for progesterone or 17-OHPC in the prevention or treatment of PE has been debated but never clarified. A recent Cochrane Collaboration review demonstrated that there is insufficient evidence for reliable conclusions about the effects of progesterone for preventing PE and its complications.[14] Alternatively, a review by Sammour et al[15] concluded that

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progesterone appears to be effective for the treatment of PE. We hypothesize that decreased endogenous progesterone could serve as a stimulus for elevated inflammatory cytokines as a mechanism to chronically activate endothelial cells to secrete ET-1 in PE women compared to NP women.

The RUPP rat model of PE induces a state of chronic placental ischemia that is associated with inflammatory cytokines and activation of the ET-1 system.[16] As shown in prior studies, RUPP-induced hypertension is associated with increases in circulating TNF-alpha, IL-6, and agonistic autoantibodies to the angiotensin II type 1 receptor, as well as decreases in endothelial-dependent relaxation factors. The overexpression of these inflammatory cytokines also occurs in placental explants from PE women.[17] Previously our laboratory has demonstrated that hypertension in response to TNF-alpha excess in pregnant rats is associated with increased ET-1 that is mediated through activation of ET_A.[6-8] Blockade of ET_A receptors in rats with placental ischemia abolishes hypertension in response to RUPP.[7] Furthermore, we showed that 17-OHPC blunts hypertension and inflammatory cytokines, TNF-alpha and IL-6, associated with RUPP. Administration of 17-OHPC blunted renal ET-1 but had no effect on placental ET-1.[18] In addition, administration of 17-OHPC attenuated TNF-alpha-induced hypertension and decreased renal ET-1, without affecting ET-1 in the placenta.[19] In the present study we first sought to determine differences in endogenous progesterone among NP and PE women. We next wanted to determine if progesterone supplementation in vitro would affect endothelial cell activation as measured by stimulated ET-1. This set of studies was designed to further investigate beneficial effects of progesterone or 17-OHPC supplementation to suppress endothelial cell activation that occurs in response to chronic placental ischemia in PE.

Materials and Methods

All studies were performed in timed pregnant Sprague-Dawley rats purchased from Harlan (Indianapolis, IN). Animals were housed in a temperature-controlled room (23°C) with a 12-/12-hour light/dark cycle. All of the experimental procedures executed in this study were in accordance with National Institutes of Health guidelines for use and care of animals, and the Institutional Animal Care and Use Committee at the University of Mississippi Medical Center approved all protocols.

The human serum protocol was submitted and approved by the Committee on Human Investigation at the University of Mississippi Medical Center. Obstetric patients were admitted to Winfred Wiser Hospital for Women and Infants (Jackson, MS) with evidence of mild or severe PE, either primary disease or secondary to underlying essential hypertension. Patients were consented to have blood obtained for PE markers under investigation. The patients underwent impedance cardiography (BioZ, Cardiodynamics Sonosite; Cardiodynamics International, San Diego, CA), followed immediately by collection of blood via venipuncture in collection tubes for serum and plasma and then patients were prepared for delivery. The sample was immediately centrifuged for 10 minutes and the serum stored at −20°C.

NP women had blood drawn immediately before scheduled cesarean section. Circulating progesterone was isolated from plasma collected from NP and PE patients. Briefly, 100 μL of plasma was pipetted into a glass tube and 1 mL of petroleum ether was added, vortexed for 30 seconds, and then allowed to separate into phases. The organic phase was transferred into a clean glass tube and the solvent evaporated with a stream of N_2. The residue was dissolved in 500 μL of diluted extraction buffer, vortexed, and assayed in 1:4 dilution with appropriate extraction buffer supplied by the manufacturer and measured in duplicate via enzyme-linked immunosorbent assay (ELISA) (Oxford Biomedical Research, Rochester Hills, MI).

Effect of progesterone on arterial pressure in RUPP rat model (protocol I)

Experiments were performed in the following groups of rats: NP (n = 9) and RUPP pregnant rats (n = 12). All of the pregnant rats undergoing surgical procedures were anesthetized with 2% isoflurane (W.A. Butler Co., Middletown, PA) delivered by an anesthesia apparatus (vaporizer for isoflorane anesthetic; Ohio Medical Products, Gurnee, IL). The 17-OHPC (Marty's Compounding Pharmacy, Jackson, MS) was diluted in normal saline and administered intraperitoneally as 0.5 cm^3 solution of 3.32 mg/kg 17-OHPC to pregnant rats. We chose the 1-time 17-OHPC dose to be the weight equivalent of a typical human dose for the prevention of preterm labor. The rats were anesthetized on day 14 of pregnancy and underwent one of the following: (1) examination under anesthesia; (2) RUPP (the lower abdominal aorta was isolated and clipped [0.203 mm in diameter] above the iliac bifurcation—branches of both the right and left ovarian arteries were clipped [0.100 mm in diameter]); (3) injection of intraperitoneal 17-OHPC; or (4) RUPP + injection of intraperitoneal 17-OHPC. On day 18, carotid artery catheters were placed in each rat; on day 19 mean arterial pressure, maternal weight, and pup weight were measured and serum and plasma were collected. Study groups were: NP, NP + 17-OHPC, RUPP, and RUPP + 17-OHPC.

Effect of progesterone on ET-1 secretion from human umbilical vein endothelial cell

In vitro experimental protocols

To determine the properties of progesterone on ET-1 synthesis, we directly examined the effect of progesterone (Sigma Aldrich, St. Louis, MO) after the human umbilical vein endothelial cell (HUVEC) culture was initially exposed to serum media collected from women diagnosed with PE and NP controls (protocol II), and to serum media from NP and RUPP rats (protocol III).

Protocol II, sera of women with PE vs NP. The experimental media contained 50%/50% culture media with and without 1 μmol/L progesterone/10% human serum. Study groups (n = 11 each)