17-hydroxyprogesterone blunts the hypertensive response associated with reductions in uterine perfusion pressure in pregnant rats

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OBJECTIVE: Reduction in uteroplacental perfusion (RUPP) in pregnant rats is associated with hypertension, elevated cytokines, and activation of the endothelin (ET-1) system. Our objective was to determine whether the antiinflammatory properties of 17-alpha-hydroxyprogesterone caproate (17 OHP) reduce cytokine-stimulated vasoactive pathways that are associated with hypertension in response to placental ischemia.

STUDY DESIGN: Mean arterial pressure (MAP), tumor necrosis factor-α (TNF-α), interleukin (IL)-6, and renal ET-1 were measured in the following: pregnant controls, pregnant controls plus 17 OHP (6.6 mg/kg), RUPP rats, and RUPP rats plus 17 OHP.

RESULTS: MAP increased 29 mm Hg in RUPP rats compared with pregnant controls ($P < .001$), whereas in RUPP plus 17 OHP rats, MAP increased only 19 mm Hg ($P < .05$). TNF-α and IL-6 increased 2- to 3-fold, respectively, in response to placental ischemia but was normalized in RUPP rats treated with 17 OHP. ET-1 increased 3-fold in RUPP rats but was markedly less in RUPP plus 17 OHP rats.

CONCLUSION: 17 OHP blunts hypertension associated with RUPP, possibly via suppression of cytokine-stimulated ET-1 activation.

Key words: endothelin, 17-hydroxyprogesterone caproate, hypertension, preeclampsia, reduced uterine perfusion

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Preeclampsia/eclampsia remains a leading cause of maternal and neonatal morbidity and mortality. Vascular complications from preeclampsia are a leading cause of maternal morbidity and death and increase the need for premature delivery. Although great strides have been made in elucidating the etiology of preeclampsia, a complete understanding of the mechanisms responsible for disease pathogenesis remains elusive. Correlating these findings with modes of early identification and prevention represents a foremost challenge for contemporary obstetric research.

Over the past 30 years, there have been few substantial changes in the treatment of preeclamptic women. The current treatment includes blood pressure control, seizure prophylaxis, and delivery of the fetus, depending on gestational age and disease severity. There have been a host of agents touted as potential therapies for the prevention or treatment of preeclampsia; however, none has proven effective.

The focus of our study was to test a potential use for progesterone, or, specifically, 17α-hydroxyprogesterone caproate (17 OHP), for the treatment of hypertension in response to placental ischemia. Whereas recent studies have demonstrated that 17 OHP aids in the prevention of recurrent preterm birth, literature on any role for this agent in patients with preeclampsia is sparse and conflicting.[1-3] Salas et al[4] suggested that an early rise in maternally derived progesterone might have a pathogenic role in the development of preeclampsia.

In contrast, a review article by Sammour et al[5] suggested that progesterone is a viable therapeutic agent for the treatment of preeclampsia. He cited Kristiansson and Wang,[6] who demonstrated that high progesterone in early pregnancy was associated with lower maternal blood pressures. Ragab et al[7] performed the only known randomized controlled trial in humans and demonstrated that parenteral administration of 200 mg of progesterone significantly reduced blood pressure, improved urine output, and reduced uric acid levels in more than 80% of the women with preeclampsia. However, in this study no mechanism for such vast improvement during preeclampsia was investigated.

Placental ischemia appears to play a pivotal role in the pathogenesis of preeclampsia. This is clinically demonstrated by those medical conditions that predispose to preeclampsia, such as chronic hypertension, diabetes mellitus, lupus, and thrombophilia, which involve some form of chronic vascular insufficiency.[8] In recent years, animal models have been developed that induce a preeclampsia-like state in various pregnant species, thereby facilitating research into potential mechanisms contributing to the observed characteristics of preeclampsia.[9-12]

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The reduced uterine perfusion pressure (RUPP) model, as demonstrated by Granger et al,[13] induces a state of chronic ischemia/hypoxia in the placenta of the pregnant rat. Chronic reductions in uterine perfusion pressure increase arterial pressure and impair endothelial function.[13,14] RUPP-induced hypertension is associated with increases in circulating tumor necrosis factor (TNF)-α, interleukin (IL)-6, and agonistic autoantibodies to the angiotensin II type 1 receptor, as well as decreases in endothelial dependent relaxation factors.

Whereas the exact mechanism whereby 17 OHP may improve vascular function and lower blood pressure is unclear, it is believed that its antiinflammatory properties are responsible for the reduction in subsequent preterm delivery in appropriately treated women. Studies in humans elucidating the mechanism of action of 17 OHP cite its potential role as an inhibitor of prostaglandins and inflammatory cytokines, such as TNF-α.[15,16]

One of the well-known modes of action of TNF-α during preeclampsia is mediating endothelial dysfunction characterized by activation of the endothelin (ET-1) system. Our laboratory has previously demonstrated that hypertension in response to TNF-α excess in pregnant rats is associated with increased ET-1 and is mediated through activation of endothelin type A (ET_A) receptors.[17] Furthermore, we have shown that blockade of ET_A receptors in rats with RUPP-induced placental ischemia abolishes the hypertensive response.[18] Therefore, we examined the potential mechanism of 17 OHP as an antiinflammatory agent to decrease ET-1 production in the renal cortices of RUPP rats. A prospective controlled animal study was designed to measure mean arterial pressure (MAP), fetal/placental outcome, inflammatory cytokines, and ET-1 in pregnant RUPP rats following 17 OHP administration.

Materials and Methods

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

Experimental design

Experiments were performed in the following 4 groups of rats: pregnant controls (n = 9), pregnant controls plus 17 OHP (n = 4), RUPP pregnant rats (n = 10), and RUPP pregnant rats plus 17 OHP (n = 5). On day 14 of gestation, all rats were anesthetized with 2% isoflurane (W. A. Butler Co., Dublin, OH) delivered by an anesthesia apparatus (Vaporizer for Forane Anesthetic; Ohmeda, West Yorkshire, England). The pregnant controls underwent an abdominal examination under anesthesia to confirm pregnancy. Pregnant rats entering the RUPP group underwent the reduced uterine perfusion pressure partial vessel occlusion procedure on day 14.[14] After a vertical midline incision, the lower abdominal aorta was isolated and a silver clip (0.230-mm inner diameter) was placed around the aorta above the iliac bifurcation. Branches of both the right and left ovarian arteries were also constricted with silver clips (0.100-mm inner diameter), as previously described.

The 17 OHP (Marty's Compounding Pharmacy, Flowood, MS) was diluted in normal saline and administered intraperitoneally as a 0.5-mL solution. We chose the 1-time 17 OHP dose to be the weight equivalent of a typical human dose for the prevention of recurrent preterm labor (250 mg). Our pilot study demonstrated only a mild decrease in MAP with administration of 3.32 mg/kg 17 OHP; therefore, we administered an increased dose of 6.6 mg/kg 17 OHP, which is the dose represented in the results of this study.

The pregnant control rats receiving 17 OHP were anesthetized on day 14 and underwent a vertical laparotomy. 17 OHP was then delivered via sterile syringe through the incision into the peritoneal cavity. In the RUPP plus 17 OHP group, the 17 OHP was administered in a similar fashion immediately following the RUPP surgical procedure and prior to closure of the laparotomy. On day 18 all rats were surgically instrumented with a carotid catheter for subsequent arterial pressure measurement. On day 19 of gestation, arterial pressure was recorded, pups and placentas were counted and weighed, and blood samples and kidneys were collected for molecular analysis.

Determination of serum IL-6 levels

Colorimetric enzyme-linked immunosorbent assay (ELISA) (R&D Systems, Minneapolis, MN) was used for quantification of serum IL-6 levels. Inter- and intraassay precision was 10.0% and 8.8%, respectively. This assay displayed a sensitivity level of less than 10 pg/mL.

Determination of serum TNF-α levels

A rat TNF-α colorimetric sandwich ELISA (R&D Systems) was used for quantification of serum TNF-α levels between 12.5-800 pg/mL. This assay displayed a sensitivity level of 5 pg/mL, with inter- and intraassay variability of 10% and 5.1%, respectively.

Determination of plasma progesterone levels

Plasma progesterone was extracted following instructions outlined by the manufacturer and determined using an ELISA provided by Oxford Biomedical Research (Oxford, MI). This test kit operates on the basis of competition between the enzyme conjugate and the progesterone for a limited number of binding sites on the antibody coated plate. The assay is used for quantification of progesterone levels between 0.1-100 ng/mL.

RNA isolation and analysis of tissue endothelin

Placentas and renal cortices were collected immediately after harvesting and quickly frozen in liquid nitrogen and stored at −80°C. Total ribonucleic acid (RNA) was extracted using the RNeasy kit (Qiagen, Valencia, CA) after the tissue was crushed in liquid nitrogen with a mortar and pestle. The isolation procedure was performed as