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17 OH Progesterone Blunts the Hypertensive Response Associated with Reductions in Uterine Perfusion Pressure in Pregnant Rats

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Abstract

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 if the anti-inflammatory properties of 17-alpha hydroxyprogesterone caproate (17OHP) reduces cytokine stimulated vasoactive pathways that are associated with hypertension in response to placental ischemia.

Study Design—MAP, TNF- α , IL-6, and renal ET-1 were measured in the following: pregnant controls, pregnant controls + 17OHP (6.6mg/kg), RUPP rats, RUPP rats + 17OHP.

Results—MAP increased 29mmHg in RUPP rats compared to pregnant controls ($P<0.001$); while in RUPP+17OHP rats MAP increased only 19mmHg ($P<0.05$). TNF- α and IL-6 increased 2-3 fold, respectively, in response to placental ischemia but was normalized in RUPPs treated with 17 OHP. ET-1 increased 3 fold in RUPP rats but was markedly less in RUPP+17 OHP rats.

Conclusion—17OHP blunts hypertension associated with RUPP possibly via suppression of cytokine stimulated ET-1 activation.

Keywords

17 hydroxyprogesterone caproate; endothelin; hypertension; preeclampsia; reduced uterine perfusion

Introduction

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. While 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

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Effects of 17 OHP on mean arterial pressure, inflammatory cytokines and tissue endothelin in a pregnant rat model of placental ischemia.

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 have proven effective. The focus of our study was to test a potential use for progesterone, or specifically 17 α -hydroxyprogesterone caproate (17 OHP), for the treatment of hypertension in response to placental ischemia. While recent studies have demonstrated 17 OHP to aid 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. (2006) suggested that an early rise in maternally derived progesterone might have a pathogenic role in the development of preeclampsia.⁴ In contrast, a review article by Sammour et al. (2005) suggests that progesterone is a viable therapeutic agent for the treatment of preeclampsia.⁵ He cited Kristiansson et al. (2001) who demonstrated that high progesterone in early pregnancy was associated with lower maternal blood pressures.⁶ Ragab et al. (1971) performed the only known randomized controlled trial in humans and demonstrated that parenteral administration of 200mg of progesterone significantly reduced blood pressure, improved urine output, and reduced uric acid levels in over 80% of the preeclamptic women.⁷ 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 The reduced uterine perfusion pressure (RUPP) model, as demonstrated by Granger et al. (2006), induces a state of chronic ischemia/hypoxia in the placenta of the pregnant rat.¹³ Chronic reductions in uterine perfusion pressure increases arterial pressure and impairs endothelial function.^{13,14} RUPP induced hypertension is associated with increases in circulating TNF- α , IL-6, and agonistic autoantibodies to the angiotensin II type 1 receptor, as well as decreases in endothelial dependent relaxation factors.

While the exact mechanism whereby 17 OHP may improve vascular function and lower blood pressure is unclear, it is believed that its anti-inflammatory 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 anti-inflammatory 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 12:12-hour light/dark cycle. All experimental procedures executed in this study were in accordance with the National Institute of Health guidelines for use and care of animals. All protocols were approved by the Institutional Animal Care and Use Committee (IACUC) 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 + 17 OHP (n=4), RUPP pregnant rats (n=10), and RUPP pregnant rats + 17 OHP (n=5). On day 14 of gestation all rats were anesthetized with 2% isoflurane (W. A. Butler Co) 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 ID) was placed around the aorta above the iliac bifurcation. Branches of both the right and left ovarian arteries were also constricted with silver clips (0.100mm ID) as previously described.

The 17 OHP (Marty's Compounding Pharmacy, Flowood, MS) was diluted in normal saline and administered intraperitoneally as a 0.5cc solution. We chose the one time 17 OHP dose to be the weight equivalent of a typical human dose for the prevention of recurrent preterm labor (250mg). Our pilot study demonstrated only a mild decrease in MAP with administration of 3.32mg/kg 17 OHP, therefore we administered an increased dose of 6.6mg/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 + 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 ELISA (R&D Systems, Minneapolis, MN) was used for quantification of serum IL-6 levels. Inter- and intra-assay 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 Enzyme-linked Immunosorbent Assay (ELISA) (R&D Systems, Minneapolis, MN) was used for quantification of serum TNF- α levels between 12.5-800pg/ml. This assay displayed a sensitivity level of 5 pg/ml with inter- and intra-assay 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 and 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 RNA was extracted using the RNeasy kit (Quiagen, Valencia, CA) after the tissue was crushed in liquid nitrogen with a mortar and pestle. The isolation procedure was performed as outlined in the instructions provided by the manufacturer.

Genomic DNA was digested with DNaseI following instructions outlined by Ambion (Austin, TX). RNA was quantified spectrophotometrically using an Eppendorf BioPhotometer. cDNA was synthesized from 1 microgram of RNA with BioRad Iscript cDNA reverse transcriptase. Real time PCR was performed following procedures outlined previously using the BioRad Syber Green supermix and iCycler.^{17,19} Levels of mRNA expression were calculated using the mathematical formula for delta/delta CT recommended by Applied Biosystems²⁰.

Statistical Analysis

All data are expressed as mean $\pm$ standard error of the mean. Difference between control and experimental groups were analyzed using ANOVA with Tukey-Kramer multiple comparison tests. Data were considered statistically different at p values < 0.05 . Statistical analysis of real time PCR results was performed using the mean normalized cycle threshold (delta/delta CT) values and standard deviations analyzed by One-Way ANOVA.

Results

Administration of 17 OHP blunts the hypertension in response to placental ischemia

To determine whether 17 OHP attenuates the blood pressure increase in response to placental ischemia we analyzed MAP during pregnancy in the presence and absence of 17 OHP. MAP was 129 ± 2 mmHg in control RUPP rats but was significantly less in RUPP + 17 OHP rats (119 ± 2 mmHg, $P < 0.05$). Administration of 17 OHP had no effect on MAP in normal pregnant rats (100 ± 2 mmHg, control vs 99 ± 2 mmHg, 17 OHP) (Figure 1).

There was no significant difference in pup or placenta weights between the normal pregnant controls and the normal pregnant rats that received 17 OHP. The decrease in pup weight in response to placental ischemia was unchanged in RUPP + 17 OHP compared to RUPP controls (Figure 2).

The effect of 17 OHP on circulating cytokines

Consistent with our previous research, circulating TNF- α and IL-6 levels are elevated approximately 2-3 times above normal in response to placental ischemia in pregnant rats.²¹ However, administration of 17 OHP to RUPP rats markedly decreased the levels of these two cytokines, although the difference did not reach statistical significance (Figure 3).

Plasma progesterone in response to 17 OHP administration

Consistent with previous observations from our laboratory, plasma progesterone levels in RUPP rats was not statistically different compared to normal pregnant rats. However, administration of 17 OHP to RUPP rats increased circulating progesterone, albeit not significantly (Figure 4).

The effect of 17 OHP on ET-1 expression

Preproendothelin (ppET-1) mRNA levels increased 4 fold in the renal cortex from RUPP rats compared to normal pregnant rats ($P < 0.02$). Administration of 17 OHP markedly decreased ET-1 transcription in the renal cortex of RUPP rats (Figure 5) and could be a mechanism for the blunted hypertension in this group.

As with previous studies, placental ischemia is associated with significant increases in placental ET-1 in RUPP rats (Figure 5).^{17,19} Interestingly, administration of 17 OHP had no effect on placental ET-1 in RUPP rats, indicating the importance of additional stimuli within the ischemic placenta that contributes to ET-1 synthesis.

Comment

In this study we demonstrated that 17 OHP blunted the hypertension in a model of placental ischemia in pregnant rats. Reduced uterine perfusion pressure (RUPP) has been previously shown to induce a condition similar to preeclampsia in the pregnant rat. This promotes the release of inflammatory cytokines, TNF- α and IL-6, which in turn results in endothelial cell activation – a known stimulus for ET-1 synthesis, ET_A receptor activation, and subsequent vasoconstriction.

Our findings support previous conclusions that endothelin synthesis may participate in the pathophysiology of hypertension induced by placental ischemia.^{17,19} A study by Alexander et al. (2001) concluded that ET-1 plays a major role in mediating hypertension in rats with placental ischemia.¹⁸ These authors demonstrated that renal expression of ET-1 was significantly elevated in both the medulla and cortex of pregnant RUPP rats. They also reported that chronic administration of a selective ET_A receptor antagonist (ABT627) attenuated the increase in MAP in pregnant rats with RUPP. In contrast, ET_A receptor blockade had no significant effect on blood pressure in the normal pregnant rat. Furthermore, sera from pregnant rats exposed to RUPP have been shown to increase ET-1 production from cultured human umbilical vein endothelial cells.²² Finally, we have shown that TNF- α induced hypertension in pregnant rats is associated with increased ET-1 synthesis in the renal cortices and placenta, but can be abolished by administration of the ET_A receptor antagonist. Although these studies suggest the importance of ET-1 activation of the ET_A receptor in mediating hypertension; the limitations of clinical studies using selective ET_A receptor antagonists in humans has hindered our understanding of the pathophysiology of ET-1 in preeclamptic women.

Administration of 17 OHP has routinely been used for prevention of preterm birth, therefore accentuating the importance of our in vivo study. We illustrated that in addition to having no deleterious outcome on pup weight, 17 OHP might exert anti-inflammatory effects to protect against endothelial cell activation and the release of the vasoconstrictor ET-1. Many studies have shown that inflammatory cytokines activate the endothelin system. In this study we demonstrated that administration of 17 OHP markedly decreased circulating TNF- α and IL-6 in response to placental ischemia. Furthermore, we demonstrated that administration of 17 OHP to RUPP rats is associated with decreased transcription of ET-1 in the renal cortices, thus suggesting one possible mechanism for the decrease in arterial pressure in RUPP rats receiving 17 OHP. However, administration of 17 OHP had no effect on ET-1 in placentas of RUPP rats, thus illustrating the importance of other factors released in the ischemic placenta that may activate the endothelin system during pregnancy. We have previously demonstrated that placental ischemia is also associated with increases in the soluble antagonist of vascular endothelial growth factor (sFlt-1), agonistic autoantibodies to the angiotensin type 1 receptor (AT1-AA), as well as with decreases in nitric oxide availability.²¹ Each of these factors could play a role in increased ET-1 levels in the placenta and may contribute to the remaining increase in blood pressure in the RUPP rats treated with 17 OHP. These factors were not evaluated in

this study, but could be the subject of further investigation into the role of 17 OHP as possible anti-hypertensive therapy in the setting of placental ischemia. Nevertheless, we conclude that administration of 17 OHP blunts the hypertension associated with RUPP possibly via suppression of the renal ET-1 system. Future *in vitro* and *in vivo* studies may involve further examination of the effects of 17 OHP administration on other potential mediators of ischemia induced hypertension.

References

1. Dodd JM, Flenady V, Cincotta R, Crowther CA. Prenatal administration of progesterone for preventing preterm birth. *Cochrane Database Syst Rev* 2006;(1):CD004947. [PubMed: 16437505]
2. da Fonseca EB, Bittar RE, Carvalho MH, Zugaib M. Prophylactic administration of progesterone by vaginal suppository to reduce the incidence of spontaneous preterm birth in women at increased risk: A randomized placebo-controlled double-blind study. *Am J Obstet Gynecol* 2003;188:419–24. [PubMed: 12592250]
3. Meis PJ, Klebanoff M, Thom E, et al. Prevention of recurrent preterm delivery by 17 alpha-hydroxyprogesterone caproate. *N Engl J Med* 2003;348:2379–85. [PubMed: 12802023]
4. Salas SP, Marshall G, Gutiérrez BL, Rosso P. Time course of maternal plasma volume and hormonal changes in women with preeclampsia or fetal growth restriction. *Hypertension* 2006;47(2):203–8. [PubMed: 16380519]
5. Sammour MB, El-Kabarity H, Fawzy MM, Schindler AE. Prevention and treatment of pregnancy-induced hypertension (preeclampsia) with progestogens. *J Steroid Biochem Mol Biol* 2005;97(5):439–40. [PubMed: 16236493]
6. Kristiansson P, Wang JX. Reproductive hormones and blood pressure during pregnancy. *Human Reproduction* 2001;16(1):13–7. [PubMed: 11139529]
7. Ragab MI, Sammour H, El-Kabarity H, Hegazi R. Progesterone a treatment for pre-eclamptic toxemia. *Ain-Shams Med J* 1971;22:9–24.
8. *Obstet Gynecol*. Vol. 99. American College of Obstetricians and Gynecologists; 2002. Diagnosis and management of preeclampsia and eclampsia. ACOG Practice Bulletin No. 33; p. 159-167.
9. Alexander BT, Kassab SE, Miller MT, et al. Reduced uterine perfusion pressure during pregnancy in the rat is associated with increases in arterial pressure and changes in renal nitric oxide. *Hypertension* 2001;37:1191–5. [PubMed: 11304523]
10. Nienartowicz A, Link S, Moll W. Adaptation of the uterine arcade in rats during pregnancy. *J Develop Physiol* 1989;21:101–8.
11. Combs CA, Katz MA, Kitzmiller JL, Brescia RJ. Experimental preeclampsia produced by chronic constriction of the lower aorta: Validation with longitudinal blood pressure measurements in conscious rhesus monkeys. *Am J Obstet Gynecol* 1993;169:215–23. [PubMed: 8333460]
12. Losonczy G, Brown G, Venuto RC. Increased peripheral resistance during reduced uterine perfusion pressure hypertension in pregnant rabbits. *Am J Med Sci* 1992;303:233–40. [PubMed: 1562040]
13. Granger JP, LaMarca BB, Cockrell K, et al. Reduced uterine perfusion pressure (RUPP) model for studying cardiovascular-renal dysfunction in response to placental ischemia. *Methods Mol Med* 2006;122:383–92. [PubMed: 16511995]
14. Granger JP, Alexander BT, Llinas MT, Bennett WA, Khalil RA. Pathophysiology of hypertension during preeclampsia: Linking placental ischemia with endothelial dysfunction. *Hypertension* 2001;38:718–22. [PubMed: 11566964]
15. Leite RS, Brown AG, Strauss JF 3rd. Tumor necrosis factor-alpha suppresses the expression of steroid receptor coactivator-1 and -2: a possible mechanism contributing to changes in steroid hormone responsiveness. *FASEB J* 2004;18(12):1418–20. [PubMed: 15231721]
16. Sfakianaki AK, Norwitz ER. Mechanisms of progesterone action in inhibiting prematurity [review]. *J Matern Fetal Neonatal Med* 2006;19(12):763–72. [PubMed: 17190686]
17. LaMarca BB, Cockrell K, Sullivan E, Bennett W, Granger JP. Role of endothelin in mediating tumor necrosis factor-induced hypertension in pregnant rats. *Hypertension* 2005;46(1):82–6. [PubMed: 15928030]

18. Alexander BT, Rinewalt AN, Cockrell KL, Massey MB, Bennett WA, Granger JP. Endothelin type A receptor blockade attenuates the hypertension in response to chronic reductions in uterine perfusion pressure. *Hypertension* 2001;37:485–9. part 2. [PubMed: 11230323]
19. LaMarca BB, Speed J, Fournier L, Babcock SA, Berry H, Cockrell K, Granger JP. Hypertension in response to chronic reductions in uterine perfusion in pregnant rats: Effect of TNF alpha blockade. *Hypertension* 2008;52:1–5. [PubMed: 18497371]
20. Applied Biosystems user bulletin, No2. Applera Corporation; Foster City, CA: 1997.
21. LaMarca BB, Gilbert J, Granger JP. Recent Progress Toward the Understanding of the Pathophysiology of Hypertension during Preeclampsia. *Hypertension* 2008;51(4):982–8. 2. [PubMed: 18259004]
22. Roberts L, LaMarca B, Fournier L, Bain J, Cockrell K, Gregory JP. Enhanced endothelin synthesis by endothelial cells exposed to sera from pregnant rats with decreased uterine perfusion. *Hypertension* 2006;47:615–8. [PubMed: 16391174]

Figure 1. Effect of RUPP and Progesterone on Arterial Pressure

Mean arterial pressure in normal pregnant control rats, reduced uterine perfusion pressure (RUPP) rats, normal pregnant control rats + 17 OHP, and RUPP rats + 17 OHP at day 19 of gestation. * $P < 0.001$ vs. normal pregnant control rats, † $P < 0.05$ normal pregnant control rats treated with 17 OHP versus RUPP rats treated with 17 OHP, ‡ $P < 0.05$ control RUPP versus RUPP rats treated with 17 OHP. All data are expressed as mean $\pm$ SEM.

Figure 2. Effect of RUPP and Progesterone on pup weight

Pup weights in normal pregnant control rats, reduced uterine perfusion pressure (RUPP) rats, normal pregnant control rats + 17 OHP, and RUPP rats + 17 OHP at day 19 of gestation. All data are expressed as mean $\pm$ SEM.

Figure 3. Administration of 17 OHP decreased circulating levels of TNF alpha and IL-6 in RUPP rats

Serum TNF- α (TOP) and IL-6 (BOTTOM) levels in normal pregnant, RUPP, and RUPP + 17OHP rats.

Figure 4. Progesterone was not significantly different among the RUPP groups in the presence or absence of 17OHP

Plasma progesterone in normal pregnant (NP), normal pregnant + 17OHP, RUPP, and RUPP + 17OHP rats.

Figure 5. Effect of Progesterone on tissue preproendothelin

a. Preproendothelin (ppET-1) levels in the renal cortex of normal pregnant control rats (n=6) versus reduced uterine perfusion pressure (RUPP) rats (n=6, * P<0.02) and RUPP rats + 17 OHP (n=4) at day 19 of gestation. All data are expressed as mean $\pm$ SEM.

b. Preproendothelin (ppET-1) levels in the placenta of normal pregnant control rats (n=6) versus reduced uterine perfusion pressure (RUPP) rats (n=7, † P<0.011) and versus RUPP rats + 17 OHP (n=6, ‡ P<0.011) at day 19 of gestation. All data are expressed as mean $\pm$ SEM.