OBSTETRICS

Progesterone supplementation attenuates hypertension and the autoantibody to the angiotensin II type I receptor in response to elevated interleukin-6 during pregnancy

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OBJECTIVE: Preeclampsia is a multisystem disorder recognized as hypertension with proteinuria developing >20 weeks' gestation. Preeclampsia is associated with chronic immune activation characterized by increased T and B lymphocytes, cytokines, and antibodies activating the angiotensin II type I receptor (AT1-AA). Hypertension in response to elevated interleukin (IL)-6 during pregnancy occurs with increased renin activity and AT1-AA, and reduced kidney function.

STUDY DESIGN: We aim to determine whether 17-alpha-hydroxyprogesterone caproate (17-OHPC), progesterone, improved inflammatory pathways during elevated IL-6 in pregnant rats. IL-6 (5 ng/d) was infused via miniosmotic pumps into normal pregnant (NP) rats beginning on day 14 of gestation and 17-OHPC (3.32 mg/kg) was diluted in normal saline and injected on day 18. Blood pressure (mean arterial pressure [MAP]) determination and serum collection were performed on day 19 of gestation.

RESULTS: MAP in NP was 100 ± 3 mm Hg, which increased with IL-6 to 112 ± 4 mm Hg ($P < .05$). Pregnant rats given 17-OHPC alone had a MAP of 99 ± 3 mm Hg and MAP increased to 103 ± 2 mm Hg in IL-6+17-OHPC. AT1-AA was 1.2 ± 0.5 bpm in NP rats, increased to 17 ± 9 bpm with IL-6 infusion but administration of 17-OHPC significantly blunted AT1-AA to 4 ± 0.8 bpm in NP+IL-6+17-OHPC. Total circulating nitrate/nitrite was significantly decreased and placental Ser^{1177}-phosporylated-eNOS/eNOS was lowered with IL-6 infusion. Supplementation of 17-OHPC significantly improved placental Ser^{1177}-phosporylated-eNOS/eNOS however, circulating nitrate/nitrite was unchanged with 17-OHPC supplementation.

CONCLUSION: This study illustrates that 17-OHPC attenuated hypertension, decreased AT1-AA activity, and improved placental nitric oxide in response to elevated IL-6 during pregnancy and could lend hope to a new potential therapeutic for preeclampsia.

Key words: cytokines, hypertension, inflammation, nitric oxide, pregnancy, progesterone, renin angiotensin system

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Preeclampsia is an important complication of pregnancy and is defined as new-onset hypertension usually with proteinuria or other evidence of maternal organ dysfunction that develops >20 weeks' gestation.[1] While preeclampsia and other hypertensive disorders of pregnancy are a leading cause of maternal and neonatal morbidity and mortality, a complete understanding of the mechanisms responsible for disease pathogenesis remains elusive.[2-4] Furthermore, improvements in treatment strategies are dampened by the lack of knowledge of the underlying cause of this disease.

Many investigators have proposed preeclampsia as a disease of immunological origin. These women exhibit a chronic innate inflammatory response and have increased circulating and placental levels of tumor necrosis factor (TNF) alpha and interleukin (IL)-6, and endothelial activation and dysfunction exhibiting high levels of endothelin (ET)-1, intracellular and vascular adhesion molecules, IL-8, and monocyte chemotactic protein.[4-7] Furthermore, women with preeclampsia display marked levels of autoantibodies that activate the angiotensin II type 1 receptor (AT1-AA).[8-10] We reported that chronic infusion of rat AT1-AA into pregnant rats from day 12-19 of gestation activates many systems, such as ET-1, reactive oxygen species, sFlt-1, and endoglin during pregnancy, all of which are suspected of playing an important role in the pathogenesis of preeclampsia.[11-13]

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Moreover, it is expected that vasodilators, such as nitric oxide (NO), are decreased and thus may have an important role in this process as well. Mechanisms involving altered formation of NO have been implicated in preeclampsia.[14-17]

Research efforts throughout the world have focused on effective low-cost alternative therapies for the treatment of preeclampsia that may help to prolong the pregnancy and avoid early delivery of the fetus and placenta. Seventeen-alpha-hydroxyprogesterone caproate (17-OHPC) is a synthetic metabolite of progesterone used effectively for the prevention of recurrent preterm birth in singleton pregnancies.[18-20] Furthermore, 17-OHPC is suspected to have vasodilatory and antiinflammatory effects, both of which could prove beneficial during preeclampsia. We have shown circulating progesterone to be decreased in preeclamptic patients.[21] Interestingly, progesterone supplementation of cell culture media decreased ET-1 secretion from vascular endothelial cells activated by serum from preeclamptic patients.[21] Previously, we identified a potential role for 17-OHPC in vivo. The 17-OHPC acted like an antiinflammatory substance that decreased hypertension, inflammatory cytokines (TNF alpha and IL-6), and ET-1 in response to placental ischemia in the reduced uterine perfusion pressure (RUPP) rat model of preeclampsia.[21,22] Although certainly not the only culprit, production of AT1-AA during pregnancy is a common pathway mediating hypertension in RUPP rats as well as in response to elevated cytokines, TNF alpha, IL-6, or IL-17.[23] Therefore, in the present study, we examined a role for 17-OHPC supplementation to decrease AT1-AA and blood pressure during IL-6-induced hypertension in pregnant rats. While the exact mechanism whereby 17-OHPC may decrease blood pressure is unclear, it has been suggested that progesterone can improve NO availability. Progesterone affects function of eNOS by both genomic and nongenomic mechanisms, involvement of PI3K/Akt leading to eNOS activation through phosphorylation of eNOS at serine 1177 (Ser^{1177}-P-eNOS), resulting in increased enzyme activity.[24-27] An up-regulation of eNOS, resulting in increased NO production has been shown to contribute to increases in uteroplacental blood flow via changes in vascular tone.[28] For these reasons circulating nitrate/nitrite and placental expression of eNOS and ser177 phosphorylated eNOS was evaluated during IL-6-induced hypertension during pregnancy with and without 17-OHPC supplementation.

Materials and Methods

All studies were performed in age-matched, timed pregnant 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 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.

Experimental design

Effect of progesterone on mean arterial pressure in response to IL-6 in pregnant rats

This experimental protocol was performed to determine whether progesterone supplementation blunts hypertension in response to elevated IL-6 during pregnancy. Experiments were performed in 4 groups of pregnant rats: normal pregnant (NP) (n = 5); NP+17-OHPC (n = 6); NP+IL-6 (n = 10); NP+IL-6+17-OHPC (n = 10). IL-6 (5 ng/d) (Recombinant Rat IL-6; R&D Systems, Minneapolis, MN) was infused via miniosmotic pumps (model 2002; Alzet Scientific Corp, Palo Alto, CA) for 5 days into NP rats during days 14-19 of gestation. 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 and what was previously shown to be effective in RUPP rats. Carotid catheters were inserted on day 18 of gestation and mean arterial pressure (MAP) was determined on day 19 as described previously.[29]

Effect of progesterone on AT1-AA in pregnant rats

This experimental protocol was performed to determine the role of progesterone to lower activity of AT1-AA in response to IL-6 in pregnant rats. Maternal serum was analyzed for AT1-AA by cardiomyocyte assay. Antibodies were detected by the chronotropic responses to AT1 receptor—mediated stimulation of cultured neonatal rat cardiomyocytes coupled with receptor-specific antagonists as previously described.[11,12] Chronotropic responses were measured and expressed in beats per minute (bpm).

Effect of progesterone on placental eNOS and Ser^{1177}-P-eNOS

This experimental protocol was performed to determine whether the improvements seen due to progesterone administration are due to an increase in eNOS. Briefly, placental extracts were homogenized in cold radioimmunoprecipitation assay buffer. A total of 100 μg of protein extracts were separated by SDS-PAGE using a polyacrylamide gel (4-20%). The proteins were transferred onto nitrocellulose membranes (BioRad, Hercules, CA). Different membranes were blocked with Blocking Buffer (LI-COR Biosciences, Lincoln, NE) for 1 hour at room temperature and incubated overnight at 4°C with primary antibody directed against eNOS (1:250; BD Transduction Laboratories, San Jose, CA) and Ser^{1177}-P-eNOS (1:250; BD Transduction Laboratories), respectively. Then the membranes were incubated with secondary antibody (IRDeye700-conjugated affinity-purified antimouse IgG, 1:5000; Rockland, Gilbertsville, PA) and scanned using Odyssey Infrared Imaging System (LI-COR Biosciences). The intensity of specific bands was quantified by densitometry using Image J (National Institutes of Health, Washington, DC) and placental eNOS and eNOS phosphorylated expression were normalized with respect to β-actin expression, respectively (1:5000, Cambridge).