Pravastatin Attenuates Hypertension, Oxidative Stress, and Angiogenic Imbalance in Rat Model of Placental Ischemia-Induced Hypertension

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Abstract—Preeclampsia is a pregnancy-specific condition characterized by an imbalance of circulating angiogenic factors and new-onset hypertension. Although current treatment options are limited, recent studies suggest that pravastatin may improve angiogenic profile and reduce blood pressure in preeclampsia. We hypothesized pravastatin would restore angiogenic balance and reduce mean arterial pressure (MAP) in rats with reduced utero-placental perfusion pressure (RUPP)-induced hypertension. Pravastatin was administered intraperitoneally (1 mg/kg per day) in RUPP (RUPP+P) and normal pregnant rats (NP+P) from day 14 to 19 of pregnancy. On day 19, MAP was measured via catheter, conceptus data were recorded, and tissues collected. MAP was increased (P<0.05) in RUPP compared with NP dams, and pravastatin ameliorated this difference. Pravastatin attenuated decreased fetal weight and plasma vascular endothelial growth factor and the RUPP-induced increased soluble fms-like tyrosine kinase-1 when compared with NP dams. Pravastatin treatment did not improve angiogenic potential in RUPP serum and decreased (P<0.05) endothelial tube formation in NP rats. RUPP rats presented with indices of oxidative stress, such as increased placental catalase activity and plasma thiobarbituric acid reactive substances along with decreased plasma total antioxidant capacity compared with NP controls, and pravastatin attenuated these effects. MAP, fetal weight, plasma vascular endothelial growth factor, and plasma soluble fms-like tyrosine kinase-1 were unchanged in NP+P compared with NP controls. The present data indicate that treatment with pravastatin attenuates oxidative stress and lowers MAP in placental ischemia-induced hypertension, but may have negative effects on circulating angiogenic potential during pregnancy. Further studies are needed to determine whether there are long-term deleterious effects on maternal or fetal health after pravastatin treatment during pregnancy-induced hypertension or preeclampsia. (Hypertension. 2013;61:00-00.)

Key Words: angiogenic factors ■ oxidative stress ■ pravastatin ■ preeclampsia

Preeclampsia is a pregnancy-specific condition characterized by new-onset hypertension, proteinuria, and intrauterine growth restriction and is a leading cause of maternal and fetal morbidity and mortality worldwide.1,2 Treatment options are currently lacking, and delivery of the placenta is the only effective treatment of preeclampsia, making it the leading cause of preterm delivery. Despite the significance of preeclampsia as a women’s health concern, the mechanisms underlying the pathogenesis of this condition are not well understood. Therefore, treatment of this condition remains a challenge.

In preeclampsia, impaired remodeling of the maternal uterine vasculature, leading to placental ischemia and inappropriate hypoxia, is believed to result in an angiogenic imbalance with increased soluble fms-like tyrosine kinase-1 (sFlt-1) and decreased vascular endothelial growth factor (VEGF).3,4 This angiogenic imbalance, in concert with dysregulation of a variety of other inflammatory mediators, including the complement system, is thought to contribute to widespread maternal inflammation and oxidative stress ultimately resulting in endothelial dysfunction and high blood pressure.5,6 These characteristics of preeclampsia are also exhibited in animal models of preeclampsia.7-12 An area of intense investigation is identification of potential therapeutic strategies that may be directed toward the amelioration of maternal inflammation, oxidative stress, and endothelial dysfunction, which in turn will ameliorate hypertension and permit gestation to continue to term. To this end, recent interest has turned to the potential role of 3-hydroxyl-3-methylglutaryl coenzyme A reductase inhibitors, or statins, to promote vascular endothelial function and mitigate manifestations of preeclampsia. Recent studies have revealed that this class of drugs may have a number of pleiotropic effects that are independent of the modification of cholesterol metabolism, including antioxidant, antiinflammatory, and antiapoptotic effects both in vivo and in vitro.13,14
Although several recent studies have revealed the potential for 3-hydroxyl-3-methylglutaryl coenzyme A reductase inhibitors (ie, statins) to attenuate characteristics of preeclampsia present in several animal and cell culture models used to study pregnancy-induced hypertension, none of these experiments has used a model of preeclampsia in which angiogenic imbalance and hypertension arise spontaneously after placental ischemia, such as the reduced utero-placental perfusion pressure (RUPP) rat or baboon with uterine ischemia via partial uterine artery ligation.15-17 These studies have generally reported positive outcomes such as lowering blood pressure, increases of proangiogenic factors, and the promotion of vasculature function. Furthermore, these studies have not reported any deleterious effects on the fetus. However, statin use remains contraindicated during pregnancy because of unknown, but potentially teratogenic, effects on the fetus. Considering the substantial controversy regarding the putative role of statin use in pregnancy, there is need for further study on this matter. Therefore, the purpose of this study was to test the hypothesis that pravastatin administration would restore angiogenic balance, ameliorate oxidative stress, and attenuate high blood pressure in rats with RUPP-induced hypertension without any deleterious effects on fetal outcome.

**Methods and Apparatus**

**Animals**

Studies were performed in timed-pregnant Sprague-Dawley rats purchased from Charles River (Portage, MI). Animals were housed in a temperature-controlled room (23°C) with a 12:12 light:dark cycle. The experimental procedures in this study were performed strictly in accordance with National Institutes of Health guidelines for use and care of animals, and the protocols used in this study were approved by the Institutional Animal Care and Use Committee of the Universities of Minnesota and Oregon. On day 14 of gestation, rat dams were randomly assigned to RUPP (n=14), RUPP+pravastatin (RUPP+P; n=7), normal pregnant (NP; n=12), or NP+pravastatin (NP+P; n=7) control groups.

**RUPP Procedure and Determination of Mean Arterial Pressure**

RUPP procedure is a well-established model for studying the link between placental ischemia and hypertension in pregnant rat and has been described in detail previously. NP rats underwent a sham surgery, which included the midline incision and suture, on the same day of pregnancy. On days 14 to 19 of pregnancy, pravastatin was administered at a dose, 1 mg/kg per day (IP QD), that has previously been reported positive outcomes such as lowering blood pressure, increases of proangiogenic factors, and the promotion of vasculature function. Furthermore, these studies have not reported any deleterious effects on the fetus. However, statin use remains contraindicated during pregnancy because of unknown, but potentially teratogenic, effects on the fetus. Considering the substantial controversy regarding the putative role of statin use in pregnancy, there is need for further study on this matter. Therefore, the purpose of this study was to test the hypothesis that pravastatin administration would restore angiogenic balance, ameliorate oxidative stress, and attenuate high blood pressure in rats with RUPP-induced hypertension without any deleterious effects on fetal outcome.

**Conceptus Measurements**

After measurement of mean arterial pressure, dams were anesthetized with isoflurane and a midline ventral incision was made to isolate the abdominal aorta for plasma and serum collection as reported previously. Pups and placentas that seemed viable were excised at term. Other tissues were excised, rinsed in saline, and stored at −80 °C for analysis.

**Plasma and Tissue Assays**

Plasma was collected in BD Vacutainer EDTA-containing tubes. Free plasma VEGF, sFlt-1, and tumor necrosis factor (TNF)-α concentrations were determined using commercially available ELISA kits (Quantikine, R&D Systems, Minneapolis, MN) according to the manufacturer’s instructions as described previously. Trolox-equivalent antioxidant capacity of the plasma was determined with a total antioxidant assay (Cayman Chemical, Ann Arbor, MI), and a thiobarbituric acid reactive substances assay (Cayman Chemical, Ann Arbor, MI) was performed to assess oxidative stress with an end measurement of plasma malondialdehyde according to the manufacturer’s instructions as described previously. Bilirubin assay (BioAssays; Harward, CA) was performed according to manufacturer’s specifications. Total soluble protein extracts from placentas were evaluated for catalase activity as per the manufacturer’s (Cayman Chemical, Ann Arbor, MI) specifications.

**Western Blot**

Western blots were performed as previously described. Briefly, 50 μg of protein was separated by electrophoresis on 4% to 20% SDS polyacrylamide gels, transferred to nitrocellulose membranes, and Ponceau stained to confirm even transfer across each gel. After 1 hour in casein blocking solution, membranes were incubated in blocking solution containing a commercially available antibody (from Abcam, Cambridge, MA unless noted otherwise) for heat shock protein (HSP) (ab12351, 1:5000), HSP 70 (ab6535, 1:5000), heme oxygenase-1 (HO-1) (Assay Designs, Ann Arbor, MI, OSA-110, 1:250) overnight at 4°C. β-actin (ab20272, 1:5000; MA5-15739, 1:10000, Thermo Pierce) was incubated at 1 hour at room temperature. Membranes were washed and incubated 1 hour with the appropriate horseradish peroxidase conjugated secondary antibodies (Cell Signaling. 1:10000–1:20000) or fluorophore conjugated antibodies (Lycor 926–8171.1:15000) and incubated in chemiluminescent substrate (West-Femto, Pierce, Rockford, IL) when required. The immunoreactive bands were digitized using an Alpha-Innotech digital imaging system or a LiCor Odyssey infrared imaging system. All digitized images were quantified using Un-Scan It gel 6.1 software (Silk Scientific, Orem, UT).

**Endothelial Tube Formation Assay**

Angiogenic potential was assessed in the serum of pregnant rats in vitro using the method of Banek et al. Two separate experiments (group 1 and group 2) were performed with this assay, and both were performed in duplicate. Group 1: Tube formation was measured in cells treated with serum from each rat in the 4 treatment groups: NP, RUPP, NP+P, and RUPP+P. Group 2: Tube formation was measured in cells treated with serum from NP or RUPP rats with and without the addition of 20 μM pravastatin added directly to the serum during the angiogenesis experiment.

**In Vitro Experiments**

BeWo and JEG cells (ATCC, Manassass, VA) were chosen to study the effects of pravastatin on fusigenic (ie, syncytium forming) extravillous type trophoblast cells (BeWo) and nonfusigenic (JEG) early pregnancy trophoblast cells. Cells were plated at 1×10^6 cells/mL in 6-well plates and treated with Ham’s F12 medium with L-glutamine (Cellgro Mediatech -10 to 080-CA), 10% fetal bovine serum and 1% penicillin/streptomycin. After 48 hours, cells were placed in an incubator chamber (Biospherix, Lacona, NY) fitted with oxygen and carbon dioxide sensors. Chambers were maintained at atmospheric control (20% O2, 5% CO2, 75% N2), physiological normoxic (8% O2, 5% CO2, 87% N2), or hypoxic (1.5% O2, 5% CO2, 93.5% N2) and cells were treated with 0 μmol/L, 10 μmol/L, or 20 μmol/L of pravastatin then harvested at 12 hours. At the end of the treatment period, cells were placed on ice and rinsed 3x with cold PBS. Laemmli sample buffer without bromophenol blue and β-mercaptoethanol was added and cell extracts collected after scraping. Conditioned media was centrifuged at 10000g for 5 minutes and the supernatant was snap frozen and stored at −80°C for analysis.

**Statistical Analysis and Calculations**

All data are presented as means±SEM and statistical significance was accepted when P<0.05. TNF-α data were square root transformed.
Results

Mean Arterial Pressure

Figure 1 shows that RUPP resulted in increased arterial pressure compared with NP controls. RUPP rats treated with pravastatin had decreased blood pressure compared with untreated RUPP rats, but had blood pressure that was higher than the NP controls (P<0.05). Although the blood pressure of the NP rats treated with pravastatin was 6 mm Hg higher than the NP rats, it was not significantly different than any of the treatment groups.

Conceptus and Maternal Morphometrics

Fetal weight was decreased in RUPP compared with NP dams (Figure 2A). Fetuses from RUPP rats treated with pravastatin were not smaller than that of the NP rats or the NP rats treated with pravastatin. Although there was a treatment effect by 1-way ANOVA for placental weight (data not shown), there were no significant differences among specific treatment groups when data were further analyzed with a Newman-Keuls post hoc test. Fetal resorptions were increased because of the RUPP procedure, and this was not altered by pravastatin (Figure 2B). Placental efficiency was decreased by RUPP and was attenuated by treatment with pravastatin (Figure 2C). Heart weight (Figure 2D) was increased (P<0.05) in the RUPP rats compared with NP rats, and this was attenuated in the RUPP+P group. Pravastatin had no adverse effect on heart weight in the NP rats. Kidney weight was not different among any of the groups (data not shown).

Circulating Factors

Figure 3A illustrates that plasma sFlt-1 was increased (P<0.05) in the RUPP compared with NP rats, and this was attenuated by treatment with pravastatin. Circulating levels of free VEGF were decreased (P<0.05) in the RUPP compared with the NP rats, and this was also attenuated by treatment with pravastatin (Figure 3B). Figure 3C shows that the sFlt-1:VEGF ratio, which was increased in RUPP compared with NP rats, was ameliorated by pravastatin treatment. There was no difference between NP and NP+P controls with respect to either sFlt-1 or VEGF. Figure 3D illustrates that TNF-α was not significantly increased in the RUPP compared with NP rats, but was increased in the RUPP+P rats compared with the NP controls. Serum total bilirubin was not different among the NP, RUPP, RUPP+P, and NP+P (1.40±0.27 versus 1.91±0.25 versus 1.10±0.21 versus 1.52±0.21 mg/dL).

Total Antioxidant Capacity and Catalase Activity

Figure 4 illustrates that thiobarbituric acid reactive substances (Figure 4A) were increased (P<0.05) in the plasma of RUPP compared with NP rats, and this increase was attenuated (P<0.05) by treatment with pravastatin. Total antioxidant capacity was decreased in RUPP rats compared with the NP rats, but was higher than normal pregnant (NP) controls. Data are expressed as mean±SEM, *P<0.05.
capacity, shown in Figure 4B, was decreased ($P<0.05$) in plasma of RUPP compared with NP controls, and pravastatin attenuated ($P<0.05$) this difference in the treated RUPP rats. There was no difference between NP and NP+P controls. Figure 5 shows that placental catalase activity was increased ($P<0.05$) in RUPP compared with NP, and this was attenuated by treatment with pravastatin. Pravastatin had no effect on catalase activity in the NP placentas.

**Western Blots for HO-1 and Heat Shock Proteins 27 and 70**

No differences were found among any of the treatment groups in regards to placental expression of HO-1 (or HSP 32), HSP 27, or HSP 70 (data not shown). In addition, hepatic expression of HO-1 relative to $\beta$-actin was not altered because of pravastatin treatment among the NP, RUPP, RUPP+P, and NP+P (2.45±1.01 versus 3.12±1.27 versus 2.83±1.06 versus 3.23±0.90) groups.

**Angiogenic Potential**

Endothelial cell tubule formation was assessed as a measure of the angiogenic potential of serum from animals in each treatment group. Figure 6A shows that angiogenic potential as determined by the tube formation assay was decreased ($P<0.05$) in the RUPP compared with the NP rats. Despite the observed increase in VEGF and decrease in sFlt-1 in pravastatin treated animals (Figure 3A–3C), there was no improvement in endothelial tube formation in the treated RUPP+P rats. Moreover, we observed a decrease ($P<0.05$) in endothelial tube formation in the NP rats that were treated with pravastatin.
To determine whether pravastatin directly affected endothelial cell tube formation, we added 20 μmol/L pravastatin to serum collected from NP and RUPP rats. Figure 6B shows that the RUPP serum decreased (P<0.05) tube formation, but addition of pravastatin to either RUPP or NP serum did not result in a statistically significant change in tube formation.

**Cell Culture Studies**

To determine whether pravastatin had differential effects on trophoblast cells compared with endothelial cells, we treated BeWo and JEG cells with varying oxygen concentrations and pravastatin doses. BeWo cells secreted increased (P<0.05) concentrations of VEGF in hypoxic (1.5% O₂) conditions with and without pravastatin, and therefore the main statistical effect was a result of O₂ concentration (Figure 7A). In contrast, Figure 7B shows JEG cells decreased (P<0.05) VEGF secretion with pravastatin treatment and showed no effect of O₂ concentrations.

**Discussion**

This study reports a combination of potentially positive and negative effects of pravastatin use during pregnancies with placental ischemia-related hypertension. Animal studies are required to investigate potential treatments for complications of pregnancy, but animal models often have limitations and ours is no different. Nevertheless, we believe that our rat model of placental ischemia-induced hypertension, which mimics many of the features of preeclampsia, is robust and contributes important findings to the volume of literature in this area.

Foremost, we report that pravastatin administration ameliorates RUPP-induced hypertension. Furthermore, we found that pravastatin increased circulating free VEGF concentrations and normalized sFlt-1:VEGF ratio, but did not restore angiogenic potential of the serum of these animals as determined by an endothelial tube formation assay. Although pravastatin treatment also improved markers of oxidative stress in the RUPP rats and resulted in no obvious adverse effects on fetal outcome, there was an increase in circulating TNF-α in the RUPP+P rats compared with the NP controls. Thus, the current study shows that pravastatin treatment during pregnancy may have effects that are specific to the concurrent presence of placental ischemia.

Our current findings are in agreement with previous studies reporting that pravastatin treatment has beneficial effects on blood pressure in hypertensive pregnant rodents. This is an important consideration as elevations in blood pressure are of primary concern in the treatment and management of preeclamptic patients. In addition, we also report that pravastatin treatment increased circulating free VEGF concentrations. Again, this is similar to other recent reports that have shown that pravastatin increases placental growth factor and VEGF in other models of pregnancy-induced hypertension or otherwise complicated pregnancies with angiogenic imbalance.

We are presently unsure of the mechanism by which pravastatin increases VEGF in the pregnant rat, but it seems to be ischemia-dependent as the same dose did not increase VEGF concentrations in the NP rats. Furthermore, it remains unclear whether the alterations in VEGF attributable to pravastatin treatment presage the reduction in blood pressure or occur because of the alteration in blood pressure, and further studies are needed to clarify the mechanisms underlying these observations. Alternatively, previous work has shown that statins may stimulate endothelial nitric oxide synthase in endothelial cells and improve vascular function via increased NO or cGMP signaling.

We also observed a decrease in endothelial tube formation in human umbilical vein endothelial cells incubated with serum from both the RUPP and NP rats treated with pravastatin when compared with NP rats. Similarly, Frick et al reported endothelial cells treated directly with 10 μmol/L simvastatin formed fewer tubes compared with cells treated with 0.1 μmol/L simvastatin. In contrast, we performed an additional study using NP and RUPP serum with pravastatin added postmortem and did not observe any direct effects on tube formation. One possibility for the differences in these observations is that factors in the rat serum interfere with the direct effects of statins on tube formation that has been reported previously. Alternatively, it is possible that TNF-α which we found to be increased by RUPP in the current study,
has a direct effect on tubule formation that is independent of pravastatin treatment. Further studies are being planned to evaluate this possibility.

Moreover, we also evaluated the effects of pravastatin on VEGF secretion from trophoblast cells in vitro at different oxygen concentrations and found differing effects depending on the cell type. In fusigenic BeWo cells we found that hypoxia increased VEGF expression, but that there was no effect of pravastatin on VEGF expression. In contrast, we found in the nonfusigenic JEG cells that pravastatin decreased VEGF secretion and there was no effect of O₂ concentrations. Data are expressed as mean±SEM, *P<0.05.

Figure 7. Effects of oxygen concentration and pravastatin on vascular endothelial growth factor (VEGF) secretion by BeWo and JEG trophoblast cells. BeWo cells secreted increased (A) concentrations of VEGF in hypoxic (1.5% O₂) conditions with and without pravastatin (Prav), thus the main statistical effect was attributable to O₂ concentration. In contrast, JEG cells decreased (B) VEGF secretion with pravastatin treatment and showed no effect of O₂ concentrations. Data are expressed as mean±SEM, *P<0.05.

Another beneficial effect we observed in the RUPP rats treated with pravastatin was restoration of total antioxidant capacity and a decrease in oxidative stress as measured by decreases in thiobarbituric acid reactive substances and catalase activity. Increased oxidative stress has long been recognized as a key characteristic of preeclampsia, and the RUPP model has previously been shown by us and others to mimic this observation. The current study is the first to report on catalase activity in the RUPP model, and we found that, similar to reports from preeclamptic women, there was an increase in catalase activity in the RUPP rat. As catalase activity is increased in response to oxidative stress, the decrease observed in the RUPP+P rats further supports the notion that pravastatin decreases oxidative stress in this model. Further, oxidative stress is known to play a key role in RUPP hypertension, and the antioxidant effects of pravastatin may be an important mediator of the blood pressure reduction we presently report.

In addition to the beneficial effects of pravastatin treatment on blood pressure, there was no evidence of fetal distress attributable to pravastatin treatment in either RUPP or NP rats. We also observed the placental efficiency, which was decreased in the RUPP compared with NP rats, was restored by pravastatin treatment. This observation is in agreement with previous work showing improved placental development and VEGF expression in a transgenic mouse model of preeclampsia in which mice have a complement component C1q deficiency. However, our study only examined the effects of 1 dose of pravastatin. It is possible that other doses may have different effects.

In contrast to previous studies that have reported only positive outcomes associated with pravastatin use in models of preeclampsia and miscarriage, we report for the first time that pravastatin treatment is associated with potentially detrimental effects on maternal physiology. In contrast to previous studies that have reported improved endothelial function
in various models of preeclampsia and angiogenic imbalance in pregnancy.\textsuperscript{16,17,25,37} We observed that, despite increases in VEGF, pravastatin treatment did not improve endothelial tube formation in RUPP hypertensive rats. Moreover, we found that pravastatin treatment decreased the angiogenic potential of the serum from NP rats. As pravastatin did not alter VEGF expression in the NP rats, it seems that this effect is VEGF independent. In addition, a dose of pravastatin that was higher than the in vivo dose used in our studies did not have the same effect on endothelial tube formation when added to NP and RUPP serum postmortem. Thus it seems that pravastatin has a pregnancy-specific interaction with maternal cells that is not dependent on placental ischemia and results in an alteration in angiogenic potential of the maternal serum. This may represent an area of concern with respect to use of pravastatin during pregnancy and further studies are warranted to further evaluate this observation.

Perspectives
Our findings in the current study are unique in that we show that pravastatin attenuates high blood pressure in a model in which hypertension develops spontaneously after initiation of placental ischemia, a situation thought to be very similar to the manifestation of preeclampsia in women. Although previous studies have reported that pravastatin and other statin drugs have been shown to have pleiotropic effects on oxidative stress and endothelial function, our findings only support potential antioxidant effects in pregnancy. Further, pravastatin treatment had no effect on blood pressure in the NP rats, suggesting that the effects were specific to the RUPP condition. The current data suggest that a decrease in oxidative stress may play an important role in these observations and that this may be independent of angiogenic balance. Lastly, these studies reveal the need for caution as well as further study to determine whether the benefits of statin use in hypertensive pregnancies outweigh the potential risks to the mother and fetus.

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Disclosures
None.

References
5. Gilbert JS, Babcock SA, Granger JP. Hypertension produced by reduced uterine perfusion in pregnant rats is associated with increased soluble fms-like tyrosine kinase-1 expression. Hypertension. 2007;50:1142–1147.


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### Novelty and Significance

**What Is New?**

- In this study we report for the first time that pravastatin treatment reduced high blood pressure, attenuated oxidative stress, and restored angiogenic balance in the reduced utero-placental perfusion pressure rat, a robust model of preeclampsia. However, in contrast to previous reports we found that pravastatin treatment did not improve angiogenic potential in rats with placental ischemia-induced hypertension suggesting pravastatin use during pregnancy may have deleterious effects on maternal physiology.

**What Is Relevant?**

- There are currently no treatments available for women with preeclampsia except for delivery of the baby when necessary. Our findings provide important evidence that pravastatin treatment attenuates many symptoms of placental ischemia-induced hypertension including high blood pressure, angiogenic imbalance, and oxidative stress. Nevertheless, we also report pravastatin treatment may have negative effects on maternal physiology. Therefore, further consideration must be given to whether reported benefits of statin use in hypertensive pregnancies outweigh the potential risks.

**Summary**

Pravastatin treatment attenuates high blood pressure, angiogenic imbalance, and oxidative stress in placental ischemia-induced hypertension but may stimulate circulating antiangiogenic factors.
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