Role of Endothelin in Mediating Soluble fms-Like Tyrosine Kinase 1–Induced Hypertension in Pregnant Rats

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Abstract—Although soluble fms-like tyrosine kinase 1 (sFlt-1), an antagonist of vascular endothelial growth factor and placental growth factor, has been implicated in the pathogenesis of hypertension during preeclampsia, the mechanisms whereby enhanced sFlt-1 production leads to hypertension remain unclear. Both sFlt-1 and endothelin 1 productions are elevated in women with preeclampsia and in placental ischemic animal models of preeclampsia; however, the importance of endothelin 1 and sFlt-1 interactions in the control of blood pressure during pregnancy is unknown. The purpose of this study was to determine the role of endothelin 1 in mediating sFlt-1–induced hypertension in pregnant rats. To achieve this goal, sFlt-1 (3.7 μg/kg per day for 6 days) was infused into normal pregnant rats and pregnant rats treated with a selective endothelin type A receptor antagonist, ABT 627 (5 mg/kg per day for 6 days). Plasma concentration of sFlt-1 increased from 735±34 pg/mL in normal pregnant rats to 2498±645 pg/mL (P<0.05) with infusion of sFlt-1. Arterial pressure increased from 100±1 mm Hg in normal pregnant rats to 122±3 mm Hg (P<0.05) in sFlt-1–infused rats. Chronic increases in plasma sFlt-1 in normal pregnant rats increased preproendothelin mRNA expression in the renal cortices by ~3-fold. In addition, chronic endothelin type A receptor blockade completely abolished the blood pressure response to sFlt-1 in pregnant rats (104±3 versus 100±1 mm Hg; P<0.05), whereas the endothelin A receptor antagonist had no effect on arterial pressure in NP rats (105±2 versus 100±1 mm Hg). In conclusion, this study demonstrates that endothelin 1, via endothelin type A receptor activation, plays an important role in mediating the hypertension in response to excess sFlt-1 during pregnancy. (Hypertension. 2010;55:394-398.)

Key Words: pregnancy ■ preeclampsia ■ endothelial factors ■ placenta ■ NO

Preeclampsia, a pregnancy-specific disease of the maternal vasculature, occurs in 5% to 10% of pregnancies within the United States,1 with the incidence of the disease having risen 40% within the last decade.2 Although it is the leading cause of maternal and perinatal death and morbidity,3 the mechanisms underlying the pathophysiology of the disease remains unclear. Over the past decade, there has been ample animal data to suggest placental ischemia as an important initiating event of the disease,4–7 although other causes than hypoxia leading to augmented placental oxidative stress in humans are suggested recently by Burton et al.8 The hypertension in response to placental ischemia is believed to result from an imbalance of proangiogenic and antiangiogenic factors, such as vascular endothelial growth factor (VEGF) and soluble fms-like tyrosine kinase 1 (sFlt-1).9–12 In support of the angiogenic imbalance hypothesis are studies demonstrating that women with preeclampsia (PE) have increased serum sFlt-1 concentrations and decreased circulating levels of VEGF and placental growth factor as compared with women with normotensive pregnancies.11,13,14 This angiogenic imbalance is then believed to lead to endothelial dysfunction, systemic vasoconstriction, and hypertension. Further support for the angiogenic imbalance concept are studies demonstrating that adenovirus overexpression of sFlt-1 or chronic administration of sFlt-1 into normal pregnant rats produces significant endothelial dysfunction, hypertension, and proteinuria.13,15

Although sFlt-1 is thought to play an important role in increasing blood pressure and causing endothelial dysfunction in PE or in response to placental ischemia in pregnant rats, the physiological mechanism whereby this angiogenic imbalance increases arterial pressure in pregnant rats is unclear. One possible mechanism whereby chronic excess sFlt-1 may result in elevations in mean arterial pressure in pregnant rats is through the potent vasoconstrictor endothelin (ET) 1. ET-1 has been found to be elevated in women with PE.16–18 In an animal model of reduced uterine perfusion pressure, serum and placental sFlt-1 levels are significantly increased in response to reduced uterine perfusion pressure in pregnant rats.19 Associated with the increase in sFlt-1, renal cortical and medullary expressions of preproendothelin (preproET-1) are increased compared with control pregnant rats.20 Furthermore, blockade of the ET type A (ETA) receptor markedly attenuated the rise in mean arterial pressure in response to reductions in uterine perfusion pressure.20 Thus, activation of the ET system appears to play an important role.
in mediating increases in blood pressure in response to placental ischemia in pregnant rats.

Although the angiogenic imbalance is known to lead to endothelial dysfunction, the importance of ET-1 in mediating the elevations in blood pressure during sFlt-1–induced hypertension in pregnant rats is unknown. Therefore, the purpose of this study was to determine the role of ET-1 in mediating the hypertension in sFlt-1–induced hypertension in pregnant rats. To achieve this goal, we examined the effect of sFlt-1 on ET-1 production in normal pregnant and sFlt-1 hypertensive pregnant rats. We also compared the blood pressure responses in both control and experimental groups treated with a selective ET\textsubscript{A} receptor antagonist, ABT627.

Methods

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

Experimental Design

sFlt-1 (recombinant mouse VEGF R1/Flt-1 Fc Chimera) was infused at a rate of 3.7 \( \mu g \) per day for 6 days (in sterile saline) beginning on day 13 of gestation via miniosmotic pump (model 2001, ALZET Scientific Corporation) into normal pregnant rats (n=11) and in pregnant rats orally treated (drinking water) with an ETA receptor antagonist (ABT627, 5 mg/kg per day for 6 days starting on day 13 of gestation). The infusion rate used has been shown to increase plasma sFlt-1 concentrations ~3-fold and decreased free VEGF by 30%,

Determination of Kidney, Placental, and Aortic PreproET mRNA Levels

The cortex and medulla of the kidneys were separated immediately after harvesting, quickly frozen in liquid nitrogen, and stored at -80°C. Total RNA was extracted using the RNaseasy Protect Mini Kit (Qiagen) after the cortex, medulla, and placenta were crushed in a mortar and pestle. Isolation procedure was then performed as outlined in the instructions provided by the manufacturer. Genomic DNA was digested with DNase I following instructions outlined by Ambion. RNA was quantified spectrophotometrically using an Eppendorf BioPhotometer. CDNA was synthesized from 5 \( \mu g \) of RNA with Invitrogen’s Superscript II reverse transcriptase using the following primers: preproET forward 1 (GTAGGTCTAAGCGATCCTTG) and reverse 1 (TCTTT-CTAGGTCTAAGCGATCCTTG), and the reverse primer as outlined. Invitrogen’s RT-PCR primer control kit was used to amplify \( \beta \)-actin transcripts as control. Levels of mRNA expression were calculated using the mathematical formulas for \( \Delta \Delta \) cycle threshold recommended by Applied Biosystems (Applied Biosystems User Bulletin, No. 2, 1997).

Statistical Analysis

All of the data are expressed as mean±SE. Differences between control and experimental groups were analyzed using unpaired \( t \) tests. Data were considered statistically different at \( P<0.05 \). Blood pressure comparisons for multigroup and multifactorial analyses were performed using ANOVA with the Student-Newman-Keul post hoc test.

Results

Plasma sFlt-1 Levels and Arterial Pressure Responses in Control and sFlt-1–Treated Pregnant Rats

A significant elevation in sFlt-1 plasma concentrations was achieved in sFlt-1–treated pregnant rats. An \( \approx 3.5 \) fold increase in circulating levels of sFlt-1 (2498±645 pg/mL) was reached after chronic infusion compared with normal pregnant rats (735±34 pg/mL; Figure 1). Associated with the elevated plasma sFlt-1 concentrations were significant elevations in mean arterial pressure, 122±2 mm Hg, compared with control rats, at 100±1 mm Hg on day 19 of gestation.

PreproET mRNA Levels in the Kidney of Normal Pregnant and sFlt-1–Treated Pregnant Rats

PreproET in the renal medulla did not differ significantly between sFlt-1–treated pregnant rats and normal pregnant
rats. However, preproET mRNA levels increased ~3-fold in the renal cortex of sFlt-1–treated pregnant rats compared with normal pregnant rats (Figure 2).

**PreproET mRNA levels in Placenta and Aorta in Normal Pregnant and sFlt-1–Treated Pregnant Rats**

PreproET in the placenta or aorta did not differ significantly between sFlt-1–treated pregnant rats and normal pregnant rats (Figure 3).

**Arterial Pressure Responses to sFlt-1 in Normal Pregnant Rats and ETA Receptor Antagonist–Treated Pregnant Rats**

Chronic infusion of sFlt-1 into pregnant rats at a rate of 3.7 μg/kg per day for 6 days resulted in significant elevations in mean arterial pressure on day 19 of gestation. Pregnant rats treated with sFlt-1 and the ETA receptor antagonist showed significant reductions in mean arterial pressure of 104±3 mm Hg compared with sFlt-1–treated pregnant rats. Treatment of normal pregnant rats with an ETA receptor antagonist alone had no effect on mean arterial pressure (105±2 mm Hg) compared with normal pregnant rats (100±1 mm Hg; Figure 4).

**Discussion**

Inadequate invasion of trophoblasts into the maternal spiral arteries is believed to play an important role in the etiology behind pregnancy-induced hypertension. Over the past decade, there has been ample animal data to suggest placental ischemia as an important initiating event of the disease, although other causes than hypoxia leading to augmented placental oxidative stress in humans are recently suggested by Burton et al. The hypertension in response to placental oxidative stress is believed to result from the release of soluble, placental factors into the maternal circulation. These factors are then believed to act on the maternal endothelium and to result in systemic vasoconstriction. One such factor that is known to be upregulated during PE is the antangiogenic factor sFlt-1. sFlt-1 is a member of the proangiogenic VEGF family and analogous to the Flt1 receptor; however, the lack of the transmembrane C-terminus makes the sFlt-1 receptor soluble. sFlt-1 acts to sequester free VEGF and to lead to an angiogenic imbalance weighted toward an antiantiogenic state. VEGF is a regulator of normal endothelial cell function, and it is suggested that a decrease in circulating levels of free VEGF leads to the abnormal endothelial cells and resultant endothelial cell activation and dysfunction associated with PE. We and others have shown that chronic excess sFlt-1 infusion into normal pregnant rats results in significant elevations in mean arterial pressure that is associated with decreased levels of VEGF, placental growth factor, and proteinuria, as well as reductions in renal function. Consistent with these findings, we report in this study that a 2- to 3-fold increase in sFlt-1 in pregnant rats increased blood pressure ~20 mm Hg. The current study extends previous findings by examining the role of the ET-1 in increasing the blood pressure in response to sFlt-1.

Although sFlt-1 increases blood pressure, there is compelling evidence to suggest an important role for endothelin in PE. ET-1 is a known byproduct of endothelial dysfunction and has been found to be elevated ~2- to 3-fold in the plasma of some, but not all, women with PE. In addition, recent data from our laboratory show that a reduced uterine perfusion pressure model of PE in pregnant rats results in significant elevations in mean arterial pressure and renal preproET production. Furthermore, selective blockade of the ETA receptor soluble.
receptor completely abolished the hypertension in response to chronic reduced uteroplacental perfusion in these animals. Collectively, these data strongly suggest that the endothelin system may play an important role in mediating the hypertension in response to chronic excess sFlt-1 infusion into pregnant rats. To test the hypothesis we first examined whether sFlt-1–induced hypertension activated the ET-1 system. In response to chronic excess sFlt-1 infusion into pregnant rats, we observed a ∼3.5-fold increase in cortical preproET expression compared with that in normal pregnant rats. There was no significant difference in medullary, placental, or aortic preproET mRNA expression in sFlt-1 hypertensive and normal pregnant rats. Treatment with a selective ETA receptor antagonist, ABT627, significantly reduced blood pressure in sFlt-hypertensive pregnant rats while having no effect on normotensive, normal pregnant rats.

Because the endothelium is a major target organ for the actions of VEGF, it is likely that alterations in the production of endothelium-derived relaxing factors, such as NO, may also play a role in the hypertensive response to sFlt-1. Although the relative importance of NO in mediating the increase in blood pressure in response to sFlt-1 has yet to be fully elucidated, Facemire et al reported recently that administration of a specific antibody against the major VEGF receptor, VEGFR2, to normal mice caused an increase in blood pressure that was associated with significant reductions in the expression of endothelial and neuronal NO synthases in the kidney. To further establish a role for reduced NO synthesis in the hypertension caused by blocking VEGFR2, they reported that N^G-nitro-L-arginine methyl ester administration abolished the difference in blood pressure between the vehicle- and anti-VEGFR2–treated groups. Additional support for an interaction between NO and antiangiogenic factors include the clinical findings of Sandrim et al showing that NO formation is inversely related to serum levels of antiangiogenic factors sFlt-1 and soluble endoglin in PE. Although these results suggest that reducing NO production and/or availability may be one mechanism underlying hypertension caused by antiangiogenic agents targeting VEGF, it remains to be determined whether sFlt-1–induced hypertension during pregnancy is associated with reductions in NO production. Moreover, it is possible that reductions in NO synthesis may be in part responsible for the increase in ET-1 in response to sFlt-1, because NO is known to be a potent inhibitor of ET-1 production.

In summary, we found that chronic excess sFlt-1 infusion into pregnant rats was associated with significant elevations in mean arterial pressure and an ∼3.5-fold increase in the cortical preproET-1 mRNA expression. Chronic treatment with a selective ET_\text{A} receptor antagonist significantly reduced mean arterial pressure in sFlt-1 hypertensive pregnant rats while having no effect on normal pregnant rats. On the basis of these findings, ET-1 appears to play an important role in mediating the hypertension produced by chronic, excess sFlt-1 in pregnant rats.

**Perspectives**

PE, which affects 5% to 10% of all pregnancies in the United States, is a multisystemic disorder of pregnancy that is associated with hypertension and endothelial dysfunction. Despite being one of the leading causes of maternal and perinatal morbidity and mortality, the pathophysiological mechanisms underlying the hypertension during PE is unknown. Although sFlt-1, an endogenous antagonist of VEGF, and placental growth factor has been implicated in the pathogenesis of hypertension during PE, the mechanisms whereby enhanced sFlt-1 production leads to endothelial dysfunction and hypertension remain unclear. Both sFlt-1 and ET-1 production are elevated in women with PE and in placental ischemic animal models of PE; however, the importance of ET-1 and sFlt-1 interactions in the control of blood pressure during pregnancy has been unknown. The data from the current study suggest that elevated preproET-1 expression in sFlt-1 hypertensive pregnant rats appears to mediate the hypertension seen in this model.

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**Disclosures**

None.

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