Abstract—Antagonism of vascular endothelial growth factor (VEGF) signaling by soluble fms-like tyrosine kinase 1 occurs during preeclampsia and is proposed to play an important role in the pathogenesis of preeclampsia. We reported recently that hypertension associated with chronic reductions in uteroplacental perfusion pressure (RUPP) is associated with increased soluble fms-like tyrosine kinase 1 and decreased free VEGF. Whether restoration of circulating VEGF can restore renal function and chronically decrease arterial pressure associated with placental ischemia remains unknown. We hypothesized that chronic infusion of VEGF121 would attenuate hypertension, increase glomerular filtration rate, and reverse the endothelial dysfunction associated with chronic RUPP. VEGF121 (at either 90 or 180 μg/kg per day) was administered for 5 days via osmotic minipump placed IP. Mean arterial pressure, renal function, and tissues were obtained on day 19 of pregnancy from RUPP, VEGF, RUPP, and normal pregnant dams. Mean arterial pressure was increased in the RUPP (131±3 mm Hg) compared with the normal pregnant (102±1 mm Hg) rats, and infusion of VEGF121, resolved the hypertension (105±5 mm Hg). Glomerular filtration rate was decreased in the RUPP dams (1.5±0.3 mL/min) and restored to normal pregnant levels (3.1±0.5 mL/min) by VEGF121 treatment (3.1±0.4 mL/min). Effective renal plasma flow, decreased by RUPP, was also increased by VEGF121 infusion. Relaxation to acetylcholine was enhanced by the VEGF treatment (P<0.05). These data demonstrate that chronic infusion of VEGF121 during late gestation restores glomerular filtration rate and endothelial function and reduces high blood pressure associated with placental ischemia. The present results suggest that VEGF121 may be a candidate molecule for management of preeclampsia and its related complications. (Hypertension. 2010;55:380-385.)

Key Words: preeclampsia ■ gestation ■ VEGF ■ blood pressure ■ angiogenic

Preeclampsia is a major obstetric problem that affects ≈5% of all pregnancies and is a significant source of maternal and neonatal morbidity and mortality.1 Moreover, the incidence of preeclampsia has seen a 40% increase in recent years.2 Although the preeclamptic syndrome has been well characterized, and many studies indicate that hypertension, proteinuria, endothelial cell dysfunction, and insufficient placentaion are key features of this disorder,3,4 the underlying pathophysiological mechanisms of this disorder remain unclear.

Recent studies have established the existence of an imbalance between proangiogenic and antiangiogenic factors, such as vascular endothelial growth factor (VEGF), placental growth factor, and soluble fms-like tyrosine kinase 1 (sFlt-1) in preeclamptic women.5–10 Moreover, findings from several clinical studies suggest that alterations in circulating sFlt-1 concentrations may presage the clinical onset of preeclampsia symptoms.5,11–13 Viewed in concert with recent experimental studies in animals, it appears that this dysregulation of angiogenic factors may play a key role in the pathogenesis of preeclampsia.9,10,14–19

Although the mechanisms underlying the overexpression of antiangiogenic factors, such as sFlt-1, are currently unresolved, in vitro and in vivo evidence suggest that placental hypoxia/ischemia may initiate this imbalance of angiogenic factors.14,16,20 Both Gilbert et al14 and Makris et al16 have shown in vivo that chronic placental ischemia results in elevated circulating levels of sFlt-1 and hypertension. In addition, previous studies have shown that reduced uterine perfusion pressure (RUPP) is associated with decreased
endothelium-dependent vascular relaxation\textsuperscript{21} and decreased glomerular filtration rate (GFR).\textsuperscript{22} In an elegantly designed study reported several years ago, Maynard et al\textsuperscript{10} reported that exogenous administration of sFlt-1 into pregnant rats via adenovirus mediated gene transfer resulted in increased arterial pressure and proteinuria and decreased plasma-free VEGF and placental growth factor concentrations similar to those observed in the preeclamptic patients. More recently, Bridges et al\textsuperscript{18} demonstrated that chronic elevations of recombinant sFlt-1 result in endothelial dysfunction that is attenuated by the superoxide dismutase mimetic Tiron. In addition, recent studies by Li et al\textsuperscript{23} have demonstrated that delivery of recombinant VEGF\textsubscript{121}, the most soluble of the VEGF isoforms, can rescue the symptoms generated by chronic elevations of sFlt-1. Although these findings are intriguing, the mechanisms by which the hypertensive phenotype is rescued remain unclear. Moreover, the efficacy of VEGF\textsubscript{121} treatment to improve hypertension and renal function in a model of hypertension during preeclampsia with spontaneously occurring overexpression of sFlt-1 remains unknown.

Thus, the purpose of the present study was to test the hypothesis that chronic infusion of VEGF will attenuate the endothelial dysfunction, impaired renal function, and hypertension associated with RUPP in the pregnant rat. To this end, we used our established model of hypertension associated with placental ischemia in which chronic reductions of uterine perfusion pressure lead to endothelial dysfunction, decreased GFR, and hypertension in the pregnant rat.

Materials and Methods

Animals

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. On day 14 of gestation, rat dams were randomly assigned to 1 of 4 experimental groups: (1) normal pregnant (NP) rats plus vehicle, which served as the control group (n=9); (2) the RUPP plus vehicle (sterile PBS) group (n=11); (3) the RUPP+VEGF group (low dose, 90 μg/kg per day; n=8); and (4) the RUPP+VEGF group (high dose, 180 μg/kg per day; n=7).

RUPP Procedure

The RUPP procedure is a well-established model for studying the links between placental ischemia and hypertension in the pregnant rat and has been described in detail previously.\textsuperscript{21,22,24–28} In brief, surgical procedures were completed with rats under isoflurane anesthesia (Webster) delivered by an anesthesia apparatus (Vaporizer for Forane Anesthetic). Catheters were tunneled to the back of the neck and exteriorized after implantation. On day 19 of gestation, rat dams were placed in individual restraining cages for arterial pressure measurements using a pressure transducer (Cobe III Transducer CDX Sema). Mean arterial pressure (MAP) was recorded continuously for a 2-hour period after 1 hour of stabilization.

Measurement of Mean Arterial Pressure in Chronically Instrumented Conscious Rats

Animals were instrumented and arterial pressure was determined in both groups of rats at day 19 of gestation, as described previously.\textsuperscript{26} Briefly, on day 17 of gestation, rats were instrumented with carotid catheters of V-3 tubing (SCI) while under isoflurane anesthesia (Webster) delivered by an anesthesia apparatus (Vaporizer for Forane Anesthetic). Catheters were tunneled to the back of the neck and exteriorized after implantation. On day 19 of gestation, rat dams were placed in individual restraining cages for arterial pressure measurements using a pressure transducer (Cobe III Transducer CDX Sema). Mean arterial pressure (MAP) was recorded continuously for a 2-hour period after 1 hour of stabilization.

Conceptus Measurements

After the measurement of MAP, the dams were placed under isoflurane anesthesia, and a ventral midline incision was made to isolate the abdominal aorta for plasma and serum collection. The uterus was exteriorized; the number of viable and resorbed pups was counted and recorded; and the pups and placentae were excised, blotted dry, and weighed. The heaviest and the lightest placentae were collected from each uterine horn, snap frozen in liquid nitrogen, and stored at −80°C until further analyses were performed.

Plasma/Serum Assays and Recombinant Protein

Blood was collected for subsequent assays into Corvac sterile serum separator tubes (Sherwood Davis) and plasma into BD Vacutainer EDTA-containing tubes. Circulating VEGF and sFlt-1 concentrations were measured using commercial kits available from R&D Systems, as reported previously.\textsuperscript{14,18} Recombinant VEGF\textsubscript{121} has been described previously\textsuperscript{23} and was a gift from Scios, Inc (Fremont, CA).

Vascular Wire Myography Experiments

The noninstrumented carotid was removed and prepared for vessel-reactivity studies in organ-chamber baths, as described previously.\textsuperscript{18,29} Carotid arteries were chosen to be consistent with our earlier work.\textsuperscript{29} Resting tension was adjusted stepwise to reach a final tension of 0.75 g. For studies of vessel relaxation, carotid segments were precontracted with the thromboxane mimetic, U46619 (0.4 μg/mL). After the vessel reached stable tension, concentration responses to acetylcholine (ACh) and sodium nitroprusside (10⁻⁶–10⁻⁴ M) were performed to assess endothelial-dependent and smooth muscle–dependent relaxation, respectively. To evaluate the viability...
of vessel segments after the final relaxation curve, maximal contractile responses to U46619 (10^{-8} to 10^{-4} M) were also tested.

Statistical Analysis and Calculations
All of the data are presented as mean±SEM, and statistical significance was accepted when \( P<0.05 \). A Grubb test was applied to identify statistical outliers. Conceptus data were calculated as mean per pregnancy. sFlt-1:VEGF ratio data were log transformed to obtain normal distributions for subsequent statistical analysis. Comparisons between groups were made with ANOVA followed by post hoc Newman-Keuls multiple comparison test. Myography data were analyzed by nonlinear regression best-fit modeling followed by an F test for log of the EC_{50}, Hill slope, and curve fitting. Statistical calculations were made with GraphPad Prism version 4.0 for Windows (GraphPad Software).

Results
Blood Pressure During Late Gestation
Figure 1 illustrates the effects of RUPP and VEGF_{121} on MAP during late gestation. VEGF_{121} infusion reduced MAP in the RUPP rats in a dose-dependent manner with the high-dose treatment abrogating the placental ischemia induced hypertension (\( P<0.05 \), RUPP+VEGF_{121} high dose vs RUPP).

Renal Hemodynamics
Figure 2A illustrates the effects of RUPP and VEGF_{121} on GFR during late gestation. Similar to the effects observed with respect to MAP, VEGF_{121} infusion improved GFR in the RUPP rats in a dose-dependent manner with the high dose treatment abrogating the placental ischemia-induced decline in renal function (\( P<0.05 \), RUPP+VEGF_{121} high dose vs RUPP).

Endothelial Function
Because the high dose of chronic infusion of VEGF_{121} resulted in increased GFR and decreased MAP, we sought to determine whether this was because of enhanced vascular endothelial cell function. Figure 3 shows that endothelial-dependent vascular relaxation of the carotid artery to ACh was enhanced by chronic infusion of VEGF_{121} to RUPP rats (Figure 3). The log EC_{50} for ACh was decreased in the RUPP+VEGF_{121} compared with the NP rats (log: \(-6.998 \) versus \(-10.29; P<0.05\)).
Plasma Angiogenic Factors

As reported previously, RUPP resulted in a decrease of VEGF and an increase of sFlt-1 when compared with NP+ vehicle (NP) rats at day 19 of pregnancy (A and B). Infusion of VEGF_{121} restored circulating VEGF to NP levels (A). Interestingly, chronic infusion of VEGF decreased plasma sFlt-1 levels in the RUPP+VEGF_{121} group to that of NP rats (B).

Conceptus Morphometrics

Figure 5A illustrates that RUPP fetuses were smaller than the NP+ vehicle (NP) fetuses \( (P<0.05) \). Chronic infusion of VEGF_{121} at 90 \( \mu \)g/kg (low dose) in the RUPP+VEGF_{121} group resulted in fetuses that were not smaller than the NP groups. High-dose infusion of VEGF_{121} (180 \( \mu \)g/kg) into RUPP rats had no effect on fetal growth. Placental size was not altered by RUPP or VEGF_{121} infusion in the present study (B).

Discussion

The present study reports several novel findings regarding the effects of recombinant VEGF_{121} infusion in a rat model of preeclampsia whereby sFlt-1 overexpression is generated spontaneously after reduction of uteroplacental perfusion pressure. As we have reported previously, RUPP rats have increased arterial pressure, renal impairment, and endothelial dysfunction in conjunction with elevated circulating sFlt-1 and decreased plasma VEGF.\textsuperscript{15,21,22,28}

In the present work, we report several novel findings. Foremost, we report that chronic infusion of recombinant VEGF_{121} resulted in reductions of arterial pressure in a dose-dependent manner. Second, we found renal hemodynamic improvement as determined by increased GFR and ERPF. Third, we found that the reduction in arterial pressure and restoration of GFR in the high-dose VEGF_{121} infusion group was associated with significant improvements in endothelial function as determined by relaxation responses to ACh. Lastly, we found that 5 days of VEGF_{121} infusion negatively regulated sFlt-1 expression in rats with placental ischemia and restored sFlt-1 back to NP levels by day 19 of pregnancy. Thus, the present study is the first to report on the efficacy of VEGF_{121} infusion to reduce arterial pressure, improve GFR, and reduce sFlt-1 levels in a robust, reproduc-
ible, and well-characterized animal model of placental ischemia-induced hypertension in which plasma sFlt-1 concentrations are increased because of placental ischemia.

We	extsuperscript{14} and others	extsuperscript{16} have demonstrated placental ischemia results in hypertension that are associated with increases in sFlt-1. In addition, other groups have shown that sFlt-1 overexpression results in hypertension during pregnancy.	extsuperscript{3,10,23} Our results are consistent with those of Li et al	extsuperscript{23} that report that VEGF	extsubscript{121} reverses the preeclamptic phenotype of the adenovirus-induced sFlt-1 overexpression model. Moreover, both the present study and that of Li et al	extsuperscript{23} show that VEGF	extsubscript{121} infusion lowers arterial pressure in a dose-dependent manner. Taken together, these data illustrate the importance of sFlt-1 in the pathophysiology of hypertension during preeclampsia. In contrast to the work of Li et al,	extsuperscript{23} the present study is in a model in which the RUPP animals presented with elevated levels of other factors (in addition to sFlt-1) that play important roles in preeclampsia, such as tumor necrosis factor-α and soluble endoglin.	extsuperscript{26,30–32} Hence, the current findings demonstrate that chronic infusion of VEGF	extsubscript{121} lowers blood pressure and improves renal function in the presence of multiple factors involved in the preeclamptic syndrome.

Previous work in our laboratory	extsuperscript{22} has shown that the hypertension in this model is associated with a decline in renal function. Hence, we set out to determine whether the improvement observed in arterial pressure was associated with restoration of GFR in VEGF	extsubscript{121}-infused rats. Although we found that VEGF	extsubscript{121} infusion resulted in dose-dependent increases in GFR, only the high dose restored GFR to NP levels. These data suggest that VEGF antagonism by sFlt-1 plays an important role in the decline in renal function and, therefore, hypertension associated with placental ischemia.

Previous work has shown that RUPP hypertension is associated with endothelial dysfunction and impaired ACh-mediated NO production and vasorelaxation.	extsuperscript{21} Moreover, previous studies have provided evidence that VEGF antagonism by sFlt-1 results in decreased endothelial function	extsuperscript{10,18} in both renal microvessels and carotid arteries. In the present investigation, we find that high-dose VEGF	extsubscript{121} infusion results in significant improvements in ACh-mediated vasorelaxation of the carotid artery. Hence, these data suggests one possible mechanism, the restoration of endothelial function in the renal vasculature, for the increased renal function and attenuation of hypertension in this model.

In accord with our previous findings, we found that fetal weight was decreased in the present cohort of RUPP dams.	extsuperscript{15,21,22,28} Although fetal weight was not significantly increased with VEGF	extsubscript{121} infusion, the low-dose regimen attenuated the growth restriction associated with chronic RUPP. The high-dose VEGF	extsubscript{121} infusion showed no effect on fetal growth. These findings are similar to the previous report by Li et al,	extsuperscript{23} who also reported no significant improvement in fetal growth with the chronic administration of VEGF	extsubscript{121} in the sFlt-1 overexpression model. The apparent differential effects observed between the low and high doses of VEGF	extsubscript{121} infusion may relate to the extent of arterial pressure change with each dose. The high dose resulted in abrogation of RUPP-induced hypertension, whereas the low dose merely attenuated the hypertension associated with placental ischemia. Thus, the higher pressure in concert with the permeability effects of VEGF	extsubscript{121} may have resulted in greater tissue perfusion and augmented fetal growth in the low-dose VEGF	extsubscript{121} group.

Previous work suggests that the primary source of circulating sFlt-1 in preeclamptic humans derives from the uteroplacental unit	extsuperscript{10,33,34} and that excessive placental ischemia plays a role in the dysregulation of sFlt-1 in pregnancy.	extsuperscript{14,20} Viewed together, these studies suggest that the increase in circulating sFlt-1 observed in preeclampsia may be a consequence of aberrant placental perfusion and that elevated sFlt-1 is likely responsible for the maternal syndrome. Whether there is a pathogenic role for sFlt-1 in early pregnancy remains unknown at the present time. Importantly, our studies suggest that antagonism of sFlt-1 with VEGF	extsubscript{121} does appear to ameliorate maternal hypertension without significant adverse effects to the fetus.

It is important to recognize that circulating VEGF levels are much different in the pregnant rat compared with the pregnant woman. Hence, the relative contributions of VEGF compared with placental growth factor may be species specific. Nevertheless, the present studies show that restoration of the angiogenic balance has beneficial effects on blood pressure and renal function in hypertension associated with placental ischemia. Additional studies are clearly required to determine the relative contributions of VEGF and placental growth factor in this and other models.

**Perspectives**

Although it appears clear that antiangiogenic factors play a role in the pathogenesis of preeclampsia, the manner in which these factors result in the features of the preeclamptic syndrome remain unclear. The present study, which relies on data gathered by using a well-characterized and robust animal model of hypertension during pregnancy, provides further evidence that antagonism of VEGF plays an important role in the link among placental ischemia, endothelial dysfunction, and hypertension in the preeclamptic syndrome. It is difficult to directly extrapolate these findings to preeclampsia in humans largely because of the differences in the duration of hypertension and exposure to toxic factors, such as sFlt-1. Although the present results suggest that sFlt-1 antagonists, such as VEGF	extsubscript{121}, may be appropriate candidate molecules for further studies on the management of preeclampsia, careful studies will be required to determine the therapeutic range in women.

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**Disclosures**

S.A.K. is a coinventor on patents filed by the Beth Israel Deaconess Medical Center for the diagnosis and therapy of preeclampsia. S.A.K. has served as a consultant to Scios, Inc.
References
32. LaMarca BD, Gilbert J, Granger JP. Recent progress toward the understanding of the pathophysiology of hypertension during preeclampsia. Hypertension. 2008;51:982–983.
Recombinant Vascular Endothelial Growth Factor 121 Infusion Lowers Blood Pressure and Improves Renal Function in Rats With Placental Ischemia-Induced Hypertension
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