Preeclampsia

Placental Growth Factor Administration Abolishes Placental Ischemia-Induced Hypertension

Frank T. Spradley, Adelene Y. Tan, Woo S. Joo, Garrett Daniels, Paul Kussie, S. Ananth Karumanchi, Joey P. Granger

Abstract—Preeclampsia is a pregnancy-specific disorder of new-onset hypertension. Unfortunately, the most effective treatment is early delivery of the fetus and placenta. Placental ischemia appears central to the pathogenesis of preeclampsia because placental ischemia/hypoxia induced in animals by reduced uterine perfusion pressure (RUPP) or in humans stimulates release of hypertensive placent al factors into the maternal circulation. The anti-angiogenic factor soluble fms-like tyrosine kinase-1 (sFlt-1), which antagonizes and reduces bioavailable vascular endothelial growth factor and placental growth factor (PIGF), is elevated in RUPP rats and preeclampsia. Although PIGF and vascular endothelial growth factor are both natural ligands for sFlt-1, vascular endothelial growth factor also has high affinity to VEGFR2 (Flk-1) causing side effects like edema. PIGF is specific for sFlt-1. We tested the hypothesis that PIGF treatment reduces placental ischemia-induced hypertension by antagonizing sFlt-1 without adverse consequences to the mother or fetus. On gestational day 14, rats were randomized to 4 groups: normal pregnant or RUPP±infusion of recombinant human PIGF (180 μg/kg per day; AG31, a purified, recombinant human form of PIGF) for 5 days via intraperitoneal osmotic minipumps. On day 19, mean arterial blood pressure and plasma sFlt-1 were higher and glomerular filtration rate lower in RUPP than normal pregnant rats. Infusion of recombinant human PIGF abolished these changes seen with RUPP along with reducing oxidative stress. These data indicate that the increased sFlt-1 and reduced PIGF resulting from placental ischemia contribute to maternal hypertension. Our novel finding that recombinant human PIGF abolishes placental ischemia-induced hypertension, without major adverse consequences, suggests a strong therapeutic potential for this growth factor in preeclampsia. (Hypertension. 2016;67:740-747. DOI: 10.1161/HYPERTENSIONAHA.115.06783.)

Key Words: blood pressure ■ preeclampsia ■ pregnancy ■ rat ■ RUPP

Preeclampsia is a dangerous disorder of pregnancy characterized by new-onset hypertension on or after the 20th week of gestation accompanied by dysfunction of cardiovascular, cerebral, visual, or renal systems. Preeclampsia is a leading cause of maternal and fetal morbidity and mortality. This hypertensive disorder of pregnancy is the second leading cause of pregnancy-induced maternal death globally behind bleeding complications.1 Unfortunately, the most effective treatment currently for preeclampsia is early delivery of the fetus and the ischemic placenta. Therefore, identifying novel therapeutic targets and agents against this maternal disorder is the subject of intensive experimental investigation.

Placental ischemia is strongly linked to the development of hypertension during pregnancy. The clearest evidence for this comes from experiments in nonhuman primates, rats, and mice showing that experimentally induced placental ischemia elicits maternal hypertension and proteinuria.2,4 Reducing uterine blood flow by ≈40% in the reduced uterine perfusion pressure (RUPP) rat model produces pronounced hypertension and reductions in glomerular filtration rate (GFR).3,5 We and others have shown that placental ischemia-driven angiogenic imbalance is causative in this hypertensive response. RUPP rats and preeclamptic women have increased placental and circulating soluble fms-like tyrosine kinase-1 (sFlt-1), which quenches circulating free vascular endothelial growth factor (VEGF) levels and placental growth factor (PIGF).6-8 Supplementing RUPP rats with recombinant rat VEGF abolished their hypertension.6

Patient safety is a critical factor in developing a therapy, particularly for the high-risk preeclamptic population. Although PIGF and VEGF are both natural ligands for sFlt-1, VEGF also binds with high affinity to fetal liver kinase 1 receptor (Flk-1) and, therefore, may lead to side-effects related to excess Flk-1 signaling. PIGF is uniquely specific for sFlt-1. Another advantage of PIGF is that, in contrast to small molecule therapies, PIGF circulates as a dimer (molecular...
weight of 45 kDa) and is significantly above the threshold size for proteins that can cross the placental barrier (<5 kDa). PIGF concentrations are significantly lower in patients with preclampsia. Therefore, we tested the hypothesis that administration of PI GF would prevent the effects of placental ischemia on blood pressure and renal hemodynamics in RUPP rats and reduce circulating sFlt-1 and oxidative stress found in this model of preeclampsia.

**Methods**

**Studies in Experimental Animals**

All animal experiments were conducted in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals with all animal-use protocols approved by The University of Mississippi Medical Center’s Institutional Animal Care and Use Committee. Timed-pregnant Sprague–Dawley rats were purchased from Harlan Laboratories (Indianapolis, IN; barrier no 202A). Gestational day 0 was defined as the presence of vaginal plug. All measurements were collected at day 19.

**RUPP Procedure and PI GF Infusion**

On gestational day 14, the RUPP procedure was performed as previously detailed, or rats remained in the normal pregnant (NP) group. In a subset of NP or RUPP rats, Alzet osmotic minipumps were loaded with recombinant human (rh) PI GF and placed in the intraperitoneal cavity. Gestational day 14 on day 14 for infusion (180 μg/kg/day) for 5 days until day 19. Dams that had real reabsorptions were excluded from the study.

**Mean Arterial Blood Pressure**

On gestational day 19, mean arterial blood pressure and heart rates were collected as described. We performed blood pressure measurements in a separate set of 6 NP rats infused with Dulbecco’s phosphate-buffered saline vehicle. Blood pressure was similar between NP+vehicle pump and NP without pump (data not shown), the latter was included in the comparisons for hemodynamic and biochemical measurements. RUPP untreated rats were not infused with vehicle, but they did have a similar incision site as to those RUPP rats treated with PI GF; this is because the intraperitoneal osmotic minipumps were placed in the abdominal cavity via the incision already made for the RUPP surgery.

At gestational day 19, blood was collected for isolation of plasma, and the sum of total and average fetal and placental weights were calculated per rat, then averaged per group. Total viable or reabsorbed fetuses were noted. Percent fetal absorption=(number of absorbed fetuses/total number of numbers)×100. Maternal body weight=mother weight−(fetal and placental weight).

**Glomerular Filtration Rate**

A subset of the rats used for the blood pressure measurements were included for the GFR measurements. We did not include the NP+rhPI GF group in the GFR measurements because there was no effect of rhPI GF on blood pressure in this group. We used a noninvasive-clearance measurement for transcutaneously assessing GFR, as developed by Mannheim Pharma & Diagnostics GmbH (Mannheim, Germany). On gestational day 18, a jugular catheter was implanted. On day 19, rats were briefly anesthetized with 3% to 5% isoflurane to remove hair from the nap of the neck with depilatory cream; USB device and attached battery placed on on the skin using doubled-sided adhesive tape that was immobilized and protected using an infusion jacket (Kent Scientific, Torrington, CT); and the jugular catheter extended. The rats were allowed to recover from anesthesia for 15 to 20 minutes followed by a bolus dose of 3 mg/100 g body weight FITC-Sinistrin in 0.2 mL sterile irrigation saline (Baxter Healthcare Corporation, Deerfield, IL) via jugular catheter while rat moved freely in cage. Data were collected, in units of mL/min per 100 g body weight, from the USB device per manufacturer’s instructions. This value for each rat was multiplied by its absolute body weight to present data as absolute GFR as mL/min.

**Drug**

The rhPI GF protein that was used in our infusion studies was generated by Aggamin Biologics (New York, NY). Briefly, cDNA encoding human PI GF131 (GenBank Accession number P49763-2) was cloned into an expression vector and electroporated into Chinese Hamster Ovary cells. The rhPI GF-1 protein was purified to >90% purity via column chromatography and formulated in Dulbecco’s phosphate-buffered saline pH 7.4.

**Quantification of Circulating rhPI GF and sFlt-1 Levels**

Circulating levels of rhPI GF and sFlt-1 in plasma samples were quantitated using an enzyme-linked immunosorbent assay detecting human PI GF or mouse Flt-1, respectively (R&D Systems, Minneapolis, MN) as described. Mouse Flt-1 kit measures free sFlt-1 as described elsewhere.

**Marker of Oxidative Stress**

Circulating levels of 8-iso-prostanates were quantified in plasma using ELISA kit (Cayman Chemicals, Ann Arbor, MI).

**Statistics**

Data were graphed and statistics performed using GraphPad Prism version 6.0. Data are presented as mean±standard error of the mean (SEM). For all data, a 1-way analysis of variance was conducted followed by a Holm–Sidak multiple comparisons test. The exception was data in Figure 1 where a nonparametric 1-way analysis of variance with an experimental design not assuming Gaussian distribution was conducted followed by a Dunn’s post hoc test. Statistical significant differences were defined as P<0.05.

**Results**

The in vivo study demonstrated that, at gestational day 19, circulating levels of rhPI GF were readily detectable but not significantly different after 5 days of infusion at the dose of 180 μg/kg per day in NP and RUPP rats (Figure 1). This recombinant human protein was not detected in the noninfused NP and RUPP controls.
Mean arterial blood pressure was increased in response to RUPP compared with NP controls by gestational day 19 (Figure 2A). Administration of 180 μg/kg per day rhPlGF abolished the development of this placental ischemia-induced hypertension. However, this dose of rhPlGF had no effect on blood pressure regulation in RUPP rats but did not alter the hypertensive response to RUPP (data not shown). Heart rates were similar between the NP, RUPP, RUPP+180 μg/kg per day rhPlGF and NP+180 μg/kg per day rhPlGF groups (Figure 2B). Figure 3 illustrates that the reduced GFR induced during placental ischemia in the RUPP rats was significantly abrogated by rhPlGF treatment.

Maternal body weights were significantly lower in RUPP versus NP rats, and these weights were not altered by rhPlGF administration (Figure 4A). This reduced maternal body weight in RUPP was largely because of the reductions in total fetal (Figure 4B) and placental weight (Figure 4C), and rhPlGF had no effect on these weights either. Average pup weights were reduced (P<0.05) in RUPP (2.08±0.06 g) versus NP (2.37±0.06 g) but was not affected by rhPlGF treatment in either group (RUPP+180 rhPlGF: 2.12±0.05 g or NP+180 rhPlGF: 2.25±0.05 g). Average placental weight was similar between all groups: NP, 0.51±0.02 g; RUPP, 0.47±0.02 g; RUPP+180 rhPlGF, 0.52±0.10 g; and NP+180 rhPlGF, 0.48±0.02 g. The number of viable fetuses was reduced (P<0.05) in RUPP (4±0.6) versus NP (13±0.7) with no influence of rhPlGF on these numbers (RUPP+180 rhPlGF: 6±0.9 or NP+180 rhPlGF: 14±0.4). Likewise, the number of reabsorptions was greater (P<0.05) in RUPP (9.3±0.9) versus NP (1.3±0.6) and was not altered by rhPlGF (RUPP+180 rhPlGF: 8.2±1.1 or NP+180 rhPlGF: 0.5±0.3). The number of pregnancies used for these fetal biometrics were N=10 (NP), N=16 (RUPP), N=14 (RUPP+180 rhPlGF), and N=6 (NP+180 rhPlGF). Thus, percent fetal absorption rate was significantly greater in RUPP versus NP, and rhPlGF did not influence this measure in the 2 pregnant groups (Figure 4D).

To assess the anti-angiogenic (Figure 5A) and reactive oxygen species (Figure 5B) profiles in the NP and RUPP after rhPlGF, we examined plasma levels of sFlt-1 and 8-iso-prostanes, respectively. Circulating levels of this anti-angiogenic factor was significantly greater in response to placental ischemia in untreated RUPP rats. Administration of rhPlGF dramatically reduced these levels back to NP values. The levels of sFlt-1 were similar between NP and NP+180 μg/kg per day rhPlGF. Similarly, 8-iso-prostanes was significantly increased in RUPP over NP, with rhPlGF treatment bringing these levels in RUPP qualitatively closer to NP values. Thus, there was not a significant difference in these values between the NP and RUPP+180 μg/kg per day rhPlGF groups. The rhPlGF did not have any effect on these levels in NP rats.

**Discussion**

Here we provide evidence that pharmacologically increasing circulating levels of PlGF has potential as an effective therapeutic strategy to reduce blood pressure during placental ischemia-induced hypertension. Administration of purified recombinant human PlGF abolished hypertension and
the reductions in GFR induced in RUPP pregnant rats. There was no effect on maternal weights or fetal biometrics, as assessed by fetal/placental weights and fetal absorption rates in response to RUPP. We also show that PlGF administration in RUPP pregnant rats prevented the development of hypertension and dramatically reduced the placental ischemia-driven rise in bioavailable levels of free sFlt-1, postulated to be a major pathogenic mediator of the anti-angiogenic state in preeclampsia.

The motivation for this project comes from the unmet need for interventions in preeclampsia. The need is urgent to develop safe, effective, and targeted therapies for preeclampsia to reduce maternal and fetal morbidity and mortality. This is unquestionably a global public health priority. A wealth of studies in humans indicate that anti-angiogenic mechanisms are involved in the pathogenesis of preeclampsia. Elevated sFlt-1 levels precede the onset of preeclampsia and correlate with disease severity. Moreover, well over 100 reports implicate increased sFlt-1 in reducing bioavailable VEGF and PlGF, both in humans and experimental models of preeclampsia. Notably, the study performed by Maynard et al demonstrated a dose–response effect of increasing sFlt-1 levels and reductions in VEGF and PlGF corresponding to the degree of hypertension found in their sample of preeclamptic women. These findings suggest that targeting this sFlt1-mediated anti-angiogenic pathway may be a promising strategy to treat preeclampsia.

A direct role for sFlt-1 in causing hypertension during pregnancy was demonstrated by infusion or adenoviral overexpression of this factor, which resulted in the development of hypertension by the end of pregnancy in both mice and rats. The levels of sFlt-1 are increased in RUPP rodents with placental ischemia-induced hypertension compared with their NP counterparts. Our laboratory previously showed that there are significant reductions in free VEGF and PlGF levels in RUPP rats. Supplementing these RUPP rats with recombinant VEGF121 for the duration of the placental ischemia abolished their hypertension and restored GFR to NP levels. The rationale for administering the rhPlGF via intraperitoneal minipump was based on these previous studies, which demonstrated that infusion of VEGF via intraperitoneal infusion significantly increased circulating VEGF levels. Until now, it was not known whether PlGF can also attenuate the heightened blood pressure levels occurring in response to placental ischemia, without the risk of off-target effects associated with VEGF. Moreover, as the reductions in circulating PlGF are a better predictor than VEGF levels for the onset of preeclampsia, we set out to examine whether PlGF has a beneficial effect on the RUPP animal model of preeclampsia.

In this study, we showed that rhPlGF abolished the development of hypertension and prevented the reductions in GFR resulting from placental ischemia. These findings implicate...
alternatives for measuring GFR showing similar reduction in GFR with RUPP (delta fall = 1.2 mL/min) compared with our previous studies using urinary clearance of radioactive inulin (delta fall = 1.6 mL/min). However, we did find that the absolute values for GFR were greater in both of these groups with using the transcutaneous technique. With this technique, a continuous disappearance curve is generated; therefore, the number of measurement points is much higher and the 95% confidence intervals narrower compared with blood or urinary sample measurements. This results in more robust curve fitting and thus seems more sensitive than other clearance techniques for assessing GFR.15

Alternative splicing of PIGF results in 4 different isoforms in human.22 PIGF-1 and PIGF-3 are diffusible because they lack heparin-binding sites. Aggamin Biologics expressed and purified a recombinant form of PIGF. We hypothesized that administration of rhPIGF for the duration of the RUPP protocol would reduce the hypertensive response to placental ischemia in rats. The dose of 180 μg/kg per day of rhPIGF is same as the dose of VEGF used in the supplementation study referenced above.2 In preliminary dose response experiments using doses of 90, 180, 300, 420 μg/kg per day, the maximal blood pressure lowering at 180 μg/kg per day was not any different than the 2 higher doses (data not shown). We think that the reason rhPIGF administration at the dose of 90 μg/kg per day did not reduce the RUPP hypertensive blood pressure levels is because it did not result in a significant rise in circulating PIGF levels (6±3 ng/mL), whereas those that were infused with double the dose had 21±3 ng/mL plasma levels of rhPIGF. This small rise in rhPIGF in the lower dose group did not change RUPP blood pressure levels at all compared with RUPP untreated, whereas the higher dose abolished the RUPP hypertension. This is similar to the findings in our previous VEGF supplementation paper.9 Furthermore, because we used the same infusion dose of VEGF that we previously showed brought the hypertensive blood pressure levels in RUPP back down to NP levels, controlling proper PIGF availability seems just as important as VEGF in blood pressure regulation during healthy pregnancies. However, in favor of PIGF as the angiogenic therapy of choice for preeclampsia, we propose that PIGF would have fewer side effects because it does not bind VEGF receptor 2 (or Flk-1) which is thought to mediate vascular leak. In contrast, VEGF binds both Flt-1 and Flk-1, the latter of which promotes edema when pathophysiologic increases in VEGF occur. Thus, we propose that PIGF administration exerts its effects by binding to Flt-1 and displacing endogenous VEGF to act on Flk-1 without causing potential side effects that may arise with exogenously increasing VEGF levels. Therefore, based on our dose–response data, we propose 180 μg/kg per day is the optimal dose for biological efficacy.

In the mother, circulating levels of PIGF increase and peak at 30 weeks of gestation, whereas in preeclampsia, it peaks before 25 weeks and then falls.23 This is similar to the timing of the onset of the hypertension in preeclampsia.24 These findings and our data highlight the importance of PIGF on maternal blood pressure regulation during pregnancy and preeclampsia. It is interesting that PIGF restored blood pressure to normal pregnancy levels as we had previously observed with infusion of VEGF.9 It is possible that these 2
angiogenic factors act cooperatively to regulate blood pressure during pregnancy. Mechanistically, PIGF only binds the Flt-1 (VEGFR1), whereas VEGF binds both VEGFR1 and VEGFR2 (Flk-1). Therefore, it is thought that increased PIGF binding to Flt-1 shunts more VEGF to VEGFR2. The VEGFR2 receptor elicits most of the angiogenic and vasoprotective actions of VEGF. Furthermore, our data indicate that exogenously increasing circulating levels of PIGF also quenches bioavailable sFlt-1. Future studies should examine the relative importance of each of these pathways and placental production of soluble factors on the effects of rhPIGF to reduce blood pressure and increase GFR during placental ischemia. Furthermore, it is not yet clear whether rhPIGF administration had any beneficial effect on the fetus, even though maternal blood pressure was reduced without changing fetal weight at gestational day 19. Future studies should examine long-term cardiovascular and metabolic outcomes in these offspring to determine if this intervention serves to benefit both mother and the baby of preeclamptic pregnancies.

Because no effective treatment is available, it is urgent to develop a novel and effective drug therapy for preeclampsia. Premature delivery by induced labor or Caesarean section is the only recourse to ensure mother’s safety, but it puts the neonate at risk for multiple complications and results in poor neonatal outcomes. Important clinical trials are directing us toward development of more rational and targeted therapies. For instance, trials with pravastatin are underway to ameliorate preeclampsia by inhibiting HMG CoA reductase, but because statins are contraindicated in pregnancy, this class of drugs has a high risk of teratogenic potential. Interestingly, the mechanistic rationale for pravastatin use is its potential to upregulate VEGF and PIGF and correct the angiogenic imbalance of excess sFlt-1 in RUPP rats. Although this rationale is consistent with our therapeutic approach, administering rhPIGF is likely to be a more direct and a safer strategy to treat preeclampsia. We believe PIGF therapy to be superior to the current treatment strategies because they are wrought with issues of drugs losing potency because of tolerance and their potential teratogenic effects. This latter point is especially true for endothelin A antagonists during pregnancy. However, based on previous studies and our results showing plasma 8-iso-prostanate, a measure of reactive oxygen species, is reduced by PIGF infusion support that vascular dysfunction and oxidative stress occurs downstream of placental ischemia and increased sFlt-1 levels to elicit hypertension. Therefore, we think that initial alterations in angiogenic balance, such as increased sFlt-1, results in these prohypertensive mechanisms and that rhPIGF therapy protects against this vascular dysfunction and hypertension by binding to sFlt-1 and improving oxidative stress.

Perspectives

Hypertensive disorders of pregnancy not only increase the risk for maternal/fetal morbidity and mortality, but it also predisposes the mother for a higher risk of developing hypertension later in life. Therefore, it is important to find novel therapeutic strategies to maintain healthy blood pressure regulation during preeclamptic events. As patient safety is a critical factor in developing a therapy for this high-risk population, it is important to mitigate any risk of off-target effects. Although PIGF and VEGF are both natural ligands for sFlt-1, only PIGF is specific for the Flt-1 receptor, whereas VEGF also binds to VEGFR2 and can promote side-effects related to excess VEGF signaling, such as increased vascular permeability and edema. Furthermore, PIGF-1 has the ability to form heterodimers with VEGF, and this may prevent such adverse effects of increasing VEGF signaling through VEGFR2 after exogenous application of PIGF. In models of inflammation, PIGF (but not VEGF) has been shown to protect the vasculature, which is particularly relevant in preeclampsia, a profoundly proinflammatory state. In conclusion, with readily-available assays to identify women most likely to benefit from PIGF therapy, our goal is to be able to guide appropriate timing of therapy initiation and discontinuation, minimizing maternal and fetal risk as clinical development progresses. We have previously shown that angiogenic imbalance with reduced PIGF and increased sFlt-1, that is, high sFlt-1 to PIGF ratio, is a robust biomarker predicting the development of preeclampsia and its related complications. Indeed, among high-risk individuals, this imbalance is present as early as 12 to 15 weeks of gestation, which is several months before the clinical manifestations of this disorder.

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Disclosures

S.A. Karumanchi is a coinventor on patents related to the use of angiogenic factors for the diagnosis and treatment of preeclampsia that are held by the Beth Israel Deaconess Medical Center. S.A. Karumanchi has financial interest in Aggamin LLC and reports serving as a consultant to Roche, Siemens, and Thermofisher Scientific. A.Y. Tan, W.S. Joo, G. Daniels, and P. Kussie are employees of Aggamin LLC. All other authors disclose no conflict.

References


What Is New?

- The role of placental growth factor (PlGF), an endogenous ligand for Flt-1 on the endothelium, in pregnancy-induced hypertension and preeclampsia is unclear.
- Our novel finding is that administration of recombinant human PlGF abolished placental ischemia-related hypertension in a pregnant rat model of preeclampsia that was accompanied by reductions in circulating free soluble fms-like tyrosine kinase-1 (sFlt-1).
- Elevations of the anti-angiogenic factor sFlt-1 occur before the onset of preeclampsia in humans; are increased in reduced uterine perfusion pressure rats; and are known to elicit hypertension in pregnant experimental animals on its own.

What Is Significant?

- In the past, interventions evaluated for their ability to prolong pregnancy in patients with preeclampsia (e.g., treatment for mild hypertension, plasma volume expansion, digibind, heparin, and corticosteroid use) have been unsuccessful or are not recommended for prevention of preeclampsia because of adverse effects to the fetus.36–41
- Our data have significant relevance toward the development of the novel treatment strategies for preeclampsia.

Summary

PlGF is abundantly made in normal pregnancy, but because of abnormally high circulating levels of sFlt-1 in preeclampsia, levels of free PlGF are insufficient. Correcting this angiogenic imbalance with a naturally occurring protein may limit the occurrence of unwanted systemic side effects. As recombinant human PlGF protein is identical in structure and function to human PlGF, it is unlikely to be antigenic or induce an immune reaction. Our data support the development of a treatment strategy for preeclampsia using naturally occurring human PlGF.
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