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 maternal and fetal morbidity and mortality. This placental imbalance is causative in this hypertensive response. PlGF 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. PlGF is specific for sFlt-1. We tested the hypothesis that PlGF 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 PlGF (180 μg/kg per day; AG31, a purified, recombinant human form of PlGF) 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 PlGF abolished these changes seen with RUPP along with reducing oxidative stress. These data indicate that the increased sFlt-1 and reduced PlGF resulting from placental ischemia contribute to maternal hypertension. Our novel finding that recombinant human PlGF 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.)
weight of 45 kDa) and is significantly above the threshold size for proteins that can cross the placental barrier (<5 kDa). PlGF concentrations are significantly lower in pregnant rats and decrease in preeclamptic rats.

Therefore, we tested the hypothesis that administration of PlGF 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 PlGF 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) PlGF and placed in the intraperitoneal cavity on gestational day 14 for infusion (180 μg/kg/day) for 5 days until day 19. Dams that had total 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. Present data as absolute GFR as mL/min. This value for each rat was multiplied by its absolute body weight to present data as absolute GFR as mL/min.

Quantification of Circulating rhPlGF and sFlt-1 Levels

Circulating levels of rhPlGF and sFlt-1 in plasma samples were quantitated using an enzyme-linked immunosorbent assay detecting human PlGF or mouse Fli-1, respectively (R&D Systems, Minneapolis, MN) as described.

Marker of Oxidative Stress

Circulating levels of 8-iso-prostanates were quantified in plasma using an 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 Dunn’s post hoc test. Statistical significance 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 rhPlGF 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.

Figure 1. Detection of recombinant human placental growth factor (rhPlGF) levels in normal pregnant (NP; N=13), reduced uterine perfusion pressure (RUPP; N=18), RUPP+180 μg/kg per day rhPlGF (N=13), and NP+180 μg/kg per day rhPlGF rats (N=6). *P<0.05 vs corresponding untreated control pregnant rats.
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 levels in the NP group. Studies were also conducted to examine the effects of 90 μg/kg per day rhPlGF 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-prostanates 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.

A direct role for sFlt-1 in causing hypertension during pregnancy was demonstrated by infusion or adenoviral over-expression 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.6,12 Our laboratory previously showed that there are significant reductions in free VEGF and PlGF levels in RUPP rats.6 Supplementing these RUPP rats with recombinant VEGF121 for the duration of the placental ischemia abolished their hypertension and restored GFR to NP levels.9 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,10 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

Figure 4. Maternal body weight (A), total fetal weight (B), total placental weight (C), and fetal absorption (D) as a percent in normal pregnant (NP; N=11), reduced uterine perfusion pressure (RUPP; N=15), RUPP+180 μg/kg per day recombinant human placental growth factor (rhPlGF; N=12), and NP+180 μg/kg per day rhPlGF rats (N=5). *P<0.05 vs untreated NP rats.

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.7,13 Moreover, well over 100 reports implicated increased sFlt-1 in reducing bioavailable VEGF and PlGF, both in humans and experimental models of preeclampsia.19 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.7 These findings suggest that targeting this sFlt1-mediated anti-angiogenic pathway may be a promising strategy to treat preeclampsia.
alternatives 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.9 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 pathophysiological 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, PlGF only binds the Flt-1 (VEGFR1), whereas VEGF binds both VEGFR1 and VEGFR2 (Flk-1). Therefore, it is thought that increased PlGF 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 PlGF 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 rhPlGF to reduce blood pressure and increase GFR during placental ischemia. Furthermore, it is not yet clear whether rhPlGF 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 PlGF and correct the angiogenic imbalance of excess sFlt-1 in RUPP rats. Although this rationale is consistent with our therapeutic approach, administering rhPlGF is likely to be a more direct and a safer strategy to treat preeclampsia. We believe PlGF 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-isoprostanate, a measure of reactive oxygen species, is reduced by PlGF 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 rhPlGF 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 and neonate 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 PlGF and VEGF are both natural ligands for sFlt-1, only PlGF is specific for the Flt-1 receptor, whereas VEGF also binds to VEGFR2 and can promote side-effects related to excess VEGFR2 signaling, such as increased vascular permeability and edema. Furthermore, PlGF-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 PlGF. In models of inflammation, PlGF (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 PlGF 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 PlGF and increased sFlt-1, that is, high sFlt-1 to PlGF 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. Kusie are employees of Aggamin LLC. All other authors disclose no conflict.

References


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**Novelty and Significance**

**What Is New?**
- The role of placental growth factor (PIGF), 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 PIGF 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**

PIGF is abundantly made in normal pregnancy, but because of abnormally high circulating levels of sFlt-1 in preeclampsia, levels of free PIGF are insufficient. Correcting this angiogenic imbalance with a naturally occurring protein may limit the occurrence of unwanted systemic side effects. As recombinant human PIGF protein is identical in structure and function to human PIGF, 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 PIGF.
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 and Joey P. Granger

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