Placental Growth Factor Reduces Blood Pressure in a Uteroplacental Ischemia Model of Preeclampsia in Nonhuman Primates

Angela Makris, Kristen R. Yeung, Shirlene M. Lim, Neroli Sunderland, Scott Heffernan, John F. Thompson, Jim Iliopoulos, Murray C. Killingsworth, Jim Yong, Bei Xu, Robert F. Ogle, Ravi Thadhani, S. Ananth Karumanchi, Annemarie Hennessy

See Editorial Commentary, pp 1114–1116

Abstract—An imbalance in the angiogenesis axis during pregnancy manifests as clinical preeclampsia because of endothelial dysfunction. Circulating soluble fms-like tyrosine kinase 1 (sFlt-1) increases and placental growth factor (PIGF) reduces before and during disease. We investigated the clinical and biochemical effects of replenishing the reduced circulating PIGF with recombinant human PIGF (rhPIGF) and thus restoring the angiogenic balance. Hypertensive proteinuria was induced in a nonhuman primate (Papio hamadryas) by uterine artery ligation at 136 days gestation (of a 182-day pregnancy). Two weeks after uteroplacental ischemia, rhPIGF (rhPIGF, n=3) or normal saline (control, n=4) was administered by subcutaneous injection (100 μg/kg per day) for 5 days. Blood pressure was monitored by intra-arterial radiotelemetry and sFlt-1 and PIGF by ELISA. Uteroplacental ischemia resulted in experimental preeclampsia evidenced by increased blood pressure, proteinuria, and endotheliosis on renal biopsy and elevated sFlt-1. PIGF significantly reduced after uteroplacental ischemia. rhPIGF reduced systolic blood pressure in the treated group (−5.2±0.8 mm Hg; from 132.6±6.6 mm Hg to 124.1±7.6 mm Hg) compared with an increase in systolic blood pressure in controls (6.5±3 mm Hg; from 131.3±1.5 mm Hg to 138.6±1.5 mm Hg). Proteinuria reduced in the treated group (−72.7±55.7 mg/mmol) but increased in the control group. Circulating levels of total sFLT-1 were not affected by the administration of PIGF; however, a reduction in placental sFlt-1 mRNA expression was demonstrated. There was no significant difference between the weights or lengths of the neonates in the rhPIGF or control group; however, this study was not designed to assess fetal safety or outcomes. Increasing circulating PIGF by the administration of rhPIGF improves clinical parameters in a primate animal model of experimental preeclampsia. (Hypertension. 2016;67:1263-1272. DOI: 10.1161/HYPERTENSIONAHA.116.07286.)

Key Words: animal model ■ hypertension ■ placental growth factor ■ preeclampsia/pregnancy

Preeclampsia is a pregnancy-related disorder resultant, in part, from imbalance of the angiogenesis axis.1 It has been shown that before the disease is clinically apparent, there is a reduction in the circulating PIGF and an increase in the circulating soluble fms-like tyrosine kinase 1 (sFlt-1).2 The reduction in PIGF has been noted as early as the first trimester in those women who will go on to develop preeclampsia subsequently in pregnancy.3,4 The reduced PIGF has been used in multifactorial assessments in predicting preeclampsia.5 Preeclampsia occurs in ≈2% to 5% of pregnancies,6,7 and the clinical manifestations are resultant from endothelial dysfunction and include proteinuria, hypertension, and fetal growth restriction among other signs and symptoms. Treatment thus far is expectant management, essentially prolongation of pregnancy by controlling blood pressure (BP) while balancing the risks of worsening endothelial dysfunction to mother and baby compared with the benefits of continued intrauterine growth.8 Ultimately, disease resolution requires the removal of the placenta—although many methods and agents have been evaluated as potential therapies9,10

Several agents have been tested to reverse the angiogenic imbalance in preclinical or animal models of preeclampsia: vascular endothelial growth factor (VEGF),11 sildenafil,10 statins,12 metformin,13 and Kraussianone-2.14 Most of the agents reduce the available or secreted sFlt-1; however, the effect on PIGF has not been assessed in all studies. Statins have been shown to increase PIGF;15,16 Sildenafil has been tested clinically, and despite its biochemical benefits, it has not been shown to improve clinical outcomes.17

Received February 4, 2016; first decision February 14, 2016; revision accepted March 4, 2016.

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Hypertension is available at http://hyper.ahajournals.org

DOI: 10.1161/HYPERTENSIONAHA.116.07286

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Pregnancy and preeclampsia work has been undertaken in rodent\(^a\) and sheep\(^b\) models; however, nonhuman primate (Papio hamadryas hamadryas) animal models of preeclampsia have many advantages. The placenta is hemomonochorial, implantation is interstitial, the uterine blood flow is antigravity (similarly to humans), and typically each pregnancy involves a single fetus and a long gestation as compared with rodents (normal baboon gestation, 182 days).\(^{20}\) The baboon placenta differs from human placenta: trophoblast invasion is more superficial and typically does not involve the myometrial segment of the uterine arteries and multinuclear giant cells are not present.\(^{21}\) Preeclampsia does not occur frequently in baboons, although there are case reports describing spontaneous preeclampsia.\(^{22}\) Inducing uteroplacental ischemia (UPI; by ligating unilateral, nondominant uterine artery) during pregnancy results in proteinuric hypertension, renal endotheliosis, and an associated rise in sFLT-1\(^{23}\); the effect on circulating PlGF has not been previously assessed.

Rising PlGF correlates to improved placental perfusion in human pregnancies.\(^{24}\) Although UPI has been shown to be pivotal to the development of preeclampsia,\(^{25}\) the change in PlGF concentrations resultant from placental ischemia has not been investigated. Using a nonhuman primate model of UPI,\(^{23}\) we investigated the effect of the ischemia on PlGF concentrations. Furthermore, because PlGF does not bind VEGF receptor 2, it may have fewer side-effects than VEGF, such as excess vascular permeability and edema. We hypothesized that by infusing PlGF, which binds and inactivates sFLT-1, there would be an improvement in clinical signs of an established preeclampsia syndrome (animal model of experimental preeclampsia), including BP and proteinuria, as well as biochemical changes, circulating sFLT-1, and PlGF.

**Methods**

**Animals**

Female pregnant baboons (Papio hamadryas, \(n=7\)) from the National Baboon Colony, New South Wales, Australia, were provided food and water ad libitum. At the beginning of the protocol, the animals were at 130 (±1.8) days’ gestation of a normally 182-day gestation. Animals were anesthetized using ketamine infusion (0.2 mg/kg per min) with a premedication of metoclopramide (5 mg intramuscularly) and clonazepam (0.01 mg/kg intravenously for seizure prophylaxis). All animals were anesthetized using ketamine infusion (0.2 mg/kg per min) with a premedication of metoclopramide (5 mg intramuscularly) and clonazepam (0.01 mg/kg intravenously for seizure prophylaxis). All animals received procedural antibiotics benzylpenicillin and tobramycin for 48 hours before the UPI). Drift in BP measurement was measured and readings were censored for activity.

After 1-week recovery, UPI was induced experimentally as described previously.\(^{22}\) Briefly, animals underwent nondominant unilateral uterine artery complete ligation through a midline abdominal transperitoneal incision. The iliac vessels were visualized, and the uterine artery identified at its origin from the internal iliac artery. The uterine artery was ligated with 1% lignocaine solution to reduce arterial spasm and then ligated with 4.0 silk sutures. Complete ligation was verified by performing repeat uterine artery duplex ultrasonography after the procedure that demonstrated no flow in the artery where previously flow had been demonstrated. The peritoneum, muscle, and skin were closed in layers. Animals were then observed for 2 weeks. Blood, urine, and chorionic villous samples were collected on days 3, 6, 10, and 14 of UPI. BP was monitored continuously by telemetry. After 2 weeks, recombinant human placental growth factor (rhPlGF; R & D, Minneapolis, MN) reconstituted in 0.9% normal saline and was administered to 3 animals subcutaneously at a dose of 100 μg/kg per day (similarly to Suzuki et al)\(^{27}\) as a single injection for 5 days administered between 0600 and 0700 hours each day. The control group (n=4) received an equal volume saline, 10 mL 0.9% normal saline. Blood, urine, and chorionic villous samples were taken at days 3 and 5 of rhPlGF (or vehicle) administration. A timeline (Figure 1) demonstrates when the procedures were performed.

**Specimen Collection**

Blood was taken from the cubital vein and centrifuged for 10 min at 3000 rpm. The plasma was aliquoted (250 μL aliquots) and stored at −80°C for future analysis of sFLT-1 and PlGF by ELISA. The peripheral blood mononuclear cells were isolated from blood using standard methods\(^{28}\) for polymerase chain reaction (PCR) of sFLT-1 and PlGF. Catheterized spot urinary protein excretion was adjusted for creatinine excretion and expressed as mg/mmol. Placental chorionic villous samples were performed transabdominally as described previously\(^{29}\) and underwent PCR for sFLT-1 and PlGF. Term placentas could not be collected because the animals ingest these immediately.

**Renal Biopsy**

Percutaneous renal biopsies were performed sequentially in each animal, resulting in 3 biopsies in each animal. The baseline biopsy was performed immediately after the insertion of the telemeter. The second biopsy was performed 2 weeks after UPI. The last biopsy was performed after the 5 days of rhPlGF or 0.9% normal saline injections. Each time the procedure was identical. The animals were placed in the right or left recumbent position on a soft wedge, and a 3 to 4 cm subcostal skin incision anterior to the erector spinale muscle was made. Blunt dissection was performed till the renal capsule kidney was identified without disrupting the peritoneum. A small wedge biopsy was taken from the lower pole (0.5 cm×0.5 cm×0.5 cm), the specimen was divided, and the portions immediately placed in 2% glutaraldehyde for electron microscopy or 10% formalin for later paraffin block embedding or snap-frozen in liquid nitrogen. Gelfoam (Upjohn, WA, Australia) was placed in the renal defect to ensure adequate hemostasis and then closure in layers.

**ELISA**

Baboon plasma sFLT-1 and PlGF and urinary cyclic guanosine monophosphate (cGMP) was measured by a commercially available human ELISA (R&D, Minneapolis, MN) in duplicate. The coefficient of variation for all assays was <5%, and the lower limit of detection for the sFLT-1 ELISA was 20 pg/mL, for PlGF ELISA was 2 pg/mL, and for the cGMP was 2 pmol/mL. Human sFLT-1 ELISA measures total sFLT-1, and human PlGF ELISA kit measures free PlGF concentrations as described elsewhere.\(^{29}\)

**Quantitative PCR**

RNA was extracted from isolated peripheral blood mononuclear cells and chorionic villous tissue using a commercially available kit (NucleoSpin RNAI; Macherey-Nagel) which has been described previously.\(^{30}\) One microgram of RNA was reverse transcribed to cDNA using Superscript III RNase Reverse Transcriptase (Invitrogen). Quantitative PCR was performed by the comparative threshold cycle method and normalized to \(β\) microglobulin expression. The primers used for sFLT-1, PlGF, and \(β\) microglobulin have been described.\(^{31}\)
RNA expression is expressed as the percentage change compared with baseline (pre-UPI) samples.

**Statistical Analysis**

Statistical analysis was performed (using SPSS v22.0) with general linear model repeated measures, Students t test and a bonferroni correction was applied where applicable to allow for adjustment of multiple comparisons. Data are expressed as mean±SEM. Significance was set at P<0.05.

**Results**

UPI resulted in a significant elevation in BP (P<0.001), which was evident within 3 days of the UPI, as has been demonstrated previously.23 There was no significant difference in baseline maternal weight (rhPlGF group: 14.9±1.0 kg and control group: 13.9±0.2 kg), BP, proteinuria, sFLT-1, or PlGF concentrations between the rhPlGF and control groups. After the UPI, there was a significant rise in BP from a baseline of 120±3.9/80±3.1 mm Hg (awake) and 121±3.3/81±3.4 mm Hg (sleep) to a BP of 132±3.0/91±3.6 mm Hg (awake) and 132±3.7/89±3.4 mm Hg (sleep) after 14 days of UPI. The changes in BP were evident at awake and asleep (Figure 2A–2D). Proteinuria was not present at baseline (spot urinary protein:creatinine concentration ratio <5 mg/mmol), but significantly (P<0.05) increased as a result of the UPI from day 6 UPI onwards (day 14 UPI spot urinary protein: creatinine concentration ratio 57.4±16.7 mg/mmol; Figure 2E). There was a significant change in plasma PlGF after the induction of UPI (P=0.025, general linear model repeated measures; Figure 3A). There was no significant difference between the 2 groups. Taken together, the PIGF levels were notably lower after 3 days of UPI compared with baseline (baseline: 14.1±4.9 pg/mL; day 3: 3.1±1.7 pg/mL; P<0.05). The PIGF levels increased after day 10 UPI back to baseline levels without intervention other than sampling as described earlier.

UPI resulted in a significant elevation in total plasma sFLT-1 concentration (n=7, all animals grouped; baseline: 1508±250 pg/mL and day 14 UPI: 21077±2209 pg/mL; P<0.0001) that remained elevated for the duration of UPI. At baseline and after 14 days UPI, there was no significant difference between the rhPIGF and control groups (day 14 UPI: 22860±4312.5 pg/mL and 19294±1617 pg/mL, respectively; P=0.48; Figure 3B). The urinary cGMP did not change from baseline either with an adjustment for urinary creatinine concentration (baseline ratio: 78.9±9.7 nmol/mmol and day 14 UPI: 85.6±24.7 nmol/mmol; P=0.80) or without a urinary creatinine concentration adjustment (P=0.25).

**Administration of rhPLGF**

The administration of PIGF resulted in a rise in plasma PIGF levels in the rhPIGF group. The change in plasma PIGF in the rhPIGF and the control group was 2116.6±1610 pg/mL and −0.3±2.6 pg/mL, respectively, after 5 days of subcutaneous injection. The difference between the 2 groups was statistically significant at day 3 of injection (Figure 4A; P=0.027). The urinary PIGF (either adjusted or not adjusted for the urinary creatinine excretion) did not differ statistically significantly between the groups after the administration of the rhPIGF (P=0.076); however, there was a trend to higher levels in the rhPIGF group (P=0.06; Figure 4B). The urinary concentrations of PIGF were markedly lower than the contemporaneous circulating PIGF concentrations. Although the concentrations in the circulation remained elevated till day 5 of injection, the urinary PIGF had reduced at day 5, suggesting a significant proportion was bound and still circulating. There was no significant change in urinary cGMP after the administration of rhPIGF (P=0.29).

The total sFLT-1 concentrations increased significantly more in the rhPIGF group at day 3 of injection compared with the control group (sFLT-1 rhPIGF: baseline, 22860±4312 and day 3, 33133±3624 pg/mL; control: 18997±1181 and day 3, 19965±2112 pg/mL; P=0.016, general linear model repeated measures, Bonferroni correction repeated measures; Figure 4C). Although there was a reduction in sFLT-1 concentration after day 3 in the control group, this was not statistically significant.

The administration of recombinant human PIGF for 5 days resulted in a reduction in the BP in the rhPIGF group compared with the control group (Figure 5A–5D). The reduction in BP was evident after 3 days of rhPIGF injections. There was a reduction in systolic (SBP) and diastolic (DBP) BP both while awake and during sleep in the rhPIGF compared with an elevation of the BP in the control group (SBP awake: P=0.015; DBP awake: P=0.026; SBP sleep: P=0.025; and DBP sleep: P=0.012). The awake SBP and DBP in the rhPIGF and the control groups altered by SBP −8.5±2.6 and 7.2±2.7 mm Hg, respectively, and DBP −5.5±2.0 and 5.4±1.6 mm Hg, respectively.

There was a significant reduction in proteinuria with the administration of rhPIGF (P=0.036). The change in proteinuria in the rhPIGF and control group was −72.7±55.7 mg/mmol and 16.7±4.8 mg/mmol, respectively, at day 5 of rhPIGF compared with at day 14 of UPI (Figure 6). Renal biopsies, after UPI, demonstrated changes consistent with
human-like preeclampsia. These findings were not clearly evident on the light microscopy. However, on electron microscopy, there is apparent endotheliosis, subendothelial fibrin deposition, as well as fibrin and fibrinoid deposition in the mesangium. These changes were evident in all animals. Qualitatively, there was an improvement in the changes in

Figure 2. Effects of uteroplacental ischemia (UPI) on systolic (SBP; A) and diastolic (DBP; B) blood pressure (mmHg) during sleep and systolic (C) and diastolic (D) blood pressure during awake periods and proteinuria (E) (expressed as the spot urinary protein:urinary creatinine ratio) on protocol days 3, 6, 10, and 14 after uteroplacental ischemia. Data for the control (n=4, green line), treated (n=3, red line), and total group (n=7, black line) is demonstrated on each graph. * indicates a significant difference of the grouped result compared with baseline blood pressure $P<0.001$. 
the rhPlGF group (Figure 7). The control group (Figure 7A–7C) renal biopsy electron microscopy did not appreciably change compared with the biopsy undertaken at the end of the UPI. There were still significant changes consistent with human-like preeclampsia. The changes were most marked in the animals given rhPlGF where there was a reduction in fibrin deposition and a reduction in subendothelial deposits (Figure 7D–7F).

rhPlGF administration resulted in a reduction in the sFLT-1 mRNA expressed in the placenta as measured by serial choriocord villous sampling compared with the control group ($P=0.021$; Figure 8). The animals given rhPlGF demonstrated a reduction in the placental mRNA expression over time. This was in contrast to the control group, where the mRNA expression increased after 3 days of rhPlGF. There was no significant change in the placental expression of PIgf. The expression

Figure 3. A, Effects of uteroplacental ischemia (UPI) on plasma placental growth factor (PIGF) grouped data (n=7) plasma PIGF at protocol days 3, 6, 10, and 14 after UPI. There is a significant change over time ($P=0.025$). B, Plasma total soluble fms-like tyrosine kinase 1 (sFLT-1) after UPI at protocol days 3, 6, 10, and 14 after UPI for the control (n=4, green line), treated (n=5, red line), and total group (n=7, black line) is demonstrated. * indicates a significant difference of the grouped result compared with baseline sFLT-1 concentration $P<0.001$.

Figure 4. Changes in placental growth factor (PIGF) and soluble fms-like tyrosine kinase 1 (sFLT-1) after recombinant human PIGF (rhPlGF). A, Plasma PIGF concentrations (pg/mL) in control (n=4, green line) and treated (n=3, red line) groups after the administration of rhPlGF 100 μg/kg or 0.9% saline subcutaneously in equal volumes, respectively. *The rhPlGF concentrations at day 3 of injection are significantly higher in the rhPlGF group compared with the control group ($P=0.027$). B, Urinary PIGF concentrations (pg/mL) in control (n=4, green line) and treated (n=3, red line) groups after the administration of rhPlGF 100 μg/kg or 0.9% saline subcutaneously in equal volumes, respectively. *The total sFLT-1 concentrations at day 3 of injection are significantly higher in the rhPlGF group compared with the control group ($P=0.016$).
of PlGF and sFLT-1 did not vary in isolated peripheral blood mononuclear cells as measured by real-time PCR.

All animals delivered at term. The neonates appeared normal on physical examination. There were no significant differences in the weights of the offspring of the 2 groups (rhPlGF group: 643±14 g and control group: 650±25 g).

**Discussion**

This study has demonstrated that the induction of UPI results in a reduction in circulating PlGF. This supports one of the etiological theories of preeclampsia—that reduced placental perfusion and, consequently, placental hypoxia or ischemia has a role in the clinical manifestation of the disease. Furthermore, this correlates with indirect evidence that women with reduced PlGF in early pregnancy have an increased risk of placental dysfunction (either as preeclampsia or growth restriction) later in pregnancy. These women have also been noted to develop smaller placentae with evidence of insufficient perfusion. The circulating PlGF in the current study increased at day 14, having reached a nadir at day 6 of ischemia. We postulate that the PlGF increase was secondary to partial reperfusion of the ischemic portion of the placenta. Improved placental perfusion, although indirectly, has been shown to be associated with increased circulating PlGF—in humans, an increase in PlGF has been shown to correlate with improved uterine perfusion as measured by uterine Doppler. Conversely, women with demonstrated histopathologic evidence of placental hypoperfusion had reduced PlGF compared with women with no placental hypoperfusion demonstrable. Gilbert et al have shown a similar magnitude reduction in PlGF in a rat reduced uterine placenta perfusion model.

The administration of exogenous PlGF in an experimental model of preeclampsia reduced the BP, proteinuria, and sFLT-1 placental mRNA expression. The circulating PlGF, as would be expected, increased after the administration of PlGF; however, the total sFLT-1 also increased. The increase in sFlt-1 may have been because of release of preformed sFLT-1 from tissues, as demonstrated by Zhao et al. The reduction in placental mRNA sFLT-1 may thus represent a feedback reduction secondary to the increased circulating levels of sFLT-1. Despite the increase in total sFLT-1, there was a reduction in BP during this time. The increase in sFLT-1 we suggest would

**Figure 5.** Change in systolic blood pressure (A), diastolic blood pressure (B) while asleep and systolic blood pressure (C) and diastolic blood pressure (D) awake (mmHg) after 3 and 5 days of recombinant human placental growth factor (rhPlGF) 100 μg/kg or control (0.9% normal saline) injections. The change in blood pressure in the control group (n=4, green line) and treated group (n=3, red line) is compared with the blood pressure after 14 days of uteroplacental ischemia (UPI; baseline PlGF). * indicates a significant difference compared with baseline PlGF, P<0.05.
hypoxic conditions, such as in the placenta, the formation of mRNA sequence as assessed by BLAST alignment. Although homologous with the human PlGF (Genbank: BC 007789.2) dated. However, we have sequenced part of the PlGF molecule means by which PlGF could have a positive proangiogenic effect, other than binding to the free sFLT-1.

There is a wide range of what is considered normal circulating PIGF in uncomplicated pregnancy,

be bound to the increased available PIGF and, thus, unable to exert its anti-angiogenic effect. There are other instances where the circulating sFLT-1 increases without a concomitant elevation in BP, such as with administration of heparin.

The therapeutic benefits of exogenous PIGF demonstrated reduction in BP and reduction in proteinuria, may be because of other factors as well. The rhPlGF, via activation of FLT-1 and sFLT-1 to a lesser extent, is potentiating the angiogenic response to VEGF. PIGF has also been shown to behave in an autocrine manner with VEGF, as well as to behave independently in activating endothelial cells.

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Although there is a wide range of what is considered normal circulating PIGF in uncomplicated pregnancy, the levels of PIGF after administration of rhPlGF in the current study were at the upper extreme of physiological levels. Others have demonstrated PIGF concentrations at the lower end of the uncomplicated human pregnancy range in the setting of lentivirus and adenoviral-induced experimental preeclampsia.

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Suzuki et al administered a similar dose of PIGF to a mouse adenoviral vector model of preeclampsia and demonstrated the improvement in BP but no change in proteinuria. They gave PIGF for 2 days, intraperitoneally. Kumawasa et al increased the concentrations of PIGF in a lentiviral model of preeclampsia indirectly by administering pravastatin but did not demonstrate a change in BP or proteinuria when administration was commenced late in pregnancy (embryonic day 16.5). If the pravastatin administration was commenced early in pregnancy (embryonic day 7.5), there was a significant reduction in proteinuria and BP at E16.5. However, with early administration of pravastatin, the magnitude increase in circulating PIGF at E16.5 was more modest than that seen in the current study (60.6–116.6 pg/mL). When the PIGF concentration was increased to a greater extent (using lentiviral transfection of the blastocyst), Kumawasa did demonstrate a reduction in BP, proteinuria, and improved glomerular endotheliosis in the mouse renal biopsies. Finally, Spradley et al administered human recombinant PIGF to a rat UPI model and showed a reduction in BP, but proteinuria was not assessed.

In each case, the sFLT-1 concentrations fell, which was in contrast to the current study; however, in this study, kits that measured murine-free sFlt-1 were used in contrast to our studies in which we used a human total sFlt-1 assay.

The improvement of PlGF in our study may also have resulted in the release of preformed membrane-bound sFLT-1, which has been described previously with proteases.

Although there is a wide range of what is considered normal circulating PIGF in uncomplicated pregnancy, the levels of PIGF after administration of rhPlGF in the current study were at the upper extreme of physiological levels. Others have demonstrated PIGF concentrations at the lower end of the uncomplicated human pregnancy range in the setting of lentivirus and adenoviral-induced experimental preeclampsia. This may have a role in the differing sFLT-1 levels seen after the increase in PIGF. Furthermore, the circulating sFLT-1 also differed between studies before the increase in PIGF. The adenoviral model developed levels of sFLT-1 that were physiologically high (113 ng/mL) and higher than the lentiviral model (5.84 ng/mL) whose levels were comparatively at the lower end of the range for women with preeclampsia. The sFLT-1 levels in our model were between the 2: at the end of 14 days of UPI (22 ng/mL).

Changes in the urinary cGMP excretion in women with preeclampsia compared with controls have been inconsistent. No change in cGMP either with UPI or with the administration of rhPlGF was demonstrated. Conrad et al and others found no significant change, yet Baksu et al found a reduction in urinary cGMP and other metabolites of nitric oxide in women with preeclampsia compared with controls.

In UPI rat models, George et al have shown a reduction in renal medullary cGMP expression; however, urinary excretion of cGMP was not assayed. Although we did not find a difference in the cGMP, the concentrations were similar to those published in humans, for example, Conrad et al found women with preeclampsia to have urinary cGMP concentrations of 1.2 nmol/mg, which approximates the concentration at day 14 UPI (0.98 mmol/mg). The concentrations may not differ either because cGMP in the urine does not actually...

Figure 6. Change in proteinuria in spot urinary protein:creatinine ratio (mg/mmol) after 3 and 5 days of recombinant human placental growth factor (rhPlGF) 100 μg/kg (n=3, red line) or control (0.9% normal saline: n=4, green line) injections. The change in proteinuria is compared with the concentration after 14 days of uteroplacental ischemia (UPI; baseline PlGF). * indicates a significant difference compared baseline PIGF, P<0.05.
change, which is in contrast to changes in cGMP seen in vascular smooth muscles,\textsuperscript{51,52} or because the syndrome produced by UPI is not severe enough to detect a change in cGMP in the urine.

The teratogenicity of any agent given during pregnancy both with regards to birth anomalies and future fetal metabolic or physiological consequences must be considered. Although our study was not powered to assess the teratogenicity of PlGF, no birth anomalies were noted at birth. Excessive PlGF has been shown to increase survival gene expression and inhibit apoptosis.\textsuperscript{53} When PlGF is constitutively expressed (in transgenic mice that overexpress PlGF), there is evidence of induced vascularization starting from fetal development and continuing into adulthood. This is associated with enhanced vessel permeability.\textsuperscript{54} Circulating PlGF levels were higher in the transgenic mice both in the fetal period and in the adulthood. Interestingly, Odorisio et al did show that the over expression of PlGF was associated with upregulation of FLT-1 and FLK-1, although the levels of sFLT-1 were not investigated.\textsuperscript{54}

**Perspectives**

Clinical features typical of human-like preeclampsia, that is, proteinuric hypertension and associated renal histological changes, can be reduced without terminating a pregnancy in primates. Placental growth factor is demonstrated to be part of the physiological sequence controlling BP and proteinuria as a consequence of placental ischemia. It may well be that if endogenous ischemia is further proven as a mechanism for preeclampsia, that targeting effector proteins such as PLGF and correcting the angiogenic imbalance may limit the BP changes and the target end organ effects, such as those seen in the kidney.

**Sources of Funding**

NHMRC Project Grant (APP1025258), Australian Postgraduate Award (K. Yeung), Howard Hughes Medical Institute (S.A. Karumanchi).
Disclosures

R. Thadhani is coinventor of patents related to diagnostics in the prediction of preeclampsia that have been out-licensed to diagnostic companies and has financial interest in Aggamin LLC. Ravi Thadhani also reports serving as a consultant to Roche Diagnostics, Siemens, and Thermo Fisher and has financial interest in Aggamin LLC. The other authors report no conflicts.

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Novelty and Significance

What Is New?

- This study shows that induced placental ischemia reduces circulating placental growth factor, already demonstrated in humans but presumed to be placental ischemia in origin.
- In a nonhuman primate animal model of preeclampsia, infusing a proangiogenic molecule, such as placental growth factor, improves hypertension, proteinuria, and renal biopsy–proven endotheliosis.

What Is Relevant?

- Placental ischemia causes endothelial dysfunction as evidenced by hypotension and proteinuria.

Placental growth factor can improve these clinical signs by improving the endothelial dysfunction.

Summary

Reversing the angiogenic imbalance in preeclampsia by increasing placental growth factor concentrations improves the endothelial dysfunction as evidenced by reductions in blood pressure and proteinuria.
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*Hypertension*. 2016;67:1263-1272; originally published online April 18, 2016;
doi: 10.1161/HYPERTENSIONAHA.116.07286

*Hypertension* is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
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Print ISSN: 0194-911X. Online ISSN: 1524-4563

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