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Placental Growth Factor Blunts Uterine Artery Responses to Angiotensin II

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Abstract

Objective: Changes in maternal serum concentration of placental growth factor (PIGF) and vascular response to intravascular infusion of Angiotensin II (Ang II) follow a bell-shaped curve pattern during gestation. This study evaluates the effects of PIGF and soluble vascular endothelial growth factor receptor-1 (sFlt-1) on responses of human uterine arteries (UA) to Ang II.

Design: Experimental

Setting: Baylor College of Medicine and Texas Children's Hospital-Pavilion for Women

Sample: UA samples (n=14) were obtained from normotensive women undergoing cesarean-hysterectomy at 32 weeks

Methods: UA rings were incubated with: 1) Krebs solution; 2) PIGF at 1.45, 14.5 and 500 pg/ml; 3) sFlt-1 at 2 ng/ml; and 4) a combination of sFlt-1 and PIGF. Dose-contraction responses to Ang II were determined in UA rings incubated in the above-mentioned conditions. Responses were also measured in presence of L-NAME or inhibitors of Endothelium-Derived Hyperpolarizing Factor: Apamine and Charybdotoxin. T-test was used for comparisons.

Main Outcome Measure: Changes in vascular reactivity of UA rings

Results: 1) PIGF blunted ($p=0.03$) and sFlt-1 increased ($p<0.01$) the UA maximum responses to Ang II; 2) A combination of sFlt-1 and PIGF blunted UA responses to Ang II ($p<0.05$); 3) L-NAME, Apamine and Charybdotoxin reversed the relaxation effects of PIGF on UA responses to Ang II ($p<0.05$).

Conclusions: 1) PIGF contributes to the blunted vascular response to Angiotensin II during normotensive pregnancies and sFlt-1 appears to attenuate this effect; 2) PIGF and sFlt-1 may contribute to the regulation of vascular tone during pregnancy by altering the vascular response to Angiotensin II.

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Contribution to Authorship:

We confirm that all authors including JE, AB, MB, AS, KF and CY have met the conditions of authorship.

Disclosure of Interests

We confirm that all authors including JE, AB, MB, AS, KF and CY declared no conflicts of interests with this manuscript.

Tweetable abstract:

Placental growth factor and soluble vascular endothelial growth factor receptor-1 modulate the uterine artery response to Angiotensin II in normotensive pregnant women.

Keywords

vascular; response; placental growth factor; sFlt-1; Angiotensin II

Introduction:

Redundancy in the regulation of vascular tone during normal pregnancy provides finely tuned relay systems designed to optimize oxygen delivery to the fetus.¹ These systems involve the release of relaxin,² prostacyclin, nitric oxide, kallikrein, angiotensin-(1–7) and vascular endothelial growth factor (VEGF).¹ Experimental evidence in pregnant animals indicates that placental growth factor (PIGF) is a potent arterial vasodilator^{3;4} and it has been proposed that PIGF may participate in the mechanisms regulating the maternal vascular tone during pregnancy.⁴ Thus, reduced bioavailability of PIGF may contribute to the dysregulation of vascular tone leading to elevation of the systemic blood pressure in pregnancies complicated by preeclampsia or gestational hypertension.

Imbalances between angiogenic factors including PIGF and VEGF and anti-angiogenic factors including the soluble form of VEGF receptor-1 (sFlt-1) and soluble endoglin appear to play a central role in the pathogenesis of preeclampsia.^{5–19} These imbalances predate the clinical manifestations of preeclampsia. For example, decreased maternal serum or plasma concentrations of PIGF has been described as early as the first trimester of pregnancy in women at increased risk of developing preeclampsia in the index pregnancy.^{11;20;21} A combination of low maternal serum PIGF, increased impedance to blood flow in the uterine arteries and other maternal characteristics can identify 93% of women requiring delivery before 34 weeks due to preeclampsia.²⁰ A recent randomized controlled trial demonstrated that the latter strategy can be used to identify women at increased risk of developing preterm preeclampsia who may benefit from daily use of 150 mg of aspirin starting in the first trimester of pregnancy.²²

PIGF is an angiogenic factor produced by the placenta that facilitates the pro-angiogenic action of VEGF in the human endothelium.²³ The median maternal serum concentrations of PIGF during normal pregnancy follows a bell-shaped curve pattern with advancing gestational age.^{5;11} This pattern mimics the changes in maternal vascular response to exogenous administration of Ang II during normotensive gestations.²⁴ In contrast, changes in the maternal serum concentration of other angiogenic-related factors including soluble vascular endothelial growth factor receptor-1 (sFlt-1), soluble endoglin, endothelin, 6-Keto-PIGF 1 alpha and thromboxane B2 do not follow a bell-shaped curve.^{5;7;25} Of note, a recent paper suggested that sFlt-1 may promote Ang II sensitivity in a mouse model of preeclampsia.²⁶ This study was designed to 1) determine effects of PIGF and sFlt-1 on responses of human uterine arteries (UA) to Ang II; and 2) to explore mechanisms of PIGF influencing Ang II actions during pregnancy.

Methods:

A validated in-vitro model involving the use of organ chambers was used to evaluate the influence of PIGF and sFlt-1 on the vascular reactivity to Angiotensin II (Ang II) in segments of uterine arteries obtained from hysterectomy specimens from normotensive pregnant women (n=14).²⁷ Once the viability of vascular rings as well as endothelial integrity were confirmed,²⁷ vascular rings were incubated with PIGF (an angiogenic factor) and the anti-angiogenic factor sFlt-1 to determine their effects on responses of human uterine arteries (UA) to Ang II. The protocol for this study was approved by Baylor College of Medicine Institutional Review Board (IRB No. H-33918). Samples from the ascending branch of the uterine artery (UA) were obtained as described before²⁷ from normotensive women undergoing cesarean hysterectomy at 32 weeks due to abnormally adherent placenta.

Samples containing a representative UA branch segment were placed in cold oxygenated physiological salt solution. A small artery (200- μ m normalized internal diameter) was cleaned, cut, and dissected. These samples were incubated for 24 hr. with 1) Krebs solution (controls); 2) PIGF (recombinant human PIGF, R&D systems) at 1.45 pg/ml (0.1 nM), 14.5 pg/ml (1nM) (sub-physiological concentrations) and 500 pg/ml (33 nM) (concentration described at 32 weeks of gestation);^{28;29} 3) sFlt-1 (Recombinant Human VEGF R1/Flt-1, R&D systems) at 2 ng/ml (physiological concentration); and 4) a combination of sFlt-1 and PIGF and at the following ratios concentrations: 1.6 (PIGF: 6 ng/ml and sFlt-1: 10 ng/ml); 5.5 (PIGF: 18 ng/ml and sFlt-1: 100 ng/ml); and 33.3 (PIGF: 30 ng/ml and sFlt-1: 1000 ng/ml).

Two-millimeter UA rings were mounted on a Multi Wire Myograph System (Model 620M; Danish Myo Technology) for isometric tension recording, as previously described.³⁰ The myograph bath contained PSS at 37°C and was bubbled with 5% CO₂ and 95% of O₂. To induce contraction, arterial rings were held at optimal tension of 2.5 millinewtons and then exposed to potassium chloride (80 mM) for 7 minutes. Cumulative dose-response curves were constructed. Dose-contraction responses to Ang II (relative to potassium chloride responses) were determined using a concentration range of 10⁻¹⁰ M to 10⁻⁵ M. Because of the characteristic response of Ang II, it was necessary to use one channel at each time. The plateau period last seconds, which prevented the simultaneous use of two or more channels. Following evaluation of dose-contraction responses to Ang II a wash out period was performed. The vascular rings rested for 10 minutes and then U46619 (10⁻⁶ M; CAYMAN) was added. When the vascular ring contraction reached a plateau, dose-contraction responses to Bradykinin were evaluated using concentration between 10⁻¹⁰ M to 10⁻⁵ M. Vascular rings from the same UAs were used to compare the different experimental conditions to reduce the influence of inter-sample variability in vascular response.

To assess the mechanisms of PIGF actions, the UA ring responses were also measured in presence of inhibitor 300 nM of nitric oxide (NO) synthase (L-NAME), or inhibitors of Endothelium-Derived Hyperpolarizing Factor (EDHF): Apamine (APA) and Charybdotoxin (CHX) (both at concentrations of 100nM). UA responses to bradykinin were evaluated to confirm endothelial integrity. Data distribution was determined using the Shapiro-Wilk Test.

Vascular rings for the same uterine artery were subjected to the two experimental condition and comparisons were performed using t-test. A $p < 0.05$ was considered significant.

Results:

The demographic and clinical characteristics of the study population are displayed in Table S1 (see online supporting material). Abnormally adherent placenta was confirmed in all women by histological evaluation demonstrating placenta accreta ($n=6$), increta ($n=4$) and percreta ($n=4$). Normal data distribution was confirmed by the Shapiro-Wilk Test.

UA rings incubated for 24 hours with 1.45 pg/ml nM PIGF had lower maximum responses (E_{max}) to Ang II compared to UA rings incubated with Krebs solution (E_{max} PIGF: 28.2 ± 7.2 vs. E_{max} control: 91.9 ± 27.4 ; $p=0.037$) (Figure 1a). Lower maximum responses to Ang II were also observed on UA rings incubated for 24 hr. with 14.5 pg/ml (Figure 1b) but the differences did not reach statistical significance. In contrast, UA rings incubated for 24 hours with 500 pg/ml PIGF (physiologic concentrations) had lower maximum responses (E_{max}) to Ang II compared to UA rings incubated with Krebs solution (E_{max} PIGF: 50.14 ± 2.16 vs. E_{max} control: 76.63 ± 14.48 ; $p=0.027$) (Figure 1c) UA rings responses to bradykinin confirmed endothelial integrity (Figure 1d).

UA rings incubated for 24 hr. with a physiological concentration of sFlt-1 had higher maximum responses to Ang II compared to controls (E_{max} sFlt-1: 124.9 ± 4.12 vs. E_{max} control: 75.3 ± 12.2 ; $p < 0.001$). (Figure 2).

High concentrations of sFlt-1 attenuated the PIGF effects on UA ring responses to Ang II (Figures 3a, 3b and 3c). Although the concentrations of PIGF and sFlt-1 used in these set of experiments are higher than those described in normotensive pregnant women at 32 weeks of gestation,^{5,12} their corresponding sFlt-1/PIGF ratios (1.6, 5.5 and 33.3, respectively) are below the cut-off of 38, which has been proposed to identify women at low risk to develop preeclampsia regardless of the gestational age.¹² Thus, high sFlt-1 concentrations in-vitro attenuates the PIGF actions on UA responses to Ang II regardless of the sFlt-1/PIGF ratios. In all these experiments, UA responses to bradykinin confirmed endothelial integrity (Figures 3d).

L-NAME, and EDHF inhibitors, APA and CHX reversed the relaxation effects of PIGF on UA rings responses to Ang II (E_{max} : Control: 75.25 ± 12.1 ; PIGF: 60.95 ± 18.1 ; L-NAME: 98.55 ± 10.2 ; APA+ CHX: 90.9 ± 15.9 ; $p < 0.05$ for all comparisons) (Figure 4). The number of uterine artery samples in Figures 1a, Figure 2 and Figure 4 differs because not all the vascular rings had adequate response to Angiotensin II and no data could be derived from these vascular rings. Comparisons were done only among UA rings with adequate response to Ang II.

Discussion:

Main findings:

The results of this study demonstrate that PIGF reduces the UA contractile response to Ang II in vitro and that this effect is attenuated by sFlt-1. PIGF effects on UA response to Ang II

during pregnancy appear to involve both Endothelium-Derived Hyperpolarizing Factor and nitric oxide synthesis from endothelial cells.

Strengths and Limitations:

Strengths of this study include the use of a validated in-vitro model involving the use of organ chambers to evaluate vascular reactivity of segments of uterine arteries obtained from hysterectomy specimens from a relatively large number of normotensive pregnant women (n=14). Limitations of this study include that UA samples were obtained from women undergoing cesarean hysterectomy due to abnormally adherent placenta. It is possible that the vascular biology of UA rings from our samples differs from that of women without abnormally adherent placenta; however, in a different set of experiments we compared the vascular reactivity to PIGF in omental arteries from normotensive women with and without abnormally adherent placenta (see Figure S1), and found no differences. Thus, it is unlikely that UA vascular reactivity from women with abnormal placentation substantially differs from women with uncomplicated pregnancies. Another limitation of the study is that the vascular response to Ang II in organ baths are known to be associated with tachyphylaxis.³¹ However, it is unlikely that tachyphylaxis introduced bias to our results since the same degree of tachyphylaxis should be expected in UA rings incubated with the different agents and controls. Furthermore, tachyphylaxis would not explain the enhanced vascular response in UA rings incubated with sFlt-1. Also, it has been reported that the expression of Ang II receptor type 1 (AT1) in UA samples differ from that in omental arteries in pregnant sheep.³² It remains unclear if the same is true in pregnant women. There is no evidence that samples of omental arteries better represent the vascular reactivity of the systemic circulation in humans compared to UA samples.

Interpretation:

Our observations suggest that PIGF contributes to the mechanisms by which normotensive pregnant women have blunted vascular responses to exogenous administration of Ang II;^{24;33;34} and provide evidence that the interactions between PIGF and sFlt-1 contribute to the regulation of vascular tone during pregnancy. This notion is further supported by the observation that maternal plasma concentration of PIGF between 22–25 weeks of gestation has a significant negative correlation with systemic vascular resistance as measured by electrical cardiography in women at increased risk for preeclampsia.³⁵ This is also consistent with the results of in vitro studies demonstrating that PIGF can induce dilatation of resistance arteries obtained from pregnant individuals.^{3;4} Our observations provide a novel mechanism by which the interactions between PIGF and sFlt-1 may modulate vascular tone and blood flow during pregnancy by altering the vascular response to Ang II. It is also possible that Ang II may amplify this effect by increasing PIGF bioavailability. This is supported by the observation that Ang II can induce PIGF mRNA expression and protein production in human vascular endothelial and smooth muscle cells.³⁶ Our results that sFlt-1 enhances the vascular reactivity on human uterine arteries to Ang II is consistent with evidence from a mouse model of preeclampsia that sFlt-1 promotes angiotensin II sensitivity.²⁶

Clinical implications:

Uncomplicated human pregnancies are characterized by blunted vascular responses to intravenous administration of Ang II.^{24;33;34} Observations in the 1960s and 70s demonstrated that the response to intravascular administration of Ang II in normotensive pregnant women was significantly diminished in comparison to that in non-pregnant women.^{24;33;34} Of note, these authors also reported that women who later developed hypertension during pregnancy were more sensitive to the “pressor” effect of exogenous administration of Ang II compared to normotensive pregnant women.²⁴ Since these early observations there has been paucity in the study of the mechanisms by which normal pregnancy is associated with changes in the vascular response to a powerful vasoactive agent such as Ang II. Our observations provide a mechanistic explanation for some of these observations and suggest that PlGF contributes to the blunted vascular response to Ang II in normal pregnancy.

Accumulating evidence indicates that preeclampsia is associated with angiogenic imbalances characterized by low maternal serum concentrations of PlGF and VEGF as well as high serum maternal concentration of anti-angiogenic factors including sFlt-1 and soluble endoglin.^{5–19} Angiogenic imbalances appear to predate the clinical diagnosis of preeclampsia.^{5–7;11;12;15–17;19;20} To the extent that PlGF and sFlt-1 alter the vascular response to Ang II, it is possible that low maternal serum concentration of PlGF and increased circulating sFlt-1 may account for the increased vascular response to Ang II in women who later developed hypertension during pregnancy.²⁴

PlGF³⁷ and sFlt-1¹³ are produced predominantly by the placental tissues. To the extent that the latter two angiogenic factors contribute to the regulation of vascular tone during pregnancy as suggested by our findings, it is possible that the human placenta may play a role in the temporal regulation of vascular resistance during pregnancy through the release of PlGF and sFlt-1 as evidenced by the temporal changes in blood pressure and PlGF during pregnancy.⁵

Research implications:

There is experimental evidence that exogenous administration of PlGF can prevent or reverse systemic hypertension in animal models of preeclampsia.^{38–40} This evidence supports a potential therapeutic role of PlGF in women with preeclampsia. Currently, there are two clinical trials designed to determine the utility of pravastatin in pre-eclampsia prevention: “Statins to ameliorate early-onset preeclampsia” (www.controlled-trials.com/ISRCTN23410175) and “Pravastatin for prevention of preeclampsia” (www.ClinicalTrials.gov/NCT01717586). Pravastatin has been demonstrated to increase PlGF in a lentivirus human sFlt-1 transfected mouse model.⁴¹ In this study, there was reduction in blood pressure and placental and fetal weights normalized in pregnant animals exposed to pravastatin.⁴¹ These effects have been proposed to be secondary to the increase in PlGF and subsequent reduction in vascular reactivity following pravastatin administration.⁴² Our observations could also provide a mechanistic explanation for the effects of pravastatin in the prevention of preeclampsia, given the effects of PlGF in modulating the actions of Angiotensin II on vascular tone during pregnancy.

The half-life of PlGF in women following delivery is 4.8 ± 1.6 days⁴³ and that of sFlt-1 ranges from 2.2 to 94 hr.⁴⁴ It is possible that in organ baths the half-life of these vasoactive agents may be longer because vascular rings contain fewer endothelial cells compared to in vivo experiments. Thus, in vitro evaluation of the biological effects of PlGF and sFlt-1 on UA reactivity may require longer incubation periods.

Conclusions:

The collective observations of this study suggest that PlGF and sFlt-1 participate in the regulation of vascular tone during pregnancy by altering the vascular response to Ang II. We hypothesize that changes in the maternal serum concentrations of PlGF and sFlt-1 may provide a mechanistic explanation for the blunted response to exogenous administration of Ang II in normotensive pregnant women as well as increased response to Ang II in women who later develop high blood pressure during the index pregnancy.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Details of ethics approval: The protocol for this study was approved on July 14, 2014 by Baylor College of Medicine Institutional Review Board (IRB No. H-33918).

Reference List

1. Valdes G, Kaufmann P, Corthorn J, Erices R, Brosnihan KB, Joyner-Grantham J. Vasodilator factors in the systemic and local adaptations to pregnancy. *Reprod.Biol Endocrinol* 2009;7:79. [PubMed: 19646248]
2. Conrad KP. Maternal vasodilation in pregnancy: the emerging role of relaxin. *Am J Physiol Regul.Integr.Comp Physiol* 2011;301:R267–R275. [PubMed: 21613576]
3. Mandala M, Gokina N, Barron C, Osol G. Endothelial-derived hyperpolarization factor (EDHF) contributes to PlGF-induced dilation of mesenteric resistance arteries from pregnant rats. *J Vasc.Res* 2012;49:43–49. [PubMed: 21985802]
4. Osol G, Celia G, Gokina N, Barron C, Chien E, Mandala M et al. Placental growth factor is a potent vasodilator of rat and human resistance arteries. *Am J Physiol Heart Circ.Physiol* 2008;294:H1381–H1387. [PubMed: 18192215]
5. Levine RJ, Maynard SE, Qian C, Lim KH, England LJ, Yu KF et al. Circulating angiogenic factors and the risk of preeclampsia. *N.Engl.J Med* 2004;350:672–83. [PubMed: 14764923]
6. Maynard SE, Min JY, Merchan J, Lim KH, Li J, Mondal S et al. Excess placental soluble fms-like tyrosine kinase 1 (sFlt1) may contribute to endothelial dysfunction, hypertension, and proteinuria in preeclampsia. *J Clin.Invest* 2003;111:649–58. [PubMed: 12618519]
7. Levine RJ, Lam C, Qian C, Yu KF, Maynard SE, Sachs BP et al. Soluble endoglin and other circulating antiangiogenic factors in preeclampsia. *N.Engl.J Med* 2006;355:992–1005. [PubMed: 16957146]
8. Espinoza J, Uckele JE, Starr RA, Seubert DE, Espinoza AF, Berry SM. Angiogenic imbalances: the obstetric perspective. *Am J Obstet Gynecol* 2010;203:17–18. [PubMed: 20231008]
9. Korzeniewski SJ, Romero R, Chaiworapongsa T, Chaemsaitong P, Kim CJ, Kim YM et al. Maternal plasma angiogenic index-1 (placental growth factor/soluble vascular endothelial growth

factor receptor-1) is a biomarker for the burden of placental lesions consistent with uteroplacental underperfusion: a longitudinal case-cohort study. *Am J Obstet Gynecol* 2016;214:629. [PubMed: 26688491]

10. Gallo DM, Wright D, Casanova C, Campanero M, Nicolaides KH. Competing risks model in screening for preeclampsia by maternal factors and biomarkers at 19–24 weeks' gestation. *Am J Obstet Gynecol* 2016;214:619. [PubMed: 26627730]
11. Romero R, Nien JK, Espinoza J, Todem D, Fu W, Chung H et al. A longitudinal study of angiogenic (placental growth factor) and anti-angiogenic (soluble endoglin and soluble vascular endothelial growth factor receptor-1) factors in normal pregnancy and patients destined to develop preeclampsia and deliver a small for gestational age neonate. *J Matern.Fetal Neonatal Med* 2008;21:9–23. [PubMed: 18175241]
12. Stepan H, Schaarschmidt W, Jank A, Verlohren S, Kratzsch J. [Use of angiogenic factors (sFlt-1/PIGF ratio) to confirm the diagnosis of preeclampsia in clinical routine: first experience]. *Z.Geburtshilfe Neonatol* 2010;214:234–38. [PubMed: 21207323]
13. Clark DE, Smith SK, He Y, Day KA, Licence DR, Corps AN et al. A vascular endothelial growth factor antagonist is produced by the human placenta and released into the maternal circulation. *Biol Reprod*. 1998;59:1540–48. [PubMed: 9828203]
14. Redman CW, Staff AC. Preeclampsia, biomarkers, syncytiotrophoblast stress, and placental capacity. *Am J Obstet Gynecol* 2015;213:S9–11. [PubMed: 26428507]
15. Rana S, Powe CE, Salahuddin S, Verlohren S, Perschel FH, Levine RJ et al. Angiogenic factors and the risk of adverse outcomes in women with suspected preeclampsia. *Circulation* 2012;125:911–19. [PubMed: 22261192]
16. Stepan H, Herraiz I, Schlembach D, Verlohren S, Brennecke S, Chantraine F et al. Implementation of the sFlt-1/PIGF ratio for prediction and diagnosis of pre-eclampsia in singleton pregnancy: implications for clinical practice. *Ultrasound Obstet Gynecol* 2015;45:241–46. [PubMed: 25736847]
17. Verlohren S, Stepan H, Dechend R. Angiogenic growth factors in the diagnosis and prediction of pre-eclampsia. *Clin.Sci.(Lond)* 2012;122:43–52. [PubMed: 21929511]
18. Chaiworapongsa T, Romero R, Korzeniewski SJ, Kusanovic JP, Soto E, Lam J et al. Maternal plasma concentrations of angiogenic/antiangiogenic factors in the third trimester of pregnancy to identify the patient at risk for stillbirth at or near term and severe late preeclampsia. *Am J Obstet Gynecol* 2013;208:287. [PubMed: 23333542]
19. Kusanovic JP, Romero R, Chaiworapongsa T, Erez O, Mittal P, Vaisbuch E et al. A prospective cohort study of the value of maternal plasma concentrations of angiogenic and anti-angiogenic factors in early pregnancy and midtrimester in the identification of patients destined to develop preeclampsia. *J Matern.Fetal Neonatal Med* 2009;22:1021–38. [PubMed: 19900040]
20. Poon LC, Kametas NA, Maiz N, Akolekar R, Nicolaides KH. First-trimester prediction of hypertensive disorders in pregnancy. *Hypertension* 2009;53:812–18. [PubMed: 19273739]
21. Thadhani R, Mutter WP, Wolf M, Levine RJ, Taylor RN, Sukhatme VP et al. First trimester placental growth factor and soluble fms-like tyrosine kinase 1 and risk for preeclampsia. *J Clin.Endocrinol.Metab* 2004;89:770–75. [PubMed: 14764795]
22. Rolnik DL, Wright D, Poon LC, O'Gorman N, Syngelaki A, de Paco MC et al. Aspirin versus Placebo in Pregnancies at High Risk for Preterm Preeclampsia. *N.Engl.J Med* 2017;377:613–22. [PubMed: 28657417]
23. Autiero M, Waltenberger J, Communi D, Kranz A, Moons L, Lambrechts D et al. Role of PIGF in the intra- and intermolecular cross talk between the VEGF receptors Flt1 and Flk1. *Nat.Med* 2003;9:936–43. [PubMed: 12796773]
24. Gant NF, Daley GL, Chand S, Whalley PJ, MacDonald PC. A study of angiotensin II pressor response throughout primigravid pregnancy. *J Clin.Invest* 1973;52:2682–89. [PubMed: 4355997]
25. Paarlberg KM, de Jong CL, van Geijn HP, van Kamp GJ, Heinen AG, Dekker GA. Vasoactive mediators in pregnancy-induced hypertensive disorders: a longitudinal study. *Am J Obstet Gynecol* 1998;179:1559–64. [PubMed: 9855596]

26. Burke SD, Zsengeller ZK, Khankin EV, Lo AS, Rajakumar A, DuPont JJ et al. Soluble fms-like tyrosine kinase 1 promotes angiotensin II sensitivity in preeclampsia. *J Clin.Invest* 2016;126:2561–74. [PubMed: 27270170]
27. Nelson SH, Steinsland OS, Wang Y, Yallampalli C, Dong YL, Sanchez JM. Increased nitric oxide synthase activity and expression in the human uterine artery during pregnancy. *Circ.Res* 2000;87:406–11. [PubMed: 10969039]
28. Knudsen UB, Kronborg CS, von Dadelszen P, Kupfer K, Lee SW, Vittinghus E et al. A single rapid point-of-care placental growth factor determination as an aid in the diagnosis of preeclampsia. *Pregnancy Hypertens.* 2012;2:8–15. [PubMed: 26104984]
29. Saffer C, Olson G, Boggess KA, Beyerlein R, Eubank C, Sibai BM. Determination of placental growth factor (PIGF) levels in healthy pregnant women without signs or symptoms of preeclampsia. *Pregnancy Hypertens.* 2013;3:124–32. [PubMed: 26105949]
30. Dong Y, Betancourt A, Chauhan M, Balakrishnan M, Lugo F, Anderson ML et al. Pregnancy Increases Relaxation in Human Omental Arteries to the CGRP Family of Peptides. *Biol Reprod.* 2015;93:134. [PubMed: 26510864]
31. Maigaard S, Forman A, Andersson KE. Differential effects of angiotensin, vasopressin and oxytocin on various smooth muscle tissues within the human uteroplacental unit. *Acta Physiol Scand.* 1986;128:23–31. [PubMed: 3766172]
32. Bird IM, Zheng J, Cale JM, Magness RR. Pregnancy induces an increase in angiotensin II type-1 receptor expression in uterine but not systemic artery endothelium. *Endocrinology* 1997;138:490–98. [PubMed: 8977439]
33. Abdul-Karim R, Assalin S. Pressor response to angiotonin in pregnant and nonpregnant women. *Am J Obstet Gynecol* 1961;82:246–51. [PubMed: 13680954]
34. Chesley LC. Vascular reactivity in normal and toxemic pregnancy. *Clin.Obstet Gynecol* 1966;9:871–81. [PubMed: 5333789]
35. Woelkers DA, Ghashghaei R, Klisser K, Archer T. PP143. Relationship between mid-pregnancy placenta growth factor and hemodynamics in the mother, fetus, and uterus. *Pregnancy Hypertens.* 2012;2:317.
36. Pan P, Fu H, Zhang L, Huang H, Luo F, Wu W et al. Angiotensin II upregulates the expression of placental growth factor in human vascular endothelial cells and smooth muscle cells. *BMC.Cell Biol* 2010;11:36. [PubMed: 20500895]
37. De Falco S. The discovery of placenta growth factor and its biological activity. *Exp.Mol.Med* 2012;44:1–9. [PubMed: 22228176]
38. Suzuki H, Ohkuchi A, Matsubara S, Takei Y, Murakami M, Shibuya M et al. Effect of recombinant placental growth factor 2 on hypertension induced by full-length mouse soluble fms-like tyrosine kinase 1 adenoviral vector in pregnant mice. *Hypertension* 2009;54:1129–35. [PubMed: 19786649]
39. Makris A, Thornton C, Thompson J, Thomson S, Martin R, Ogle R et al. Uteroplacental ischemia results in proteinuric hypertension and elevated sFLT-1. *Kidney Int.* 2007;71:977–84. [PubMed: 17377512]
40. Spradley FT, Tan AY, Joo WS, Daniels G, Kussie P, Karumanchi SA et al. Placental Growth Factor Administration Abolishes Placental Ischemia-Induced Hypertension. *Hypertension* 2016;67:740–47. [PubMed: 26831193]
41. Kumasawa K, Ikawa M, Kidoya H, Hasuwa H, Saito-Fujita T, Morioka Y et al. Pravastatin induces placental growth factor (PGF) and ameliorates preeclampsia in a mouse model. *Proc.Natl.Acad.Sci.U.S.A* 2011;108:1451–55. [PubMed: 21187414]
42. Chau K, Hennessy A, Makris A. Placental growth factor and pre-eclampsia. *J Hum.Hypertens* 2017;31:782–86. [PubMed: 29115294]
43. Saleh L, Danser J, Van Den Meiracker A. On The Origin and Disappearance of sFlt-1 and PIGF in Preeclampsia. *Pregnancy Hypertens.* 2016;6:199.
44. Wathen KA, Sarvela J, Stenman F, Stenman UH, Vuorela P. Changes in serum concentrations of soluble vascular endothelial growth factor receptor-1 after pregnancy. *Hum.Reprod* 2011;26:221–26. [PubMed: 21097488]

Figure 1.

Uterine artery (UA) rings incubated for 24 hours with 0.1 nM and 33 nM of placental growth factor (PIGF, hollow circles and squares) had lower maximum responses to Angiotensin II (Ang II) compared to UA rings incubated with Krebs solution (solid circles) (Figures 1a and 1b); Similarly, lower maximum responses were found in UA rings incubated for 24 hours with 1 nM of PIGF (hollow triangles, Figure 1a) but the difference was not statistically significant. UA responses to bradykinin confirmed endothelial integrity (Figure 1d). All the vessels were normalized before the experiments. An average start tension of 13 micro Newtons was applied to the vessels.

Figure 2.

Uterine artery (UA) rings incubated for 24 hours with physiological concentration of the soluble form of vascular endothelial growth factor receptor-1 (sFlt-1, hollow squares) had higher maximum responses to Angiotensin II (Ang II) compared to controls (solid circles).

Figure 3.

Uterine artery (UA) rings incubated with a combination of placental growth factor (PIGF) and the soluble form of vascular endothelial growth factor receptor-1 (sFlt-1) at high concentrations, attenuated the PIGF effects on uterine artery responses to Angiotensin II (Ang II), ($p < 0.05$; Figures 3a, 3b and 3c). The sFlt-1/PIGF ratios are as follows: 1.6 (PIGF: 6 ng/ml and sFlt-1: 10 ng/ml); 5.5 (PIGF: 18 ng/ml and sFlt-1: 100 ng/ml); and 33.3 (PIGF: 30 ng/ml and sFlt-1: 1000 ng/ml). In all experiments, UA responses to bradykinin confirmed endothelial integrity (Figure 4d).

Figure 4.

Nitro L-arginine methylester (L-NAME, an inhibitor of nitric oxide synthase, hollow circles), and inhibitors of Endothelium-Derived Hyperpolarizing Factor (EDHF): Apamine (APA) and Charybdotoxin (CHX) (hollow squares) reversed the relaxation effects of PIGF on uterine artery rings responses to Angiotensin II (Ang II) (solid squares).