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New Approaches for Managing Preeclampsia: Clues from Clinical and Basic Research

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Abstract

One of the most common, and most vexing, obstetrical complications is preeclampsia—a major cause of maternal and perinatal morbidity. Hallmarked by new-onset hypertension and a myriad of other symptoms, the underlying cause of the disorder remain obscure, despite intensive research into the etiology. While the initiating events aren't clear, one common finding in preeclampsia cases is failure of the maternal arteries which supply the placenta, with resulting hypoxia/ischemia. Intensive research over the last two decades has identified several categories of molecular dysfunction resulting from placental hypoxia, which when released into the maternal circulation are responsible for the spectrum of symptoms seen in these patient; in particular angiogenic imbalance and activation of innate and adaptive immune responses. Despite these new insights, little in the way of new treatments have been advanced into clinical practice for the management of these patients. Indeed, little in the way of therapeutic options exist for the obstetrician facing a preeclampsia case. Pharmacological management is typically to seizure prophylaxis and anti-hypertensives in severe cases to try and control the worsening hypertension. Ultimately, induction of labor is indicated; making preeclampsia a leading cause of premature birth. Here, the molecular mechanisms linking placental ischemia to the maternal symptoms of preeclampsia are reviewed, and several areas of recent research suggesting new potential therapeutic approaches for the management of preeclampsia are identified.

Introduction

Preeclampsia remains one of the most prevalent complications of pregnancy, affecting ~5–7% of pregnancies in the United States.¹ While classically, preeclampsia was defined by new-onset hypertension and proteinuria, recent guidelines issued by the American Congress of Obstetricians and Gynecologists have eliminated proteinuria as an essential criterion; instead suggesting a positive diagnosis with new-onset hypertension coupled with proteinuria or any of a spectrum of other symptoms: thrombocytopenia, renal insufficiency, impaired liver function, pulmonary edema, or cerebral/visual symptoms.¹ While better obstetrical care has lessened the maternal mortality associated with preeclampsia, it remains a leading cause of peripartum morbidity, and hypertensive disorders, primarily preeclampsia, are a major cause pregnancy termination prior to term due to life threatening symptoms in

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the mother.² A number of risk factors have been implicated as being significant for the development of preeclampsia including elevated BMI, primiparity, multiparity, and ethnicity.^{3–5} However, despite the identification of these factors, the underlying cause of the disorder remains elusive. One mechanism that has been strongly implicated as central to the maternal symptoms is defective development of the placental unit, leading to placental hypoperfusion and chronic ischemia.

Early clues implicating the placenta as critical to the symptomatic manifestation of the disorder came from case reports suggesting that delivery of the fetus alone was insufficient for the remission of symptoms, but delivery of the entire placenta is necessary for resolution of the disorder.^{6, 7} It was possible then that defects in normal placental development could be at the root of the maternal symptoms. Placental development is an extraordinarily complicated process which involves not only development of the organ itself, but invasion and remodeling of the maternal uterine vasculature to ensure adequate delivery of blood. In normal placental development, placentally derived invasive cytotrophoblasts enter the convoluted maternal arteries of the uterus, termed spiral arteries, and displace the maternal endothelium. In the process, the high resistance vessels are remodeled into high capacitance, low resistance vessels; thereby ensuring free flow of blood to the placenta and adequate exchange of nutrients/waste at the fetal maternal interface.⁸ Early studies suggested that preeclampsia patients had inadequate remodeling of these spiral arteries, which retained highly muscular and narrow diameter phenotypes.⁹

The end result of this failure to remodel the spiral arteries is hypoxia in the placental tissue which ultimately causes chronic ischemia as the hemodynamic demand increases through gestation. A variety of clinical studies and experimental animal models examining the functional consequences of defective placentation or placental ischemia directly have elucidated a number of molecular pathways which are believed to play a role in the maternal manifestation of the disease. Importantly, these pathways suggest multiple therapeutic avenues which could provide new approaches for the management of the preeclampsia patient. Among the most important of these are altered angiogenic balance, activation of maternal inflammatory responses, decreased nitric oxide bioavailability, and increased production of the vasoconstrictor endothelin-1 (ET-1). Preclinical, and in some cases human, studies have suggested the viability of intervening in these pathways for the management of preeclampsia symptoms. Below, the rationale for each of these pathways is outlined, as well as potential therapeutic opportunities which could alter each.

Angiogenic Imbalance

Perhaps no area in preeclampsia research has received as much attention as altered maternal angiogenic imbalance. One of the best known, and most potent, pro-angiogenic proteins is vascular endothelial growth factor (VEGF). In addition to its activities in angiogenesis, VEGF is crucial for maintenance of endothelial cell function, and reduction of VEGF in human via inhibition by specific monoclonal antibodies results in hypertension, proteinuria, and endotheliosis; symptoms which are consistent with many preeclampsia patients.¹⁰ Insight into how the VEGF signaling system could contribute to the pathogenesis of preeclampsia came from reports identifying significant elevations of the VEGF antagonist

soluble fms-like tyrosine kinase-1 (sFlt-1) in the circulation of preeclamptic patients.¹¹ sFlt-1 is in fact an alternatively spliced variant of the full length VEGF receptor Flt-1, which consists of the extracellular domain alone, without the transmembrane and intracellular domains. This soluble form of the protein is secreted, where it binds free VEGF, making it unavailable for signaling to its endogenous receptors. Although the exact mechanisms which up-regulate sFlt-1 production are still an area of investigation, it is believed to be at least partially dependent on the activity of hypoxia inducible factor 1 α (HIF-1 α), suggesting regulation by changes in oxygen tension¹²

Besides clinical correlative studies, a number of *in vitro* and animal models of increased sFlt-1 have indicated a link between placental ischemia, sFlt-1 production, and the maternal symptoms of preeclampsia. In culture, increased production of sFlt-1 by hypoxic conditions similar to those seen in a preeclampsia patient's placenta has been reported in both cultured placental tissue and in trophoblasts cells.^{13–15} There are also a number of reports that exogenous introduction of sFlt-1 by either infusion or viral-mediated overexpression in animal models results in symptoms similar to those seen in preeclampsia, including hypertension, renal injury, and proteinuria.^{11, 16–19} Importantly, artificially inducing placental ischemia in the rodent with the Reduced Uterine Perfusion Pressure (RUPP) model increases the levels of sFlt-1 in both the maternal circulation and placental tissue, again suggesting a causative link between placental ischemia and the induction of sFlt-1.^{20, 21}

Several lines of evidence have suggested that targeting the VEGF/sFlt-1 system could have a palliative benefit in the preeclampsia patient. Li et al first demonstrated that sFlt-1 induced hypertension and proteinuria during pregnancy in the rat (via viral overexpression) could be significantly attenuated by daily infusion of VEGF₁₂₁.²² Likewise, retroviral overexpression of sFlt-1 in the mouse resulted similar symptoms and endotheliosis and vascular injury. In this model, co-administration of an adenovirus coding for the VEGF₁₂₁ construct significantly decreased the associated renal injury and proteinuria.¹⁷ Interestingly, in a related study, sFlt-1 induced hypertension in a murine model was significantly reduced by administration of either VEGF₁₆₅ or the VEGF family member placental growth factor (PlGF), which is believed to be more specific for the Flt-1 receptor compared to the other major VEGF receptor, Flk-1.²³

VEGF administration has also demonstrated effectiveness in other preclinical models which do not rely on sFlt-1 administration. Using the RUPP rodent model of placental ischemia-induced hypertensive, Gilbert et al demonstrated that continuous VEGF₁₂₁ infusion could attenuate the hypertension associated with that model. Furthermore, VEGF administration significantly improved renal hemodynamics. This was associated with an increased vascular relaxation in response to acetylcholine, suggesting a decrease in endothelial dysfunction with VEGF administration.¹⁸ Preeclampsia has also been associated with maternal immune activation (discussed in detail below), including production of agonistic autoantibodies to the angiotensin type 1 receptor (AT1-AA).²⁴ Infusion of the AT1-AA to levels mimicking those seen in preeclampsia patients results in hypertension, proteinuria, and renal injury, implicating them in the symptomatic manifestation of the disorder.^{25, 26} Intriguingly, Siddiqui et al found that co-administration of VEGF₁₂₁ with the AT1-AA in mice significantly attenuated each of these symptoms. This suggest that the AT1-AA is

contributing to the endothelial dysfunction associated with preeclampsia, which can be overcome by additional VEGF signaling activity.²⁵

There are two immediately obvious ways in which the balance between VEGF/sFlt-1 could be affected; elimination of sFlt-1 or introduction of exogenous VEGF. In the first category, an intriguing preliminary study was recently reported by Thadhani et al, who used ionic binding apheresis cassettes to remove sFlt-1 in a small cohort of preeclampsia patients. As a result, both blood pressure and proteinuria were reduced, and there was a trend, though not significant for prolongation of pregnancy; though given the small cohort size, it is not possible to determine whether this was significant.²⁷ Further clinical trials to evaluate this methodology are ongoing and should prove enlightening. While the most straightforward approach would be to administer exogenous VEGF, a number of safety and pharmacokinetic concerns have proved problematic. In the first place, free exogenous VEGF has an extremely short half life-with a plasma half-life of ~34 minutes.²⁸ Due to the poor stability, constant infusion over potentially weeks or months would likely be required for preeclampsia patients, limiting translational potential in a large patient population. Secondly, maternal infusion of VEGF in pregnant animals or genetic overexpression in the developing fetus has been strongly and repeatedly associated with cardiac malformation and fetal demise, indicating that free VEGF is diffusible across the maternal/fetal interface.^{29, 30} It is necessary then that therapies targeting VEGF increase should have two characteristics-increased pharmacokinetic half-life, and restriction to the maternal circulations. This could either be by small molecules agonists of the VEGF receptors which are maternally restricted, or VEGF and/or PlGF fusion peptide chimeras which would both stabilize the peptide and prevent its translocation across the placental barrier. While there are still a number of approaches that could be taken to target the sFlt-1/VEGF imbalance in preeclampsia, evidence strongly suggest that this could be a significant target in the treatment of the disorder.

Maternal Inflammation/Autoimmunity

It has long been postulated that maternal immune activation could play a causative role in the development of preeclampsia, possibly due to incompatibility with the developing fetus. However, it is only more recently that the role of both the maternal innate and adaptive immune responses in causing the associated maternal symptoms has begun to be fully appreciated. In particular, one mechanism consistently found up-regulated in preeclampsia patients and pre-clinical animal models of preeclampsia is the increased production of inflammatory cytokines; in particular tumor necrosis factor α (TNF- α) and interleukin-6 (IL-6).³¹⁻³³ Again supporting a central role for placental ischemia in the development of the disorder, rodents undergoing chronic placental ischemia have been shown to exhibit significantly elevated levels of both TNF- α and IL-6.^{33, 34} Furthermore, infusion of either of the cytokines to levels comparable to those seen in preeclampsia patients results in hypertension during gestation, though not to as great a degree as placental ischemia-suggesting a partial causal role for these factors in preeclampsia-induced hypertension.³⁴⁻³⁶ Perhaps most tellingly, administration of the TNF- α antagonist etanercept tended to decrease placental ischemia-induced hypertension in rats, though this did not reach statistical significance.³³ Even more striking cardiac abnormalities such as fibrosis

deposition and vascular rarefaction produced by placental ischemia in the rat could be significantly attenuated by administration of etanercept in late gestation.³⁷

At present, no prospective studies have been published to examine a potential therapeutic benefit of TNF- α inhibitor administration in either the prevention or treatment of preeclampsia, however there are several interesting and suggestive studies which suggest that this could be an interesting line of future investigation. Though large scale safety trials have not been conducted in the clinical population, the preponderance of the evidence has shown no teratogenic effects or adverse maternal consequences of TNF- α inhibitors during pregnancy, consequently the spectrum of TNF- α inhibitors (etanercept, infliximab, adalimumab, etc.) have been designated as pregnancy class B by the FDA.³⁸ It should be noted, however that while a great number of studies have found TNF- α inhibitors to be safe, these have been almost exclusively small-scale studies, and even the most recent meta analyses are insufficiently powered to draw definitive conclusions.³⁹ There is currently one ongoing industry sponsored observational clinical trial, the OTIS Autoimmune Diseases in Pregnancy Project (clinicaltrials.gov IDNCT00116272), which is to evaluate the incidence of major birth defects, pregnancy complications, and postnatal fetal outcomes in patients taking etanercept for inflammatory disease. A second trial, the Exposure to Remicade (Infliximab) During Pregnancy in Patients With Inflammatory Bowel Disease, Rheumatoid Arthritis, Psoriatic Arthritis, Ankylosing Spondylitis and Psoriasis: a Review and Analysis of Birth Outcomes From the Swedish, Danish and Finnish Medical Birth Registers (clinicaltrials.gov IDNCT00658827), has a similar set of primary and secondary outcomes, but will also include an arm examining patients taking alternative TNF- α inhibitor therapies. Finally, the Humira (Adalimumab) Pregnancy Exposure Registry: OTIS Rheumatoid arthritis in Pregnancy Project (clinicaltrials.gov ID NCT018086059), will examine adalimumab administration and its safety during pregnancy with primary outcomes of major malformation, and secondary outcomes of minor malformations, pregnancy outcome, and 1 year infant outcome. Assuming favorable outcomes of these trials, these and other safety studies should open the door for new clinical trials to evaluate the efficacy of TNF- α blockade as a potential therapy for the preeclampsia patient.

Great research enthusiasm has been generated since the discovery of AT1-AAs in the maternal circulation of preeclampsia patients.²⁴ Intriguingly, follow-up studies which examined the circulation of RUPP placental ischemic rats also found elevations in the AT1-AA levels comparable to those seen in the preeclampsia patients, suggesting a causal link between placental ischemia and the production of the AT1-AA.⁴⁰ Furthermore, infusion of TNF- α into rodents has also been shown to result in an increase in AT1-AA comparable to that seen in placental ischemia, suggesting that production of the AT1-AA may be largely secondary to the initial innate immune response mediated by TNF- α and other inflammatory cytokines.⁴⁰ Importantly, co-administration of an AT-1 antagonist in both the RUPP and TNF- α infusion rodent models resulted in attenuated increases in blood pressure. This suggests that placental ischemia generally, and the inflammatory cytokine-induced hypertension specifically, are partially dependent on AT-1 activation, perhaps partially through production of, the AT1-AA. Indeed, infusion of the AT1-AA led to ~20% increase in blood pressure which was completely abrogated by administration of AT1 receptor

antagonists.²⁶ It seems possible then that the maternal AT-1 receptors could be a viable intervention for the prevention of hypertension in the preeclampsia patient.

Unfortunately, drugs targeting the renin angiotensin system (RAS), including AT receptor antagonists and angiotensin converting enzyme inhibitors, are strongly contraindicated during pregnancy and are believed to be responsible for a range of teratogenic phenotypes.^{41, 42} However, direct inhibition of the AT1-AA itself without interfering with the endogenous RAS components could be a viable approach which would avoid adverse fetal effects. Luckily, the epitope for the AT1-AA has been mapped to a single heptapeptide stretch in the second extracellular loop of the extracellular domain.²⁴ In an intriguing report, Zhou et al co-infused the epitope peptide with the AT1-AA, and found that the hypertensive effects of the AT1-AA on pregnant mice were prevented.⁴³ While infusion of small peptides is not necessarily an ideal therapeutic approach due to stability and pharmacokinetic issues, it is possible that forms of the epitope peptide stabilized by either chemical means, or expressed as a larger more stable chimeric peptide, could have more favorable pharmacokinetics and serve as a viable therapy for chronic treatment of hypertension during pregnancy.

Maternal Endothelial Dysfunction

One common factor believed to be induced by both angiogenic imbalance and the maternal inflammatory response is widespread maternal endothelial dysfunction, hallmarked by overproduction of the endogenous vasoconstrictor peptide ET-1 and a decrease in nitric oxide (NO) bioavailability. Healthy pregnancy is generally associated with increased vascular production of NO from the vascular endothelium particularly in the uterine circulation.⁴⁴ This has primarily been attributed to increased expression of various isoforms of the nitric oxide synthase (NOS) enzymes and thence increased production of the NO secondary messenger cGMP.⁴⁵ Furthermore, studies in a number of animal models have suggested increased vascular NO production, NOS synthesis, and NO-mediated vasodilation temporally as pregnancy progresses, possibly contributing to the known maternal volume expansion that takes place during normal gestation.^{46, 47} As an abundance of studies have suggested an important role for increased NO bioavailability during gestation and the important physiological need for increased delivery of blood to the uterus, it is perhaps not surprising that alteration of NO has been hypothesized as an important mechanism in the etiology of preeclampsia. Indeed, NO metabolites in the umbilical vein and amniotic fluid of preeclampsia patients are significantly higher when compared to women with healthy pregnancies, presumably as a compensation for decreased blood flow in the placental ischemia seen during preeclampsia.⁴⁸ Interestingly the RUPP rodent model of placental ischemia has been shown to have impaired vascular relaxation in response to acetylcholine and decreased levels of NO-associated metabolites, suggesting that placental ischemia is directly responsible for the endothelial dysfunction seen in preeclampsia patients.⁴⁹ It is perhaps not surprising that inhibition of NOS activity in pregnant rodents results in a phenotype reminiscent of preeclampsia, even when compared to inhibition of NOS in non-pregnant animals; including hypertension, proteinuria, fetal growth restriction, and increased renal vasoconstriction.^{46, 50, 51} It seems apparent then, that NO signaling, and alterations in NOS activity/expression are important mediators of the symptoms of preeclampsia.

One potential approach to affecting NO signaling in the preeclampsia patient is through altering the levels of the second messenger cGMP. cGMP is metabolized by hydrolysis and inactivated by the actions of phosphodiesterase (PDE) enzymes, which differ in their tissue expression and localization, and which by their actions exert a vasoconstrictive effect. Early studies demonstrated that the PDE-5 isoform was expressed specifically in the uterine tissue of suggested that inhibition of PDE-5 could semi-specifically cause vasodilation of the vasculature supplying the uterus, and could thereby increase blood flow to the uteroplacental unit. While PDE-5 inhibitors have not been shown conclusively to be safe for administration during pregnancy, a small scale study to investigate their utility for the management of preeclampsia has been published. The study found no adverse effects on either the fetus or mother, but disappointingly found no beneficial effect for preeclampsia patients.⁵³ However, some concerns about the experimental design have been raised, and the effects in a larger clinical population have not been determined.⁵⁴ We have recently examined the role of PDE-5 inhibition in the RUPP model of placental ischemia, and found that administration of sildenafil in late gestation could significantly decrease the associated increase in blood pressure. Interestingly, this was not associated with restoration of angiogenic balance in the placenta, which would be expected with improved blood flow. Intriguingly and inexplicably, placental ischemia was associated with increased renal medullary PDE-5 expression and a subsequent decrease in medullary cGMP.⁵⁵ This was on top of increased medullary PDE-5 expression which occurs in normal pregnancy and contributes to the increased fluid volume.⁵⁶ Several clinical trials have been initiated with varying outcomes: the A Randomised, Double-Blind, Placebo-Controlled, Flexible-Dose, Parallel-Group Study to Evaluate the Efficacy, Safety and Toleration of Oral Sildenafil Citrate Administered in the Dose Range of 20–80 Mg TID for the Treatment of Pre-Eclampsia. (PET) trial (clinicaltrials.gov ID NCT00141310), which ended for statistical reasons and was never resumed, and the Phase 2 Study of Fetal Growth Retardation Treatment by Sildenafil (clinicaltrials.gov ID NCT01107782) and Improvement of Utero-Placental Perfusion and Fetal Growth in IUGR and PET by Administration of Sildenafil Citrate in Pregnancy (clinicaltrials.gov ID NCT00347867) which appear to be inactive. Given the positive results in the pre-clinical studies, as well as an increased appreciation for the role of renal PDE-5 in fluid homeostasis and blood pressure during pregnancy, further clinical studies examining the utility of these agents as therapeutics for preeclampsia are warranted.

Endothelin-a final common pathway

A number of pre-clinical and clinical studies have implicated the production of ET-1 as an important mediator of the endothelial dysfunction and hypertension associated with preeclampsia. The RUPP rodent model has strongly suggested that placental ischemia is associated with increased renal cortical production of ET-1, as assayed at the mRNA level. Furthermore, while administration of an endothelin type A receptor (ET_A) antagonist had no effect on pregnant control animals, the RUPP associated hypertension was completely abrogated.⁵⁷ Subsequently, studies utilizing sera from RUPP animals demonstrated that incubation of cultured endothelial cells with RUPP sera led to a significant increase in ET-1 production when compared to incubation with sera from control pregnant sera, again suggesting that ischemia linked, placental-derived factors in the maternal circulation are

responsible for the endothelial dysfunction which hallmark the disorder.⁵⁸ Interestingly enough, sFlt-1 induced hypertension in pregnant rats has been shown to be significantly attenuated by administration of an ET_A antagonist, indicating that sFlt-1 hypertension is dependent on ET_A activation, and that decreased VEGF bioavailability is a direct cause endothelial dysfunction through this mechanism.¹⁶

Ample evidence suggests that inflammatory cytokine and AT1-AA -induced hypertension is also associated with enhanced production of ET-1. For instance the ~20mmHg of increased blood pressure associated with TNF- α in rodents is accompanied by increased ET-1 production in the renal cortex, aorta, and placental tissue. Further, the associated hypertension was completely blocked by co-administration of an ET_A antagonist.³³ Furthermore, infusion of the AT1-AA to levels similar to those seen in preeclampsia patients results in a similar hypertensive response which was also associated with increased vascular production of ET-1. As with the TNF- α infusion model, co-administration of an ET_A antagonist could ameliorate the hypertensive effect, implicating the endothelin system in AT1-AA induced hypertension.²⁶ Taken together, these studies strongly implicate the ET-1 peptide, specifically through activation of the ET_A receptor as an important regulator of endothelial dysfunction and hypertension during preeclampsia. It stands to reason then, that the ET_A could be an important pharmacologic target for the management of preeclampsia. Unfortunately, knockout of the ET_A receptor or pharmacologic antagonists administered during pregnancy result in severe teratogenicity.^{59, 60} Two approaches remain viable however. In the first, small molecular inhibitors of the ET_A receptor which do not cross the maternal/fetal interface would presumably confer protective effects on the mother without detrimental effects to the fetus. On this line, Thaete et al recently reported a small molecule inhibitor of the receptor, ABT-546, was maternally restricted, with fetal exposure at only ~2% of maternal levels.⁶¹ Likewise, a number of small peptide-based inhibitors of the ET_A receptor have been described which, if stabilized for better pharmacologic parameters and fetal exclusion, could be a potent approach to managing the maternal symptoms of preeclampsia.⁶²

Conclusion

Preeclampsia remains one of the most enigmatic disorders of pregnancy with an incomplete understanding of its etiology and a lack of therapeutic options for the clinical practitioner. However, recent studies have shed significant light into the molecular mechanisms which are responsible for the symptomatic manifestations of the disorder and suggest a number of novel interventions which could prove beneficial for the management of these patients. Whether targeting angiogenic imbalance, maternal inflammation/autoimmunity, or their common effector ET-1, these therapeutic agents could finally provide a weapon in the obstetrician's arsenal in what has previously been an unconquerable disorder.

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