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PREECLAMPSIA (VD GAROVIC, SECTION EDITOR)

The Endothelin System: A Critical Player in the Pathophysiology of Preeclampsia

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Abstract

Purpose of Review Preeclampsia (PE) is a disorder of pregnancy typically characterized by new-onset hypertension and proteinuria after gestational week 20. Although preeclampsia is one of the leading causes of maternal and perinatal morbidity and death worldwide, the mechanisms of the pathogenesis of the disorder remain unclear and treatment options are limited. Placental ischemic events and the release of placental factors appear to play a critical role in the pathophysiology. These factors contribute to a generalized systemic vascular endothelial dysfunction and result in increased systemic vascular resistance and hypertension.

Recent Findings There is increasing evidence to suggest that endothelin-1 (ET-1) in the maternal vascular endothelium is a critical final common pathway, whereby placental ischemic factors cause cardiovascular and renal dysfunction in the mother. Multiple studies report increased levels of ET-1 in PE. A number of experimental models of PE are also associated with elevated tissue levels of prepro-ET-1 mRNA. Moreover, experimental models of PE (placental ischemia, sFlt-1 excess, TNF-α excess, and AT1-AA infusion) have proven to be responsive to ET type A receptor antagonism. Recent studies also suggest that abnormalities in ET type B receptor signaling may also play a role in PE.

Summary Although numerous studies highlight the importance of the ET system in the pathogenesis of PE, further work is needed to determine whether ET receptor antagonists could provide an effective therapy for the management of this disease.

Keywords Preeclampsia · Pregnancy · Hypertension · Endothelin · Endothelium · Placenta · Cardiovascular · Blood pressure · Vascular smooth muscle

Introduction

Preeclampsia (PE) is a pregnancy-specific disorder defined as hypertension > 140/90 mm Hg in pregnancy and proteinuria > 0.3 g/24 h after 20 weeks of gestation. Recently, the American College of Obstetricians and Gynecologists has broadened the definition of PE to blood pressure > 140/90 after 20 weeks of pregnancy and either proteinuria ≥ 300 mg/24 h or protein/creatinine ratio ≥ 0.3 or one of the following: thrombocytopenia, elevated liver transaminases, pulmonary edema, new onset renal insufficiency, or cerebral or visual disturbances [1]. Despite being a leading contributor of maternal and perinatal morbidity and death worldwide, the mechanisms of the pathogenesis of PE remain unclear and treatment options are very limited [2–4]. Current therapy for the management of PE includes antihypertensives, such as methyldopa, labetalol, and nifedipine and magnesium sulfate for prevention of eclamptic seizures [5]; however, these treatments have limited efficacy, and the only "cure" for PE is the delivery of the placenta. Early-onset PE (< 34 weeks) is more severe, and outcomes are especially serious if disease develops every preterm (< 32 weeks). Each week pregnancy is prolonged markedly reduces fetal morbidity and mortality, but only at the expense of an increased risk of maternal morbidity or even death [6]. A therapeutic approach that will prolong pregnancy yet do no harm to the fetus represents a critically important step in identifying agents for treatment of PE.

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The Pathogenesis of PE

While the exact causes of PE remain unknown, it is thought that in some cases of the disease, especially early-onset PE, abnormal placentation due to insufficient trophoblast invasion and failure of spiral artery remodeling leads to inadequate blood flow to the placenta and a continuing cycle of repeated ischemia-reperfusion injury [3, 7]. The resulting hypoxic environment within the placenta stimulates oxidative stress and the release of placental factors such as soluble fms-like tyrosine kinase 1 (sFlt-1), soluble endoglin, agonistic autoantibodies to the angiotensin type 1 receptor (AT1-AA), and inflammatory cytokines [8]. These factors, along with the presence of additional maternal risk factors for PE, such as age, obesity, and pre-existing hypertension, contribute to the vascular endothelial dysfunction, vasoconstriction, and hypertension in PE.

It is becoming increasingly evident that an important final common pathway whereby many soluble placental factors elicit cardiovascular and renal dysfunction during PE is by activation the endothelin (ET) system. In order to develop safe therapeutic interventions of this system, it is important to fully understand the role of the ET system in blood pressure control during normal pregnancy and in PE. In this review, we start by discussing biology of the ET system, its role during normal pregnancy, and end with highlighting the critical role of ET in the pathophysiology of PE by discussing the therapeutic potential and limitations for treatment of PE.

The ET System

ET-1 is the most potent vasoconstrictor substance known in the human cardiovascular system [9]. ET-1 is derived from prepro-ET-1 mRNA. The mRNA is then translated to prepro-ET-1, which is first cleaved by furin to produce big-ET-1 and then subsequently acted on by ET converting enzymes (ECEs) or other enzymes to generate bioavailable ET-1 [9]. ET-1 is 21 amino acids in length and is produced and released from endothelial and other cell types. Since approximately 80% of ET-1 is secreted from the basolateral side of endothelial cells [10], ET-1 typically acts in an autocrine fashion signaling, but under conditions of marked endothelial activation, ET-1 can spill over into the systemic circulation and affect distant organ systems [11].

Once released, ET-1 acts largely on two cell-surface G-protein-coupled receptors, the ET-1 Type A (ET_A), located primarily on vascular smooth muscle, and Type B (ET_B) receptors on endothelial, vascular smooth muscle, and renal epithelial cells [9]. Binding of the ET_A receptor elicits vasoconstriction via increased Ca^{2+} influx and generation of reactive oxygen species (ROS) [12, 13]. In contrast, ET_B receptors on the endothelium allow ET-1 to signal in an autocrine fashion and stimulate nitric oxide synthase (NOS) and NO production, which serves the vasodilatory arm of this system [14]. Therefore, it can be appreciated that there is a balance of ET-1-mediated control of vascular tone, with vasodilation promoting proper blood pressure regulation during pregnancy but aberrant signaling of these receptors encouraging vasoconstriction and hypertension in PE.

The ET System in Normal Pregnancy

Human studies have revealed changes in ET receptor expression during pregnancy, whereas most of our understanding for the functional role of ET-1 in blood pressure regulation comes from experimental animal studies. For example, ET_A and ET_B receptor expression is increased in the uterus in pregnant versus non-pregnant women and is thought to modulate contraction during normal labor [15–17]. Alternatively spliced forms of ET_A in smooth muscle cells isolated from human placenta may control the amount of function ET_A involved in constriction [18]. Unfortunately, these studies only yield correlative data and they do not provide functional information regarding the role of ET-1 in blood pressure regulation during pregnancy.

Pharmacological and genetic studies in rodents provide a better insight into the function of the ET system during normal pregnancy. ET_A receptor control of blood pressure remains similar to non-pregnant levels. Some studies show that antagonism of this receptor reduces blood pressure similarly between groups [19] and others a minimal change in response to antagonism during pregnancy [20]. With regard to ET_B, its expression is increased in decidua in humans [21] but is not altered by pregnancy in isolated blood vessels in rodents [22]. Yet, studies point to greater ET_B receptor-mediated relaxation in human and rodent arteries during pregnancy [23, 24]. This is attributed to pregnancy-related factors, including relaxin and proteinases, to convert big-ET-1 to the alternate form of ET(1–32), which signals via ET_B to stimulate NOS in endothelial cells [24, 25]. Whether ET_B receptor control of blood pressure is greater during pregnancy is debatable. Some investigators suggest that it contributes to the increased vasodilation and reduced blood pressure during pregnancy [23, 26], whereas others do not [19]. Pharmacological blockade of ET_B increases blood pressure during pregnancy, but whether this response is greater than non-pregnant conditions to implicate greater ET_B signaling in control of blood pressure during pregnancy is lacking. This requires further investigation, and genetic ET_B-deficient models have become available for this purpose.

Pregnancy-related factors allow big-ET-1 conversion to ET(1–32) that acts on ET_B receptors within the kidney [25, 27, 28]. This is thought to increase GFR during pregnancy, which is likely due to greater relaxation of the renal afferent

and efferent arterioles [29]. ET_B receptors are shown to contribute to efferent arteriole dilation via NO signaling [30, 31]. It is unknown the extent to which this pathway modulates ET_A-induced constriction in the renal circulation. Furthermore, the role of the ET_A or ET_B pathways on direct tubular handling of sodium has not been examined during pregnancy. It is possible that relaxin shunts big-ET-1 away from ET-1(1–21) and its sodium excretory ability and towards ET-1(1–32) having vasodilatory actions without direct signaling of sodium control in the tubules. This is unknown. Again, what is known is that under non-pregnant conditions from rodent studies, ET-1 promotes sodium excretion via both ET receptors in females via NO signaling, whereas pregnancy is associated with increased GFR and renal blood flow via NO signaling but reduced sodium excretion due to attenuated NO signaling. Overall, this allows for plasma volume expansion of normal pregnancy [32], but the complete understanding of ET-1 involvement in this process is not yet fully elucidated. Although urinary levels of ET-1 are higher in pregnant versus non-pregnant women and increases as pregnancy progresses [33], it is unclear the relative levels of the different ET-1 cleavage products and their actions on vascular and renal function in proper blood pressure regulation during normal pregnancy.

The ET System in PE

ET-1 Levels in PE Multiple studies have examined circulating ET-1 levels in normal pregnant and preeclamptic women, and found elevated plasma ET-1 in the preeclamptic group, with some studies indicating that circulating ET-1 correlates with the severity of the disease symptoms, though this is not a universal finding [34–39]. Verdonk et al. recently investigated the relationship between disturbed angiogenic balance, arterial pressure, and ET-1 in pregnant women with a high (≥ 85; $n = 38$) or low (< 85) soluble Fms-like tyrosine kinase-1 (sFlt-1)/placental growth factor (PlGF) ratio [40]. Plasma ET-1 levels were increased in women with a high ratio. In addition, plasma ET-1 correlated positively with sFlt-1. Aggarwal et al. also investigated the correlation between ET-1 and sFlt-1, PlGF, and soluble endoglin (sEng) levels during uncomplicated normotensive pregnancy and PE [41]. Their results also show an association between elevation of sFlt-1, sEng, and ET-1 in the maternal circulation in PE, which strengthens the possibility that ET-1 could be a mediator in pathogenesis of PE secondary to the anti-angiogenic factors, sFlt-1, and sEng released by the placenta [41].

A number of experimental models of PE are also associated with elevated tissue levels of the ET-1 precursor, prepro-ET-1 mRNA. Both the renal cortex and medulla of placental ischemic rats express significantly higher levels of prepro-ET-1 when compared to normal pregnant controls [20]. Furthermore, chronic elevation of sFlt-1 in pregnant rats directly increased prepro-ET-1 gene expression in the renal cortex [42]. It has also been shown that infusion of the pro-inflammatory cytokine, tumor necrosis factor-alpha (TNF-α), directly induced hypertension in pregnant rats and is associated with significant increases in the expression of prepro-ET-1 in the maternal vasculature, placenta, and kidney [43]. Finally, infusion of the AT1-AA into the pregnant rat results in moderate hypertension and is associated with increased prepro-ET-1 expression in both the renal cortex and placenta [44].

Another potential mechanism for increased ET-1 levels in PE is via matrix metalloproteinase (MMP) activation. MMPs are enzymes that cleave the ET-1 precursor, big-ET-1 to active ET-1. Interestingly, the expression levels of MMPs (particularly MMP-2 and MMP-1) have been shown to increase in women who subsequently develop PE. Abdalvand et al. recently hypothesized that the increased MMP-2 expression leads to increased big-ET-1 conversion, and therefore increasing vasoconstriction [45]. They reported increased vascular contractility to big-ET-1 in the reduced uterine perfusion pressure (RUPP) model of PE that was likely attributable to upstream enzymatic pathways. These data are consistent with the greater contribution of MMP to cleave big-ET-1 to ET-1 ex vivo in RUPP rats, suggesting that this enzyme could be partially responsible for increased big-ET-1-induced contractility. Nugent et al. also recently examined the potential role of MMP-1 as an activator of protease-activated receptor 1 (PAR-1), which is known to mediate the release of ET-1 in endothelial cells [46]. They reported increased serum and vascular MMP-1 in women with PE and hypothesized that the action of MMP-1 on PAR-1 might have vasoconstrictive effects. They demonstrated that MMP-1 has potent vasoconstrictor effects and the ability to enhance vascular reactivity to vasoconstrictor hormones, which are mediated by an endothelial ET-1 pathway. The authors concluded that increased circulating levels of MMP-1, and increased expression in systemic vessels of women with PE, may contribute to the development of hypertension in the mother.

ET_A Receptors in PE A number of experimental animal models have been utilized to examine the etiology and development of PE [47–49]. One of which we and others have used with great success is the RUPP model, where mechanical restriction of blood flow to the placenta causes hypoxia and ischemia [49]. This model has been used in species ranging from rats to non-human primates and mimics a number of the pathological features of human preeclampsia, such as hypertension, angiogenic imbalance, renal injury, proteinuria, and endothelial dysfunction [20, 50••, 51]. The renal cortex and medulla of RUPP rats express significantly higher prepro-ET-1, when compared to normal pregnant controls [18]. We have also

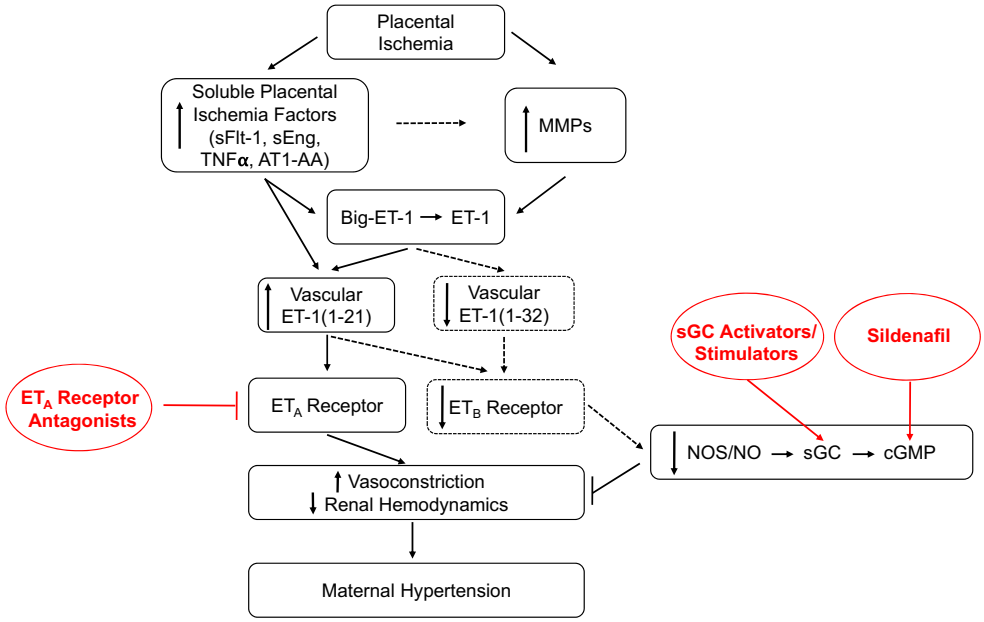

Fig. 1 Hypothetical scheme highlighting the importance of the ET-1 system in blood pressure regulation during placental ischemia and PE. Placental ischemia induces the release of soluble factors for the placenta that target the vasculature. There, they activate the endothelium to produce ET-1(1–21). This acts on the ET_A receptor on underlying vascular smooth muscle to elicit vasoconstriction and reduced renal hemodynamics to result in maternal hypertension. It has been shown that placental ischemia-induced hypertension in RUPP rats is linked to increased activity of matrix metalloproteinases (MMPs) in the blood vessel wall, which cleave big-ET-1 to active ET-1(1–21). The dashed line indicates that it is unknown if the placental factors mediate increased MMP. Furthermore, it is unknown the extent that ET-1(1–21) or its alterative form, ET-1(1–32), is produced or activates any remaining ET_B receptors during hypertensive pregnancies. Knowing the vasoprotective mechanisms that are still available for targeting in the mother will allow for a full understanding of how upcoming therapeutic approaches are beneficial in PE. These include the therapies illustrated in red, including ET_A antagonists, activators and stimulators of sGC, or compounds to increase cGMP bioavailability, like the PDE5 inhibitor, sildenafil. This understanding could allow for optimization of these pharmacological strategies in PE

reported that serum from RUPP animals significantly induces production of ET-1 from the endothelial cells when compared to those exposed to serum from normal pregnant animals, suggesting that circulating factors produced by placental ischemia are responsible for increased vascular ET-1 production [52]. When an ET_A receptor antagonist was administered to the RUPP rats, the associated hypertension was abolished, and there was a trend for improved renal function [20]. Tam Tam et al. also reported that pretreatment with ET_A attenuates both the mean arterial pressure (MAP) and uterine artery resistance index (UARI) in the RUPP group without affecting these parameters in the normal pregnant group [53]. The improvement in UARI could be one potential mechanism for the reduction in MAP in response to ET_A in pregnant dams with ischemic placentas. Collectively, these studies suggest that placental ischemia induces factors that activate the production of ET-1 in the vasculature, which acts through ET_A receptors to mediate the maternal hypertension seen in RUPP model. Zhou et al. also demonstrated that chronic hypoxia during gestation triggers PE-like symptoms in pregnant rats via heightened ET-1- and ET_A-mediated signaling, providing a molecular mechanism linking gestational hypoxia and increased risk of PE [54].

Several studies have investigated the role of the ET-1 system in models of sFlt-1 excess in pregnancy. In support of the role of ET-1 in mediating sFlt-1-induced hypertension, our group has recently reported that continuous infusion of sFlt-1 in pregnant rats directly increased the level of ET-1 in the renal cortex and caused an increase in the MAP of ~ 20 mmHg [42]. Administration of an ET_A receptor antagonist abolished this hypertension, which strongly supports that ET-1 is an important mediator of sFlt-1-induced hypertension. Kappers et al. also reported that the administration of Sunitinib, a tyrosine kinase inhibitor of the vascular endothelial growth factor (VEGF) receptor, induces a reversible rise in blood pressure in patients, and also in rats, associated with activation of the ET-1 system and generalized microvascular dysfunction [55]. Moreover, Sunitinib in swine results in ET-mediated hypertension [56]. Thus, another potential mechanism whereby VEGF blockade could increase blood pressure is by enhancing ET-1 synthesis.

The innate immune system has also been implicated in PE. For example, increased production of TNF-α is seen in both preeclamptic women and rodents undergoing chronic placental ischemia [43, 57, 58]. Studies in vitro demonstrated that production of ET-1 by endothelial cells could be mediated by exposure to TNF-α [59]. Studies from our group have

demonstrated that administration of the soluble TNF-α receptor, Etanercept, reduces hypertension associated with placental ischemia in pregnant rats [43]. This treatment is accompanied by reduced expression of prepro-ET-1 in the renal cortex and medulla as well as the placenta itself. It has also been shown that infusion of TNF-α alone induces hypertension in pregnant rats, producing an approximate 20 mmHg increase in MAP in late gestation [60]. This is also associated with a significant increase in the expression of prepro-ET-1 in the maternal vasculature, placenta, and kidney. As seen in the RUPP model, administration of an ET_A receptor antagonist in these animals completely abolished the associated hypertension [43]. LIGHT, a novel tumor necrosis factor superfamily member, is also significantly elevated in the circulation and placentas of preeclamptic women [61]. Injection of LIGHT into pregnant mice causes placental apoptosis, small fetuses, and key features of PE, with hypertension and proteinuria. The work reported in this study was the first to show that elevated LIGHT, coupled with enhanced lymphotoxin β and herpes virus entry mediator receptor activation, promotes placental damage and triggers release of potent vasoactive factors, namely sFlt-1 and ET-1. Together, these data suggest that ET-1, acting via the ET_A receptor signaling, is a crucial mediator of TNF-α-induced hypertension in pregnancy.

AT1-AA is elevated in PE women as well as in RUPP rats. Studies show that infusion of the AT1-AA directly into the pregnant rat results in moderate hypertension that is associated with increased prepro-ET-1 expression in both the renal cortex and the placenta. Administration of an ET_A receptor antagonist abrogated the hypertensive response to AT1-AA, highlighting the importance of the ET-1 system in the manifestation of AT1-AA-induced hypertension [44]. Recent studies also report that IgG from women with PE increase preproET-1 mRNA expression via angiotensin II type 1 receptor activation in kidneys and placentas in pregnant mice [62]. The ET_A receptor-specific antagonist, BQ123, significantly reduced autoantibody-induced hypertension, proteinuria, and renal damage in these mice, suggesting that autoantibody-induced ET-1 production contributes to the pathogenesis of PE.

ET_B Receptors in PE Less is known about the regulation of ET_B receptors in PE and their role in blood pressure regulation in this maternal disorder [63•]. In the RUPP model, there is reduced vascular expression of ET_B receptors compared to their normal pregnant counterparts [64], which implicates reduced ET_B receptor signaling in the vasculature during the pathogenesis of PE. Most studies have only examined this in systemic vessels and not in the kidney vasculature. Nevertheless, administration of an ET_B agonist was still able to reduce blood pressure in RUPP rats [64], and ET_B-deficient pregnant rats have exaggerated placental ischemia-induced hypertension [26]. It is hypothesized that the exaggerated placental ischemia-induced hypertension in ET_B-deficient rats is due to defective NO signaling, which suggests that ET_B are still present and act to buffer hypertension in wild-type controls. Thus, ET_B receptors could be an important target in PE, and upregulation of endothelial ET_B receptors, by using pharmacological agonists or genetic approaches, may represent novel strategies to manipulate this system in treatment of this maternal disorder. Foremost, it would be interesting to determine if ET_A blockade is capable of completely reducing hypertension in ET_B-deficient pregnant rats to determine if the latter receptor is important for the efficacy of ET_A blockade. Moreover, a novel therapeutic option to increase NO signaling in PE and downstream of defective ET_B signaling are activators and stimulators of the NO receptor, soluble guanylate cyclase (sGC) [59], or agents to increase bioavailability of the product of NO-sGC signaling, cGMP. Sildenafil, an inhibitor of phosphodiesterase enzyme 5 (PDE5) that quenches bioavailable cGMP, has been reported to attenuate hypertension and intrauterine growth restriction in a model of superimposed PE [65•]. These targeted strategies are highlighted in Fig. 1.

Targeting ET_A Receptor Signaling as a Potential Therapeutic Target in PE

ET_A receptor antagonists are used in the treatment of numerous cardiovascular diseases including pulmonary and systemic hypertension, congestive heart failure, myocardial infarction, vascular restenosis and atherosclerosis, renal failure, cerebrovascular disease, and cancer. Given the myriad of experimental models of PE, which have proven susceptible to ET_A antagonism, could the ET-1 system be a therapeutic approach in managing the hypertension associated with PE? The clinical relevance of this potential therapeutic approach is tempered by work showing that genetic knockout of the ET_A receptor leads to birth defects and eventual embryonic lethality in mice [66]. As a result, administration of ET receptor antagonists is contraindicated in pregnancy [67]. However, specific windows during early to mid-gestation have been identified in animal studies, where pharmacological antagonism of ET_A caused phenotypes similar to that seen in the knockout. Administration of the ET_A antagonist only during late gestation was not performed, and it is entirely plausible that ET_A receptor antagonists might prove safe and efficacious in later pregnancy, when the symptoms of PE are most severe [68]. Furthermore, development of ET_A receptor antagonists that do not cross the placental barrier would circumvent these problems altogether. Indeed, Thaete and colleagues reported of an ET receptor antagonist that had limited access to the fetal compartment during chronic maternal administration late in pregnancy [69]. Moreover, linking ET_A receptor antagonists with elastin-like peptides that do not cross the placental barrier could also be an approach to limit the effects of ET_A receptor

antagonism within the fetal circulation but allow beneficial effects localized to the maternal circulation in PE [70].

Conclusions

Although numerous studies highlight the importance of the ET system in the pathogenesis of PE, further work is needed to determine whether ET receptor antagonists could provide an effective therapy for the management of this disease.

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Compliance with Ethical Standards

Conflict of Interest The authors declare no conflicts of interest relevant to this manuscript.

Human and Animal Rights and Informed Consent This article does not contain any studies with human or animal subjects performed by any of the authors.

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