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Review Article

Endothelial Nitric Oxide synthase (eNOS) in Preeclampsia: An Update

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Abstract

Preeclampsia (PE) is a common pregnancy-related hypertensive disorder and is a leading cause of maternal and perinatal morbidity and mortality. The incidence of PE and its associated health care costs have been increasing in the United States over the past three decades. Pregnancies complicated by PE put both the mother and child at increased risk for chronic illnesses such as cardiovascular disease, cerebrovascular disease, and cognitive impairment later in life. To date, there is no effective treatment for PE and the etiology of PE is largely unknown. While human epidemiological studies have established an association between various genetic factors and PE, a causative link between genes associated with PE and PE development has been difficult to establish. Human studies have shown that variants in eNOS (endothelial nitric oxide synthase, also known as NOS3) gene are associated with PE, and animal experimental studies have provided evidence to show the potential functional connection between the eNOS gene and PE. Here we review several studies that investigated the role of eNOS in PE, as well as studies that described how manipulating the eNOS/NO pathway could aid in disease intervention.

Preeclampsia

Preeclampsia (PE), a pregnancy related disorder that is characterized by hypertension, proteinuria, and end-organ damage in the mother, affects 5-8% of pregnancies in the United States [1,2]. Unfortunately, the incidence of PE in the United States has increased over the past three decades despite numerous clinical and experimental studies on the disease [3]. There are two major classifications of PE: early-onset PE (EOP), which develops before 34 weeks of pregnancy, and late-onset PE (LOP) which develops on or after 34 weeks of pregnancy. About 20% of PE cases are EOP, which is most commonly associated with severe clinical symptoms, while patients with LOP usually have mild symptoms [4]. Although approximately 75% of PE cases are mild, some severe PE cases (approximately 10%) [5] can evolve into eclampsia without intervention. Eclampsia clinically manifests in symptoms like muscle pain, disturbances of vision, and seizures in the mother. Even though eclampsia is a devastating life-threatening condition, it rarely happens today [6]. Currently, there is no effective method to predict which woman could develop PE [7,8] and no real cure for PE or eclampsia; the ultimate treatment is delivery of the placenta and baby as physicians did a century ago [8].

The etiology of PE is still largely unknown. Dr. Redman's two-stage model of the disease has been progressively refined since it was first proposed in 1991, and it is still a widely accepted theory of PE pathogenesis [9,10]. In stage one placentation is impaired which results from poor trophoblast cell invasion into the maternal endometrium. This occurs before clinical manifestations of the disease. Insufficient placentation makes the placenta hypoxic or ischemic, causing the release of detrimental antiangiogenic and inflammatory factors into circulation. These soluble factors lead to systemic maternal endothelial dysfunction which subsequently causes clinical signs of PE, such as hypertension and proteinuria (stage two) [10,11].

Pre-pregnancy health conditions including chronic hypertension, obesity, diabetes mellitus, renal disease and autoimmune disorders increase risk for PE [12]. These pre-existing maternal conditions can negatively affect placentation, leading to elevated detrimental factors—i.e., soluble form of

vascular endothelial growth factor receptor 1 (sFlt1), tumor necrosis factor α (TNFα)—and decreased beneficial factors—i.e., vascular endothelial growth factor (VEGF) and placental growth factor (PLGF) in circulation [13]. Increased detrimental factors and decreased beneficial factors could synergistically exacerbate PE symptoms. (Figure 1) summarizes this hypothesis.

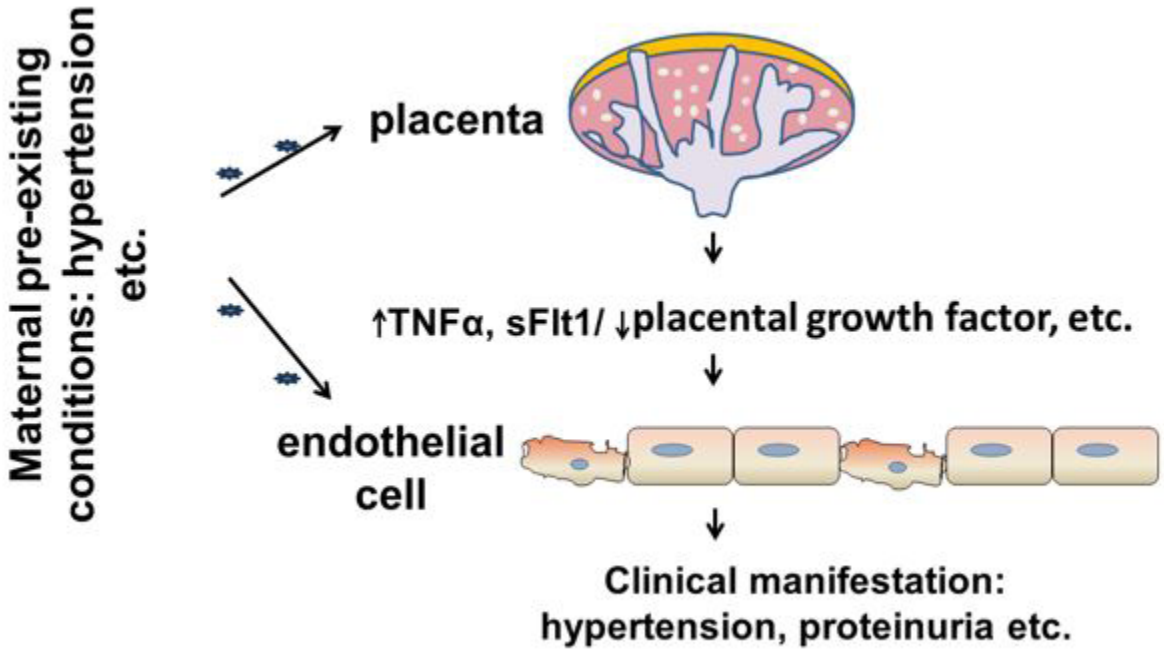

Figure 1: The potential role of pre-existing maternal conditions in risk of PE. Detrimental factors (blue stars) from low degree inflammation and hypertensive conditions caused by eNOS polymorphisms influence both endothelial cells and placentation negatively. Poor placentation increases antiangiogenic (sFlt1) and inflammatory (TNFα) factors and decreases angiogenic factors (VEGF, PLGF) in maternal circulation. Placental dysregulation of these factors worsens endothelial dysfunction and PE symptoms.

Primipaternity, advanced maternal age, multiple pregnancies, and molar pregnancy are also risk factors for PE [14]. Clustering of cases of PE within families has been reported, suggesting genetic factors play an important role in the disorder [14,15]. It is very challenging to decipher the impact of genetics in PE on humans. Current knowledge of the susceptibility genes for PE and the impact of environmental factors on susceptibility genes is limited. Fortunately, animal models act as an effective tool to study the involvement of genetic factors in PE. In this review, we focus on the eNOS gene because ofits association with PE and its key role in blood pressure (BP) regulation [16-19].

eNOS and PE

Human studies

While eNOS is mainly expressed by endothelial cells [18,20], it is also detectable in cardiomyocytes [21], adipocytes [22], kidney tubular epithelial cells [23] and syncytiotrophoblasts of human placenta [24]. Normal eNOS function is essential for maintaining appropriate blood pressure (BP) through its enzymatic conversion of L-arginine to nitric oxide (NO)—a colorless gas with short half-life [25]—and cyclic guanosine monophosphate (cGMP) is the mediator of its biological function. The role of eNOS/NO and in cardiovascular system and its mechanism are elegantly reviewed by Oliveira-Paula et al. [19].

NOS3 is a highly polymorphic gene with more than one thousand genetic variations including single nucleotide polymorphisms (SNPs), insertions/deletions, variable number of tandem repeats (VNTR), and microsatellites [19]. Many studies highlight three NOS3 polymorphisms with the most significant functional implications and clinical relevance; these polymorphisms alter NOS3 expression/activity, leading to decreased NO bioavailability [19]: 1. SNP rs2070744 (g.-786T > C) is characterized by a thymine to cytosine substitution at the position -786 and decreases NOS3 transcriptional activity. 2.

SNP rs1799983 (Glu298Asp), results from a guanine to thymine substitution at position 894 of exon 7 (G894T). This leads to a glutamine to aspartate replacement at position 298 of the protein which affects posttranslational modifications, ultimately resulting in decreased NOS3 activity and NO formation. 3. 27-base pair VNTR in intron 4 (VNTR 4a/4b polymorphism) [26,27]: a functional study showed endothelial cells with 5 copies of the 27-base pair VNTR (4b variant) not only have more small RNA than cells with 4 copies of the 27-base pair VNTR (4a variant) but also lower levels of NOS3 mRNA [19,28].

Yoshimura's group was first to report the frequency of the G894T variant (rs1799983) to be significantly higher in severe PE patients [29,30]. Since then, many investigators have reported the association of this SNP with PE in different countries and ethnic groups [31-36]. In Tunisian Arab women, Ben Ali Gannoun et al. found higher frequencies of heterozygous G894T genotypes in PE patients compared to normal pregnant women [35]. Later, a meta-analysis performed by Abbasi et al. also showed the G894T polymorphism was associated with an increased risk of PE, especially among Caucasian and Mixed populations [36]. Alpoim et al. also reported the frequency of the 894T allele was higher in late severe PE patients than in normotensive pregnant women [37]. The association between this SNP and preeclampsia is also found in Iranian women [38]. Besides higher frequencies of heterozygous G894T, Ben Ali Gannoun et al. also found higher frequencies of homozygous -786T/-786T (rs2070744) in PE [35]. g.-786T > C is also associated with increased risk for developing severe PE according to Seremak-Mrozikiewicz et al. [39]. Fondjo et al. reported pregnant Ghanaian women carrying the 4c allele had increased risk for PE [40]. Alpoirn et al. also reported that higher frequencies of aa genotype and a allele for VNTR a/b polymorphism were observed in early severe PE compared to late severe PE and normotensive pregnancies [37].

Mechanistic studies suggest that inflammation and oxidative stress resulting from decreased NO production related to eNOS polymorphisms can play a critical role in PE [41]. For example, Zhuge et al. proposed that eNOS deficiency leads to decreased red blood cell-derived NO bioactivity, subsequent vascular oxidative stress, endothelial dysfunction and PE [42]. Most recently, it is reported that preeclamptic women had reduced plasma eNOS concentrations [43] emphasizing importance of eNOS in pregnancy.

Animal studies

Because mice lacking eNOS (eNOS-/-) have hypertension and human studies have established the association between NOS3 polymorphisms and PE, it is logical to conjecture that female mice lacking eNOS would develop superimposed PE-like phenotypes during pregnancy. However, this is not the case. Shesely et al. first reported that BP decreased to wild type (WT) levels in pregnant mice lacking eNOS-/- during the second week of the pregnancy, although they had higher BP than WT female mice before pregnancy [44]. The authors did not report any other manifestations of PE such as proteinuria and fetal growth restriction in their paper [44]. Later, Adamson's group studied eNOS-/- pregnant mice more thoroughly. They demonstrated that eNOS-/- pregnant mice did not show maternal clinical signs of PE, but they developed placental ischemia and insufficient maternal spiral artery remodeling [45]. Despite apparent placental hypoxia in eNOS -/- pregnancies, plasma levels of sFlt1 did not increase; placental sFlt1 mRNA in eNOS -/- pregnant mice was even lower than that in placentas from WT pregnant mice. Authors speculated that eNOS was required to produce sFlt1 in placentas. Thus, low sFlt1 in eNOS-/- pregnant mice may explain why these dams did not have maternal phenotypes of PE. This intriguing observation brought up an unavoidable question: what would the maternal response be if the circulating sFlt1 is increased in eNOS-/- mice? Our research provided some clues: a lack of eNOS exacerbated the endothelial injury caused by sFlt1 [46]. In our study, both WT and eNOS-/- female virgin mice received an adenovirus to over-express sFlt1 (Adv-sFlt1) from the livers. Excess sFlt1 in circulation caused hypertension, proteinuria and endotheliosis in both WT and eNOS-/- virgin mice, but to greater extents in eNOS-/- mice. Additionally, eNOS-/- mice with excess sFlt1 demonstrated severe effacement of foot process of podocyte and a decreased glomerular filtration rate (estimated by creatinine clearance), which were not present in WT mice possessing the same plasma levels of sFlt1 [46]. Considering all these observations, we hypothesize that maternal systemic endothelial dysfunction and maternal phenotypes of PE could occur in eNOS-/- pregnant mice if their plasma sFlt1 increases. The high sFlt1 level combined with pre-existing endothelial problems in eNOS-/- mice could cause very severe outcomes such as malignant hypertension and severely impaired kidney function in pregnant eNOS-/- mice, even leading to the demise of fetuses and dams.

Experiments using pharmacological approaches have also provided evidence that eNOS plays an important role in PE. A study conducted by Molnár et al. reported that inhibition of eNOS by NG-nitro-L-arginine methyl ester (L-NAME) resulted in a full spectrum of PE-like phenotypes in pregnant rats [47]. The authors did not report whether the circulating sFlt1 was increased in these pregnant rats. Bahtiyar et al. reported that infusion of L-NAME to pregnant rats significantly increased serum sFlt-1 [48]. Although L-NAME is a potent non-selective inhibitor of all three isoforms of nitric oxide synthetase—eNOS, inducible nitric oxide synthase (iNOS, also known as NOS2) and neuronal nitric oxide synthase (nNOS, also known as NOS1)—it has widely been used to induce hypertension by inhibiting eNOS [49-52]. Experimental conditions using L-NAME to inhibit eNOS in animals mimic human subjects with altered eNOS better than eNOS-/- mice because there is no individual reported with complete lack of eNOS in humans.

Taken together, ischemic/hypoxic placentas with a complete lack of eNOS do not increase sFlt1 while placentas with impaired—but not completely diminished—eNOS increase sFlt1. Insufficient eNOS in maternal tissues could then cause an aggravated response to excess sFlt1 from placentas (Figure 2).

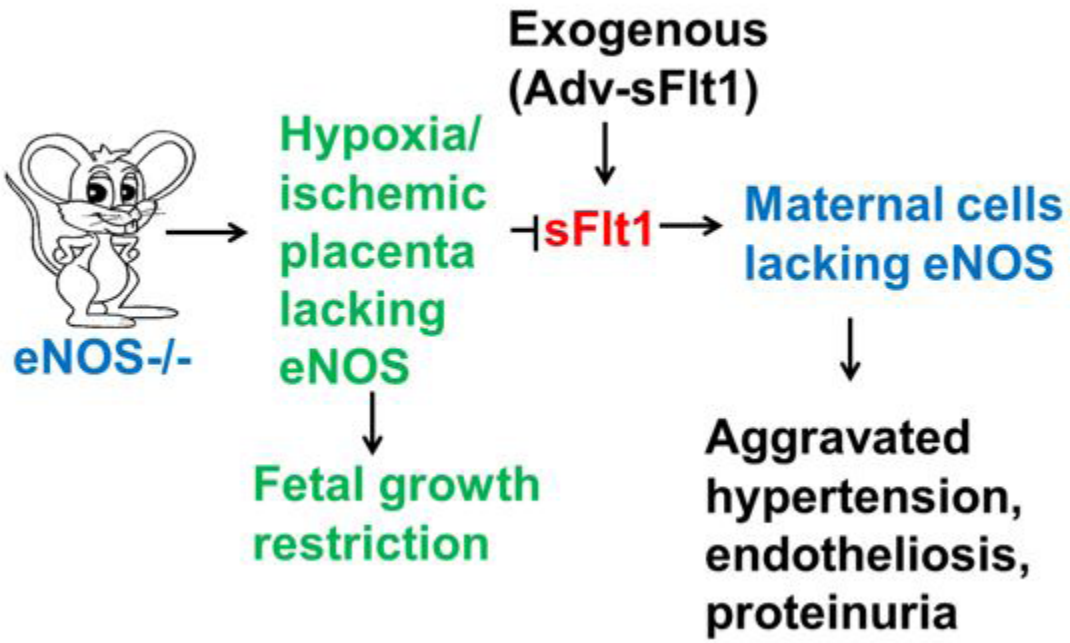

Figure 2: Mechanism illustrating the potential role of eNOS in PE. eNOS-/- placentas develop hypoxia/ischemia, which causes fetal growth restriction, while maternal symptoms of PE are not exhibited because sFlt1 production is low due to a lack of eNOS. When eNOS-/- mice overexpress sFlt1 through exogenous source (Adv-sFlt1), they exhibit aggravated endothelial dysfunction and symptoms of PE (more severe hypertension, endotheliosis, and proteinuria). ⊣: inhibiting sFlt1 production.

Studying heterozygous (eNOS+/–) pregnant mice could provide meaningful information regarding the role of eNOS in PE. eNOS+/- mice have approximately 50% as much eNOS protein as WT mice [53], and this modest decrease in eNOS is comparable to that associated with polymorphisms in the human eNOS gene [54-56]. Generally, eNOS+/- mice do not show obvious abnormalities except a slightly elevated BP without extra stress [57]. However, we have demonstrated that eNOS+/- male diabetic mice develop diabetic nephropathy and the severity of the phenotype is between WT diabetic mice and eNOS-/- diabetic mice [53]. It is also reported that eNOS+/- mice were equally hyper-responsive to mild hypoxia as eNOS-/- mice [58]. Indeed, Roberts et al. have demonstrated that eNOS+/- dams (mated with WT males) have elevated systolic blood pressure (SBP) and decreased fetal weight, though their litter size is not altered [59]. In addition, the carotid arteries from eNOS+/- dams have a reduced relaxation capability compared to WT dams [59]. However, the authors did not report whether eNOS+/- dams have kidney problems or increased sFlt1 in their circulation. Conceivably, eNOS+/- pregnant mice which are mated with eNOS-/- males (as both maternal and fetal lack of eNOS contributes to placental problems [45]) may have more severe placental problems than placentas from eNOS+/- dams mated with WT males. This would lead to an increase in circulating sFlt1 that aggravates maternal PE-like phenotypes (including kidney problems). Future experiments are needed to test this hypothesis.

Potential PE Intervention Associated with eNOS/NO Signaling

Because of the critical role of eNOS/NO in maintaining endothelial homeostasis, eNOS mutations could dysregulate any step of pregnancy from maternal pre-existing health conditions, to placentation, or even altered maternal endothelial response to soluble factors released by placentas. Therefore, screening women for eNOS gene polymorphisms could not only help physicians

identify women at risk for developing PE but also allow physicians to monitor women carrying these mutations before and throughout pregnancy. This strategy may reduce the impact of PE on women.

Therapeutic interventions that manipulate the NO signaling pathway could help ameliorate PE. A selective phosphodiesterase 5 (PDE5) inhibitor, sildenafil citrate (SC), prevents cGMP—a second messenger of eNOS/NO—degradation by PDE5, beneficially relaxing blood vessels in PE patients [60]. In the L-NAME induced rat model of PE, SC decreased SBP and proteinuria, and increased both fetal weight and survival rates [61]. Notably, SC decreased maternal BP and increased fetal weight from eNOS+/- dams [59]. After performing a systematic meta-analysis of 22 animal studies—i.e., mouse, rat, rabbit, sheep, and guinea pigs—Paauw et al. concluded that SC decreases maternal BP and improves fetal growth during preeclamptic pregnancy [62]. The first clinical trial conducted in 2011 demonstrated that SC improved fetal growth velocity in 10 women with severe, early-onset fetal growth restriction without adverse effects [63]. Following promising results from preclinical and small clinical trial studies, the international Sildenafil Therapy in Dismal Prognosis Early-Onset Fetal Growth Restriction (STRIDER) study consortium was formed to further investigate SC [64]. Unfortunately, on July 19, 2018, after evaluating the results of the first 183 patients, the Dutch arm of the trial had been advised to stop the trial due to safety concerns and a lack of evidence of positive effects [65]. Furthermore, Hitzerd et al. found an absence of PDE5 upregulation and SC-induced NO potentiation in arteries of PE placentas, which was combined with non-PDE5-mediated effects of SC [66].

Taken together, although SC has been approved and widely used for erectile dysfunction and pulmonary arterial hypertension, the future of SC for treating PE is not optimistic [64]. However, another member of PDE5 inhibitor family, tadalafil, shows promising beneficial effects in the preclinical studies [67]. A small phase II clinical trial conducted by Furuhashi et al. showed that 12 women with hypertensive disorder of pregnancy were treated with 20 mg oral tadalafil/day started at 20-33 weeks of gestation did not show adverse effects [68]. Future clinal trials are needed to establish its safety and efficacy.

Future Direction

Human and experimental studies demonstrate the important role of abnormal maternal eNOS/NO in PE. Insufficient placentas are the center of PE, and effective placentation requires both maternal and paternal contributions. The role of paternal eNOS in PE urgently needs to be studied as there are scarcely any studies on it. eNOS-/- mice are still a good platform for tackling the role of paternal eNOS in PE. For example, comparing the pregnancy outcome of WT females vs. eNOS+/- female mice mated with eNOS-/- male mice will provide valuable information.

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