Animal models of preeclampsia: investigating pathophysiology and therapeutic targets

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Introduction

Despite its position as the leading cause of maternal death and a major contributor to maternal and perinatal morbidity, there is no effective drug treatment to delay the progression of preeclampsia (PE), and current management therapies have significant limitations.[1] At present, the only effective treatment for PE is early delivery (removal of the placenta). Thus, the discovery of novel approaches for the treatment of PE is a major unmet need in the field. The identification of therapeutic targets for the treatment of PE can only result from the interplay between basic research involving animal models and clinical research in humans. The study of PE in humans is of critical importance to identify potential biomarkers and pathogenic factors that associate with the progression of PE.[2−4] However, the data obtained from human studies are often correlative and unable to establish cause-and-effect relationships. Moreover, clinical studies in humans have obvious limitations that

prevent detailed investigation of the quantitative importance of time-dependent mechanisms involved in this syndrome. In contrast, experimental studies in animal models, despite their limitations, allow investigators to perform proof-of-concept studies. In addition, studies in animal models allow investigators to examine whether certain factors found in women with PE can indeed lead to hypertension and other manifestations of this disease. In this brief review, we attempt to summarize some of the more widely studied and/or recently developed animal models used to investigate pathophysiological mechanisms that are thought to be involved in

PE. These mechanisms and resulting models include placental ischemia (reduced uterine perfusion pressure models), impaired angiogenesis (soluble fms-like tyrosine kinase-1 [sFlt-1] chronic excess model), models used to study maternal immune activation in PE, and genetic mouse models that examine specific pathogenic pathways.

Animal Models of Preeclampsia

Because the spontaneous development of PE is essentially limited to the human species, the study of PE in patients is of critical importance to identify biomarkers and potential pathogenic factors that correlate with the progression

Animal models have been critical in investigating the pathogenesis, mediators, and even therapeutic options for a number of diseases, including preeclampsia. Preeclampsia is the leading cause of maternal and fetal morbidity and mortality worldwide. The placenta is thought to play a central role in the pathogenesis of this disease because it releases antiangiogenic and proinflammatory factors into the maternal circulation, resulting in the maternal syndrome. Despite the deleterious effects preeclampsia has been shown to have on the mother and baby during pregnancy and postpartum, there is still no effective treatment for this disease. Although clinical studies in patients are crucial to identify the involvement of pathogenic factors in preeclampsia, there are obvious limitations that prevent detailed investigation of the quantitative importance of time-dependent mechanisms involved in this syndrome. Animal models allow investigators to perform proof-of-concept studies and examine whether certain factors found in women with preeclampsia mediate hypertension and other manifestations of this disease. In this brief review, we summarize some of the more widely studied models used to investigate pathophysiological mechanisms that are thought to be involved in preeclampsia. These include models of placental ischemia, angiogenic imbalance, and maternal immune activation. Infusion of preeclampsia-related factors into animals has been widely studied to understand the specific mediators of this disease. These models have been included, in addition to a number of genetic models involved in overexpression of the renin-angiotensin system, complement activation, and trophoblast differentiation. Together, these models cover multiple mechanisms of preeclampsia from trophoblast dysfunction and impaired placental vascularization to the excess circulating placental factors and clinical manifestation of this disease. Most animal studies have been performed in rats and mice; however, we have also incorporated nonhuman primate models in this review. Preclinical animal models not only have been instrumental in understanding the pathophysiology of preeclampsia but also continue to be important tools in the search for novel therapeutic options for the treatment of this disease.

Key words: animal models, hypertension, preeclampsia

of the syndrome. However, experimental studies in pregnant women have obvious limitations that prevent a complete investigation of many of the mechanisms involved. In contrast, studies in animal models, despite their limitations, allow investigators to isolate and study the many pathways involved in PE. Preclinical studies in rodent animal models have been critical in understanding the pathogenesis of this disease. In investigating therapeutic options, targets identified in small animal models must be tested in nonhuman primates for efficacy and safety before transitioning to human trials. Limited studies have been performed in nonhuman primates to study PE, most likely owing to expense and other limitations in resources. Nevertheless, these studies have contributed to a better understanding of this disease and have been included in this review.

Models used to study the relationship between placental ischemia and maternal syndrome

Experimental induction of chronic uteroplacental ischemia (UPI) seems to be a promising animal model to study potential mechanisms of PE. Uterine artery resistance is markedly increased in PE as a result of impaired spiral artery remodeling and placental ischemia.[5] Indeed, the most accurate predictive measurement of early-onset PE is the measurement of angiogenic balance and uterine artery Doppler assessment.[6] Reductions in uteroplacental blood flow in a variety of species lead to a maternal cardiovascular phenotype that closely resembles PE in women.[7–13] The chronic reduced uteroplacental perfusion pressure (RUPP) rat model was developed and characterized by our laboratory to examine potential pathophysiological mechanisms that mediate cardiovascular and endothelial dysfunction in response to placental ischemia.[7]

The RUPP surgical procedure is typically performed in timed-pregnant Sprague-Dawley rats (gestation day 14),[12] but the procedure has also been successfully performed in other rat strains, including Wistar rats.[14] Uterine perfusion pressure in the gravid rat is reduced by slipping a silver constriction clip around the aorta below the renal arteries, right above the iliac bifurcation where the uterine arteries lie.[12,15] We have found this procedure to reduce uterine perfusion pressure by approximately 40%.[16] Because compensation of blood flow to the placenta occurs in pregnant rats through an adaptive increase in ovarian blood flow, branches of both the right and left ovarian arteries that supply the uterus are also clipped.[12] The timing of the vessel constriction is an important consideration. During a series of pilot studies to determine the appropriate clip size and the ideal gestational time for reducing uterine perfusion pressure, it was found that placement of the clips before day 14 of gestation in the rat resulted in a significant increase in fetal death. However, placement of clips at gestational day 14 to reduce placental perfusion produces a consistent blood pressure effect and minimizes total fetal reabsorption.[12,17]

In response to RUPP, both uterine and placental blood flow are decreased by approximately 40%.[16] Arterial pressure increases by approximately 20 to 25 mm Hg by day 19 of pregnancy. In contrast, RUPP in virgin rats results in no significant effect on arterial pressure relative to control virgin rats.[12] The hypertension in RUPP rats is associated with increases in total peripheral resistance and decreased cardiac output, which are systemic hemodynamic characteristics consistent with PE in women.[16] Glomerular filtration rate and to a lesser extent renal blood flow decrease in the RUPP model relative to normal pregnancy at day 19.[12] Although quite variable, an increase in urinary protein excretion has also been observed in the RUPP model compared with normal pregnancy.[12] The reason for the variability is unknown but may be caused by the short time frame of exposure to placental ischemia.

Angiogenic imbalance, endothelial dysfunction, reduced production of nitric oxide (NO) in vascular tissue, and increases in vascular endothelin (ET)-1 and reactive oxygen species (ROS) production are all characteristics of PE also present in the RUPP rat.[1,18–23] Placental ischemia in rats also increases placental expression of hypoxia-inducible factor-1α and immunoreactive sFlt-1 in the placenta and plasma.[22] Soluble endoglin (sEng) levels are also elevated in the RUPP model.[23] Moreover, RUPP is associated with decreases in plasma concentrations of vascular endothelial growth factor (VEGF) and placental-derived growth factor (PlGF).[22]

Fetal growth restriction (FGR), another feature of human PE, is also evident as pup weights are decreased in RUPP rats relative to controls.[18] Alexander et al[24] have performed a series of studies using the FGR rat offspring from the RUPP model to examine the mechanisms underlying the relationship between birthweight and cardiovascular diseases later in life. Thus, offspring from RUPP rats are also an important model to examine mechanisms of fetal programming of cardiovascular disease.

The RUPP model has been proven to be a useful tool to examine the mechanisms whereby ischemia initiates a cascade of events resulting in placental inflammation and subsequent systemic inflammation and hypertension in the mother.[25–28] RUPP rats have increased placental expression and circulating levels of prohypertensive cytokines (tumor necrosis factor [TNF]-α, interleukin [IL]-6, IL-17, etc) and immune cell counts (T and B lymphocytes) and increases in the production of the agonistic autoantibody to the angiotensin II type 1 receptor autoantibody (AT1-AA) and imbalance in T helper (Th) and regulatory T cells (Tregs).[25–28] Activation of the complement system also occurs in RUPP rats, as detected in the circulation by decreased complement component 3 and increased complement activation product C3a.[29]

Cerebrovascular disturbances are now part of the diagnostic criteria for PE when accompanied by new-onset hypertension after 20 weeks' gestation.[1] Blood-brain barrier (BBB) disruption, cerebral edema, and impaired cerebral blood flow (CBF) regulation are common cerebrovascular findings in women with PE.[30–33] However, the mechanisms contributing to these cerebrovascular abnormalities during pregnancy are not completely understood. In the past few

years, the RUPP model of placental ischemia has been used to determine whether placental ischemia causes similar characteristics as the clinical syndrome and to begin to dissect the mechanisms that contribute to RUPP-induced cerebrovascular abnormalities. Placental ischemia in the RUPP model leads to increased BBB permeability and cerebral edema.[34,35] In these studies, impaired CBF and vascular myogenic reactivity were particularly evident at higher perfusion pressures.[34,36] The RUPP model also has a shorter latency to the onset of drug-induced seizures and is associated with increased concentrations of proinflammatory cytokines in the cerebrospinal fluid.[37] These findings were consistent with a study using the RUPP rat coupled with high cholesterol in which the threshold for seizures was reduced compared with normal pregnant rats.[37] Importantly, magnesium sulfate has the capability of reducing placental ischemia—induced increases in proinflammatory cytokines in the cerebrospinal fluid.[38] Although these studies demonstrated that the RUPP model can be used to study mechanisms of cerebrovascular abnormalities, it is still not known what factors are responsible for BBB disruption, cerebral edema, and impaired CBF after placental ischemia.

PE is associated with peripartum and postpartum myocardial fibrosis and heart failure.[39–41] Despite the high incidence of serious morbidity and mortality owing to postpartum heart failure after a preeclamptic pregnancy, there is a substantial gap in our knowledge regarding the cardiac dysfunction during PE and how to prevent or reverse it. Recent studies from our laboratory indicate that the RUPP model has many features of cardiac dysfunction seen in women with PE. Importantly, the RUPP model of PE also demonstrates reduced myocardial function (ejection fraction [EF] and global longitudinal strain) seen in women with PE. Furthermore, biomarkers consistent with cardiac fibrosis including cardiac troponin, increased collagen messenger RNA (mRNA) expression, and markers of pathologic hypertrophy including increased brain

natriuretic peptide, myosin heavy chain α/β, and atrial natriuretic peptide levels are present in RUPP rats.[42–44] In addition, we have data indicating that reduced EF and fractional shortening persist up to 8 weeks after delivery in the RUPP model despite blood pressure returning to normal by this time.[43] Thus, the RUPP model may also be a useful tool to provide a better understanding of the mechanisms underlying cardiac dysfunction that occurs during PE and after delivery.

Thus, RUPP-induced hypertension in pregnant rats is associated with endothelial dysfunction, angiogenic imbalance, immune activation, and cardiac and cerebrovascular dysfunction, all of which are seen in women

with PE (Table 1). A major strength of the RUPP model is that investigators have been able to assess the functional and quantitative role for each of the systems in mediating the hypertension in the RUPP model by using pharmacologic agents to disrupt their actions. The RUPP model has also been used by numerous laboratories as a valuable tool to investigate potential therapeutic targets for the treatment of preeclampsia. Importantly, the RUPP model involves mechanical constriction of vessels that feed the uteroplacental unit as a means to reduce blood flow to the placenta. As a result, the RUPP rat displays a similar phenotype to PE, where placental ischemia is also thought to play a central role. However, a

TABLE 1
Features of reduced uterine perfusion pressure model compared with PE

Features	PE	RUPP rat
Mean arterial pressure	↑	↑
Total peripheral resistance	↑	↑
Circulating sFlt-1	↑	↑
Circulating sEng	↑	↑
Circulating free PlGF	↓	↓
Circulating free VEGF	↓	↓
Oxidative stress	↑	↑
AT1-AA	↑	↑
Prohypertensive cytokines	↑	↑
T and B lymphocytes	↑	↑
ET-1	↑	↑
NO	↓	↓
Renal plasma flow	↓	↓
GFR	↓	↓
GLS	↓	↓
Ejection fraction	↓	↓
Cardiac output	↓	↓
Cerebral blood flow regulation	↓	↓
Cerebral edema	↑	↑
BBB permeability	↑	↑
FGR	↑	↑

AT1-AA, angiotensin II type I receptor autoantibody; *BBB*, blood-brain barrier; *ET-1*, endothelin-1; *GFR*, glomerular filtration rate; *GLS*, global longitudinal strain; *FGR*, fetal growth restriction; *NO*, nitric oxide; *PE*, preeclampsia; *PlGF*, placental-derived growth factor; *ROS*, reactive oxygen species; *RUPP*, reduced uterine perfusion pressure; *sEng*, soluble endoglin; *TNF-α*, tumor necrosis factor-α; *UPI*, uteroplacental ischemia; *VEGF*, vascular endothelial growth factor; ↑, increased; ↓, decreased.

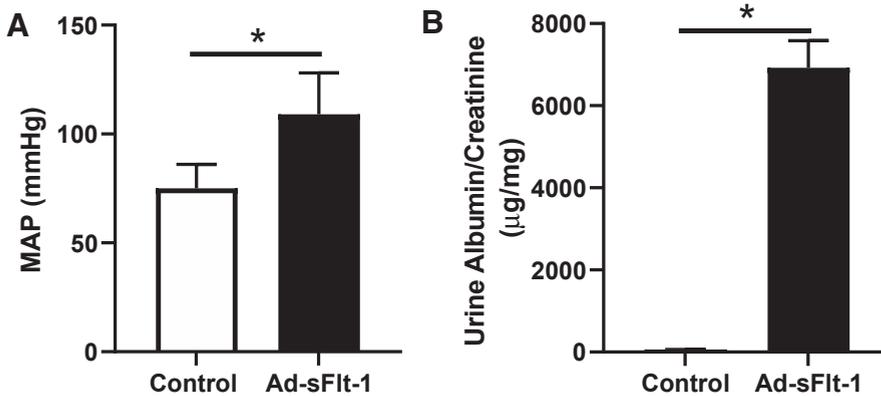*Bakrania. Animal models of preeclampsia. Am J Obstet Gynecol 2022.*

A, MAP and B, proteinuria are shown for rats after the administration of an Ad-sFlt-1 during pregnancy.[50] Sprague-Dawley rats were injected at gestational day 8 or 9 of pregnancy, and data were collected at gestational day 16 or 17. Data are presented as mean+SEM. The asterisk symbol indicates $P<.05$.

Ad-sFlt-1, adenovirus expressing soluble fms-like tyrosine kinase-1; *MAP*, mean arterial pressure; *SEM*, standard error of the mean; *sFlt-1*, soluble fms-like tyrosine kinase-1.

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limitation of this model is that it is not useful for studying the mechanisms involved in the abnormal spiral artery remodeling proposed to underlie placental ischemia in women with PE.

Although numerous laboratories have used the rat RUPP model, the placental ischemic model of PE has also been applied in mice and in nonhuman primate models. The RUPP technique has been applied in mice, which is especially beneficial in using genetic engineered mice to enhance our understanding of the pathogenesis of placental ischemia—induced hypertension.[11,45] Makris et al[46] also characterized a UPI model in radiotelemetered pregnant baboons by selective ligation of 1 uterine artery resulting in a 40% decrease in uteroplacental blood flow. Hypertension, proteinuria, and increased production of antiangiogenic markers were reported in the pregnant baboons compared with control animals that underwent a sham procedure.[46] Makris et al[46] also recently reported that RNA interference modulation of placental sFlt-1 significantly attenuated the blood pressure response to placental ischemia.

Models used to study the role of angiogenic imbalance

Angiogenic imbalance is thought to play a central role in the development of PE. Proangiogenic factors, VEGF and PlGF, are produced during pregnancy and are critical in maintaining endothelial integrity. These angiogenic factors bind to VEGF receptors (VEGFR), including VEGFR-1 or Flt-1.[47,48] sFlt-1 is an alternately spliced variant of the full-length receptor in which the transmembrane and cytosolic domains have been removed, leaving only the extracellular recognition domain.[49] This recognition domain acts as a VEGF antagonist by binding free VEGF and making it unavailable for proper signaling. Increased circulating levels of sFlt-1 and reduced PlGF levels have been documented in women during PE and before the onset of clinical symptoms.[50] Excess sFlt-1 and sEng, which are also significantly increased in PE,[51] cause depletion in the bioavailability of VEGF and PlGF at the detriment of the endothelium and blood pressure regulation.

Although there is a strong association between angiogenic imbalance and severity of PE, the relative importance of angiogenic factors in PE remains an open area of investigation. A number of experimental models have been used to uncover the mechanisms that link angiogenic imbalance and PE. Overexpression and infusion of sFlt-1 have both been shown to induce a PE-like phenotype.[50,52,53] Maynard et al[50] showed that infusion of an adenovirus (Ad) expressing sFlt-1 in Sprague-Dawley rats at day 8 or 9 of pregnancy causes the development of hypertension, proteinuria, and glomerular endotheliosis by day 16 or 17 of pregnancy (Figure 1). Similarly, infusion of sFlt-1 into pregnant rats induces PE-like symptoms, including hypertension, reduced fetal weight, and increases in vascular ROS.[52] Interestingly, excess sFlt-1 itself is not thought to cause hypertension in PE, but rather the deficiency of free proangiogenic factors such as VEGF and PlGF. In 1 study, mice infused with Ad-sFlt-1 developed hypertension, proteinuria, endotheliosis, and vascular damage. However, when Ad-VEGF was administered in conjunction, these features were alleviated.[54] These data indicate that free excess sFlt-1 and depletions of free VEGF and PlGF play a major role in PE. Importantly, studies have also investigated full-length human sFlt-1-e15a isoform, which is most abundant in placentas of women with PE. Studies by Szalai et al[55,54] show that infusion of full-length human sFlt-1 in mice also results in hypertension, proteinuria, glomerular injury, and vascular damage.

As reported in the RUPP and UPI models, placental ischemia induces significant increases in circulating sFlt-1, and the administration of PlGF in these models has been shown to improve the PE-like phenotype. In the RUPP rat, the administration of 180 μg/kg/d recombinant human (rh) PlGF for 5 days reduced blood pressure and fetal reabsorption in the RUPP rat, in addition to significant increases in placental and fetal weight.[56] Subsequently, another study showed that the administration of 100 μg/kg/d rhPlGF in the UPI nonhuman primate model results in significant improvements in hypertension and proteinuria.[10] Most recently,

Logue et al[57] found that the administration of an elastin-like polypeptide linked VEGF construct in the RUPP rat significantly reduces blood pressure. These and other data have led to the hypothesis that the ratio between sFlt-1 and PlGF/VEGF is critical to maintain a healthy endothelium and normal vascular activity. Furthermore, these studies suggest that improving the angiogenic balance in PE could be a therapeutic avenue for treatment. Current therapeutic efforts have also focused on reducing circulating sFlt-1 via apheresis and in doing so improving the angiogenic balance, with promising results.[58] These clinical data highlight the importance of angiogenic factors in the development of PE.[58,59]

Animal models of angiogenic imbalance have also been useful to study the closely associated pregnancy disorder, that is, hemolysis, elevated liver enzymes, and low platelet count (HELLP) syndrome. Although HELLP syndrome only occurs in 0.2% to 0.8% of pregnancies, it is diagnosed alongside PE in 70% to 80% of cases.[60] Therefore, it is not surprising that factors induced in animal models of PE also produce a HELLP-like phenotype. Infusion of sFlt-1 and sEng at gestational day 12 results in increased mean arterial pressure, elevated liver enzymes, and reduced platelets and proinflammatory factors such as TNF-α, IL-17, IL-6, CD4$^+$ T cells, and CD8$^+$ T cells.[61,62] Interestingly, this model is one of the few that have been studied after delivery to show BBB permeability, persistent hypertension, and indicators of anxiety.[63] The profound effect on immune factors in this model and others of angiogenic imbalance highlight the interactions between these 2 pathways.

Models used to examine role of agnostic angiotensin II type 1 receptor autoantibodies

The renin-angiotensin system (RAS) plays an important role in normal pregnancy and in PE. Normal pregnancy is associated with the activation of RAS components with diminished vascular responsiveness to angiotensin II. In contrast, women with PE have reduced angiotensinogen, plasma renin (REN) activity, and angiotensin II but typically exhibit increased vascular responsiveness to angiotensin II.[64] Herse et al previously reported that the sera from women with PE contain an immunoglobulin G (type 3) autoantibody that reacts with the AT1 receptor. A number of studies have now indicated that women with PE produce this novel agonistic autoantibody to the AT1-AA during pregnancy and up to 8 years after delivery.[65,66] One animal model used to examine the role of AT1AA is the AT1-AA chronic excess model where purified AT1-AA is infused into normal pregnant rats. A number of investigators have shown that AT1-AA signaling, via the AT1 receptor, results in a variety of physiological effects, including TNF-α and ROS generation, both of which have been implicated in PE.[67]

Zhou et al[68] demonstrated that the immunoglobulin isolated from women with PE increases systolic pressure in pregnant mice. Similar studies from LaMarca et al[67] have reported that infusion of purified rat AT1-AA, isolated from the serum collected from a pregnant transgenic rat overproducing components of the RAS, into pregnant rats from day 12 to day 19 of gestation, increased serum AT1-AA and blood pressure. Although these data suggest AT1-AA causes hypertension by direct activation of AT1 receptor, additional pathways involving ET and anti-angiogenic factors may also be involved during pregnancy. More recently, Cunningham et al[69] showed that renal natural killer cells are activated in renal tissue from AT1-AA—infused rats and that mitochondrial dysfunction is also present in this model. The blood pressure response in AT1-AA—infused animals has been blocked by AT1 receptor antagonists or coinjection of an AT1 receptor antagonist or the 7 amino acid peptide (n7AAc) that selectively blocks the actions of the AT1-AA.[70] The administration of n7AAc in the RUPP rat has also been shown to reduce blood pressure (Figure 2).[71] Another approach to block the effects of endogenous AT1-AA in the RUPP rat is B-cell depletion, which results in a

FIGURE 2

MAP of normal pregnant rats, the RUPP rat model, and inhibition of the AT1-AA in the RUPP rat (with "n7AAc")
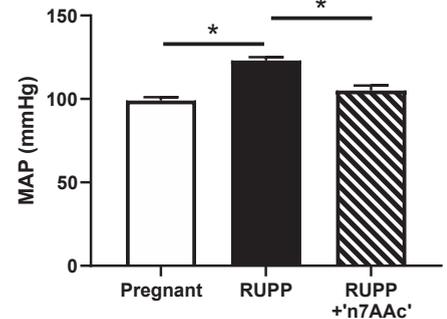
These data suggest that AT1-AA plays a role in placental ischemia—induced hypertension. Data are presented as mean+SEM and were retrieved from Cunningham et al.[71] The asterisk symbol indicates $P<.05$.

AT1-AA, angiotensin II type 1 receptor autoantibody; *MAP*, mean arterial pressure; *RUPP*, reduced uterine perfusion pressure; *SEM*, standard error of the mean.

Bakrania. Animal models of preeclampsia. Am J Obstet Gynecol 2022.

blunted blood pressure response to placental ischemia.[72]

AT1-AA infusion in rats is also associated with angiogenic imbalance and elevated tissue levels of prepro-ET-1.[67] Interestingly, blood pressure response in AT1-AA-infused animals can be blocked by ET type A (ET$_A$) receptor antagonist.[71] These data suggest that AT1-AA—induced hypertension during pregnancy is in part caused by the activation of the ET system.[71] sFlt-1 and sEng are significantly elevated in this model.[71] sFlt-1 levels measured in media from placental explants from AT1-AA—infused rats are also significantly increased.[73] Interestingly, sEng was not increased in media from placental explant, suggesting another source for its production.[73] These studies demonstrate an important interaction between inflammatory and angiogenic markers found to be produced excessively in response to placental ischemia.

Immune activation and cytokines

One of the earliest and most persistent theories about the origins of PE is that PE is a disorder of immunity and

inflammation. This maternal immune tolerance involves crucial interactions between Tregs and uterine natural killer cells that recognize and accept the fetal antigens and facilitate placental growth. Complete failure of this crucial step leads to spontaneous miscarriage, whereas partial failure leads to poor placentation and dysfunctional placental perfusion and chronic immune activation. Increase in proinflammatory cytokines and decreases in uterine natural killer cells and Tregs are observed in women with PE.[27,74] In a mouse model, acute Treg depletion from gestational day 3.5 results in fetal loss, increases in proinflammatory markers, and uterine artery resistance. Interestingly, Treg depletion alone does not increase blood pressure. However, in the presence of L-NG-nitroarginine methyl ester (L-NAME), which blocks NO production for proper endothelial function, blood pressure increases by approximately 25% in Treg-depleted mice, but only 15% in normal pregnant mice.[75] Treg populations have also been shown to be important in late gestation. Partial or total Treg depletion from gestational day 14.5 in a murine model of susceptibility to preterm birth resulted in greater fetal loss and reduced survival of pups up to 3 weeks of age.[76] The RUPP rat is another model that is induced late in pregnancy that also exhibits a reduction in Tregs[77] and increases in Th-17 cells. Cornelius et al[77,78] showed that Th-17 cells isolated from the spleen of RUPP rats, cultured, and injected interperitoneally into normal pregnant rats induced a PE-like phenotype. Meanwhile, the administration of Tregs to the RUPP rat reduces hypertension and proinflammatory factors.[79,80] These data suggest that the increased proinflammatory immune cells populations play a deleterious role in PE. These factors all act to cause pathologic activation of the maternal endothelium and directly affect multiple organ systems.

Proinflammatory cytokines are produced in abundance in PE coupled with deficiencies of antiinflammatory factors, such as IL-10. IL-10 plays an important role in normal pregnancy in regulating the polarization of T cells to a Th-2 phenotype over the proinflammatory Th-1 phenotype.[81] Women with PE have a 2-fold elevation in placental and plasma TNF-α protein levels. It is becoming increasingly evident that proinflammatory cytokines such as IL-6 and TNF-α interact with important blood pressure regulatory systems such as the RAS system, sympathetic nervous system, and endothelial factors.[27] TNF-α infusion (50 ng/d) in normal pregnant rats from gestational day 14 to 19 results in elevated blood pressure and increased expression of renal, placental, and aortic prepro-ET-1.[82] Moreover, the increase in mean arterial pressure in response to TNF-α is completely abolished in pregnant rats treated with an ET_A receptor antagonist.[83] Sera from pregnant rats exposed to chronic RUPP increase ET-1 production by cultured endothelial cells, and these levels of ET-1 production are mimicked when cells are exposed to TNF-α.[84] Further studies in this model suggest that TNF-α—induced hypertension could also be caused by decreases in renal NO synthase expression[82] and increases in AT1-AA production.[85] Most recently, TNF-α infusion in pregnant rats has been shown to exhibit impaired cerebrovascular regulation owing to reduced β-epithelial Na+ channel expression.[86]

Systemic activation of the immune cells during pregnancy has been studied as a model of PE. Faas et al[87] showed that injection of low-dose endotoxin to induce an proinflammatory state at gestational day 14 results in hypertension and proteinuria. These animals were also found to have platelet coagulopathy and glomerular injury. An alternative approach to induce systemic inflammation is through the administration of lipopolysaccharide (LPS). A low dose of LPS is adequate to induce FGR, placental nitrosative stress, reduced spiral artery area, hypertension, and proteinuria.[88] Although these models are not induced through specific PE-related factors, they have been useful in understanding how widespread activation of leukocytes in this disease contributes to its pathogenesis.

A novel model of PE was recently published where mice were treated with placenta-derived extracellular vesicles (pcEVs) at gestational days 17—18 from women with PE, which resulted in hypertension, proteinuria, and kidney injury. This was associated with increased vascular wall tension and reduced CBF. Interestingly, in virgin mice, hypertension developed within 30 minutes of pcEV infusion.[89] pcEV shedding from the placenta is considered normal throughout pregnancy. However, excess levels are seen owing to the dysfunction placenta in PE.[90]

Models used to study role of endothelin and nitric oxide in preeclampsia

Endothelial dysfunction is another known stimulus for ET-1 synthesis. Plasma concentration of ET-1 has been measured in a number of studies involving normal pregnant and women with PE. Most investigators have found higher ET-1 plasma concentrations of approximately 2- to 3-fold in women with PE.[91,92] Previous studies have reported that 2- to 3-fold elevation in plasma levels of ET-1 in animals is adequate to impart significant long-term effects on systemic hemodynamics and arterial pressure regulation. Thus, long-term elevations in plasma levels of ET-1 comparable with those measured in women with PE could play a role in mediating the reductions in renal function and elevations in arterial pressure observed in women with PE.

There are robust increases in ET-1 coupled with marked deficiencies in vasodilatory mediators, including NO, in PE.[93,94] Substantial production of ROS during PE leads to the oxidation of NO and soluble guanylate cyclase (sGC), a critical molecule involved in NO-mediated vasodilation. NO production is reduced in women with PE and in the RUPP and sFlt-1 excess models.[20] An important model used to examine the role of NO deficiency in PE is the L-NAME model. In normal pregnant rats, L-NAME administration to block NO production from gestational day 1 to 19 leads to hypertension, proteinuria, elevated sFlt-1, and reduced fetal and placental weight.[95]

Genetic models used to study preeclampsia mechanisms

BPH/5. Perhaps the best characterized genetically linked model for the study of PE is the BPH/5 mouse. The BPH/5 is a strain derivation of the BPH/2 "borderline hypertensive" mouse and exhibits mildly elevated blood pressure throughout the adult life span of the animal. However, Davisson et al[96] demonstrated that the BPH/5 strain also demonstrates late gestational acute elevations in blood pressure (up to approximately 25 mm Hg), which resolve immediately after parturition, mimicking the effects on blood pressure seen in the patient with typical PE. This was accompanied by proteinuria, glomerulosclerosis, intrauterine growth restriction, maternal endothelial dysfunction, and increased fetal mortality.[96] Work from the Davisson and Sones laboratories in subsequent years has uncovered a number of additional characteristics similar to human PE, such as altered extravillous trophoblast invasion and placental abnormalities that coincide with increased uterine artery vascular resistance. Importantly, these effects are seen before the onset of hypertension, as is postulated in the human disorder.[97] However, it should be noted that although total circulating angiogenic potential is decreased in the BPH/5, this seems to be associated with decreased VEGF and PlGF rather than increased sFlt-1, an interesting difference from the human syndrome. Recent studies from Sones et al[98] show that angiogenic imbalance precedes complement activation, which has been implicated in the pathogenesis of PE.

Angiotensinogen/renin overexpression. Although its role in long-term maintenance of blood pressure is well established, the exact pathophysiological role of the RAS in the development of PE is less clear. To investigate the role of the RAS in pregnancy-induced hypertension, several groups have used transgenic mouse and rodent strains in which females overexpressing human angiotensinogen (AGN) are crossed with males overexpressing human renin (REN).[99−101] This model has been

shown to exhibit late gestational hypertension and proteinuria. Recent studies have also showed the development of postpartum cardiac dysfunction in this model.[102] Importantly, when the gender of the transgenic animals is swapped (ie, male AGN and female REN), no phenotypic effect is noted, likely owing to species specificity of REN activity and lack of secretion of AGN from the placenta itself. A similar model has been reported in mice overexpressing both REN and AGN, which are chronically hypertensive, as a model of superimposed PE, which also develops very late gestational hypertension and FGR.[103]

STOX1 overexpression. Another new potential model that has recently been reported is transgenic overexpression of the transcription factor storkhead box 1 (STOX1) in a murine model. A significant, although often contradictory, body evidence has previously implicated STOX1 dysregulation in the etiology of PE.[104,105] Interestingly, Doridot et al[106,107] showed that STOX1 overexpression leads to increases in systolic blood pressure from very early gestation, proteinuria, renal capillary swelling, fibrin deposition, and elevated levels of sFlt-1 and sEng. The early onset of hypertension suggests abnormal placentation may not be the cause of elevated blood pressure in this model. Nevertheless, the STOX1 overexpression model has been used to study many organ systems related to PE. Recently, studies have shown that STOX1 overexpression in mice results in increased renal artery resistance, cardiac hypertrophy, FGR, and a trend toward increased umbilical resistance.[108,109] In a 2019 study, Miralles et al[110] showed that STOX1 overexpression in mice with a PE-like phenotype during pregnancy exhibited left ventricular hypertrophy, cardiac fibrosis, and markers of inflammation and cellular stress up to 8 months after delivery.

Ankyrin repeat and SOCS box containing 4 deletion. Another model that may be useful in studying vascularization of the placenta in the early stages of PE is the

ubiquitin ligase ankyrin repeat and SOCS box containing 4 (ASB4), which promotes the differentiation of vascular lineages in trophoblasts. Townley-Tilson et al[111] showed that in placentas of ASB4$^{-/-}$ mice, trophoblast-to-endothelial cell differentiation is impaired resulting in fewer mature endothelial cells and reduced placental vascularization. Consequently, these mice produced smaller litter sizes and developed hypertension and proteinuria later in gestation.[111,112]

ELABELA deficiency. ELABELA, an endogenous ligand of the apelin receptor, has recently garnered much attention as a biomarker and therapeutic target of PE. Serum levels of ELABELA have been examined during early- and late-onset PE in a number of studies with varying results.[113,114] Although some of these studies indicate that ELABELA may be increased in some cohorts, it is generally accepted that ELABELA plays a role in proper extravillous trophoblast migration and is deficient at least in the placentas of women with PE.[115,116] Animal studies found that ELABELA knockout in mice results in hypertension, proteinuria, impaired placental vascularization, and FGR.[117] Interestingly, the investigators did not see the same results in apelin knockout mice. Furthermore, exogenous ELABELA attenuated these outcomes.[117]

Complement component 1q deficiency. Complement activation has been shown to play a role in not only the clinical manifestation of PE but also spiral artery remodeling. Women with both early and late PE have decreased serum levels of complement component 1q (C1q) compared with those with uncomplicated pregnancies.[118] In a mouse model of C1q deficiency (C1q$^{-/-}$), animals develop hypertension, proteinuria, endotheliosis, decreased circulating VEGF, and elevated sFlt-1.[119] These characteristics are accompanied by increased fetal death.[119]

Dahl salt-sensitive rat. Clinically, PE can be classed as "superimposed" when a patient is hypertensive before pregnancy.

TABLE 2
Summary of pathways involved in PE that are studied using animal models

Pathway	Animal model	References
Placental ischemia	Reduced uterine perfusion pressure	Intapad et al[11]; Alexander et al[12]
	Uteroplacental ischemia	Makris et al[46]
Angiogenic imbalance	Adenovirus expressing sFlt-1 excess	Maynard et al[50]; Bergmann et al[146]
	sFlt-1 infusion	Murphy et al[52]; Szalai et al[54]
Immune activation	Regulatory T-cell depletion	Care et al[75]
	TNF-α infusion	Alexander et al[82]
	Low-dose endotoxin infusion	Faas et al[87]
	Low-dose lipopolysaccharide infusion	Cotechini et al[88]
	Placenta-derived extracellular vesicle infusion	Han et al[89]
Renin-angiotensin system activation	Agnostic angiotensin II type 1 receptor autoantibody infusion	LaMarca et al[67]; Zhou et al[68]
	Angiotensinogen/renin overexpression	Takimoto et al[101]; Denney et al[103]
Endothelial dysfunction	L-NAME infusion	Ramesar et al[95]
Abnormal placentation	STOX1 overexpression	Doridot et al[106]; Doridot et al[107]
	ASB4 deletion	Townley-Tilson et al[111]
	ELABELA deficiency	Ho et al[117]
Complement system activation	C1q$^{-/-}$ deficiency	Singh et al[119]
Superimposed or spontaneous models of PE	BPH/5 model	Davisson et al[96]
	Dahl salt-sensitive rat	Gillis et al[120]
	African green monkey	Weaver et al[124]

ASB4, ankyrin repeat and SOCS box containing 4; *C1q*, component 1q; *L-NAME*, L-NG-nitroarginine methyl ester; *PE*, preeclampsia; *sFlt-1*, soluble fms-like tyrosine kinase-1; *STOX1*, storkhead box 1; *TNF-α*, tumor necrosis factor-α.

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Similarly, the Dahl salt-sensitive (S) rat is hypertensive and develops a PE-like phenotype during pregnancy, including further elevations in blood pressure, proteinuria, glomerulomegaly, increased uterine artery resistance, FGR, and elevated circulating levels of TNF-α and sFlt-1.[120] Further studies show postpartum renal injury in these animals despite no long-term blood pressure differences.[121] Interestingly, not all strains of hypertensive rats develop a PE-like phenotype during pregnancy. In contrast to the Dahl S rat, blood pressure in the spontaneously hypertensive rat decreases toward late pregnancy. Using this model, Gillis et al[122] also demonstrated that sildenafil treatment ameliorates the maternal syndrome of PE and rescues fetal growth in the Dahl S rat.

African green monkey. The African green monkey (*Chlorocebus aethiops sabaeus*)

has been shown to develop hypertension during adulthood.[123] More recently, Weaver et al[124] showed that in nonhypertensive adults, some animals develop hypertension and FGR during pregnancy similar to PE.

Many pathways in this disease have been targeted to produce animal models of PE (Table 2). A summary of the characteristics of each of the models discussed in this review is presented in Table 3.

Use of animal models to study long-term consequences of preeclampsia
Because more recent studies have revealed the profound impact of PE later in life for both the mother and baby, long-term consequences have become an important consideration in developing animal models. Although studies in this area are limited, some models have been shown to have persistent features beyond

pregnancy. In some cases, such as in the RUPP, postpartum cardiac and renal dysfunctions are present despite return to normal blood pressure in these animals.[43] These data suggest that placental ischemia and the factors involved cause irreversible damage and therefore do substantiate further studies in this area. Long-term studies in which animals undergo multiple pregnancies may also be interesting because cardiovascular risk in women later in life increases with each pregnancy complicated by PE.

Investigating Therapeutic Options for Preeclampsia Using Animal Models
Currently, treatment for PE is limited to managing symptoms and in severe cases premature delivery of the fetus, which poses a significant risk to both the mother and baby. Because initial studies in women with PE are obviously impossible, animal models represent a

TABLE 3
Summary of features in animal models of PE during pregnancy

Model	Animal	Features	References
RUPP	Rat	↑MAP, ↑proteinuria, ↑sFlt-1, ↑sEng, ↓PlGF, ↓VEGF, ↑ROS, ↑AT1-AA, ↑TNF-α, ↑Tregs, ↑NK cells, ↑ET-1, ↓NO, ↓RPF, ↓GFR, ↑cardiac dysfunction, ↑cardiac hypertrophy, ↓CBF, ↑cerebral edema, ↑ BBB permeability, ↓placental weight, ↑FGR	Rana et al[1]; Alexander et al;[12]; Alexander et al[17]; LaMarca et al[18]; Gilbert et al[19]; Khalil et al[20]; Sedeek et al[21]; Gilbert et al[22]; Gilbert et al[23]; LaMarca et al[25]; LaMarca et al[26]; Cornelius et al[27]; LaMarca et al[28]; Lillegard et al[29]; Warrington et al[34]; Warrington et al[35]; Bakrania et al[42]; Gutkowska et al[44]
Uteroplacental ischemia	Nonhuman primate	↑MAP, ↑proteinuria, ↑sFlt-1,	Makris et al[46]; Turanov et al[147]
Ad-sFlt-1 infusion	Rat	↑MAP, ↑proteinuria, ↑glomerular endotheliosis	Maynard et al[50]
sFlt-1 infusion	Rat	↑MAP, ↑proteinuria, ↑prepro-ET-1, ↑ROS, ↓placental weight, ↑FGR	Murphy et al[52]
Regulatory T-cell depletion	Mouse	↔MAP, ↑proinflammatory markers, ↑UARI, ↑fetal loss	Care et al[75]
AT-AA infusion	Rat	↑MAP, ↑NK cells, ↑sFlt-1, ↑sEng, ↑prepro-ET-1, ↑AT1-AA	LaMarca et al[67], Cunningham et al[69]; LaMarca et al[71]
TNF-α infusion	Rat	↑MAP, ↑prepro-ET-1, ↓NOS, ↑AT1-AA, ↓CBF	Alexander et al[82]; LaMarca et al[85]; Duncan et al[86]
L-NAME infusion	Rat	↑MAP, ↑proteinuria, ↑sFlt-1, ↓placental weight, ↑FGR	Ramesar et al[95]
pcEV infusion	Mouse	↑MAP, ↑proteinuria, ↑kidney injury, ↓CBF	Han et al[89]
BPH/5	Mouse	↑MAP, ↑proteinuria, ↑endothelial dysfunction, ↑glomerulosclerosis, ↑fetal mortality, ↑UARI, ↔sFlt-1, ↓VEGF, ↓PlGF, ↓impaired cytotrophoblast invasion, ↓ placental weight, ↑FGR	Davisson et al[96]; Dokras et al[97]; Sones et al[98]
hAGTxhRen overexpression	Rat	↑MAP, ↑proteinuria, ↑cardiac hypertrophy	Bohlender et al[99]; Hering et al[100]
STOX1 overexpression	Mouse	↑systolic pressure, ↑proteinuria, ↑renal capillary swelling, ↑sFlt-1, ↑sEng, ↑cardiac hypertrophy, ↑FGR, ↑renal artery resistance	Doridot et al[106]; Doridot et al[107]; Collinot et al[108]; Ducat et al[109]
Complement C1q deficiency	Mouse	↑MAP, ↑proteinuria, ↑endotheliosis, ↑sFlt-1, ↓VEGF, ↑fetal death	Singh et al[119]
ASB4 deletion	Mouse	↓placental vascularization, ↑MAP, ↑proteinuria, ↓litter size	Townley-Tilson et al[111]; Li et al[112]
ELABELA deficiency	Mouse	↓placental vascularization, ↑MAP, ↑proteinuria, ↑FGR	Ho et al[117]
Dahl salt-sensitive rat	Rat	↑MAP, ↑proteinuria, ↑sFlt-1, glomerulomegaly, ↑UARI, ↑FGR	Gillis et al[120]; Gillis et al[122]
African green monkey	Nonhuman primate	↑MAP, ↑FGR	Rhoads et al[123]; Weaver et al[124]

ASB4, ankyrin repeat and SOCS box containing 4; *Ad-sFlt-1*, adenovirus expressing soluble fms-like tyrosine kinase-1; *AT1-AA*, angiotensin II type 1 receptor autoantibody; *BBB*, blood-brain barrier; *C1q*, component 1q; *CBF*, cerebral blood flow; *ET-1*, endothelin-1; *GFR*, glomerular filtration rate; *hAGT*, human angiotensinogen; *hREN*, human renin; *FGR*, fetal growth restriction; *L-NAME*, L-NG-nitroarginine methyl ester; *NK*, natural killer; *NO*, nitric oxide; *PE*, preeclampsia; *PlGF*, placental growth factor; *ROS*, reactive oxygen species; *RPF*, renal plasma flow; *RUPP*, reduced uterine perfusion pressure; *sEng*, soluble endoglin; *Tregs*, regulatory T cells; *TNF-α*, tumor necrosis factor-α; *VEGF*, vascular endothelial growth factor; ↑, increased; ↓, decreased; ↔, unchanged.

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FIGURE 3
Circulating levels of sFlt-1, systolic blood pressure, and proteinuria in the UPI nonhuman primate model of PE
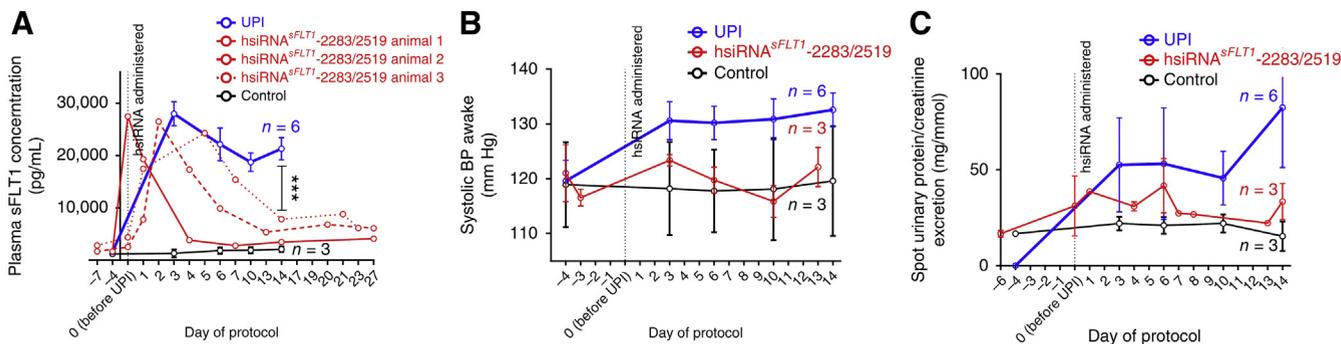
Circulating levels of **A,** sFlt-1, **B,** systolic blood pressure, and **C,** proteinuria in the UPI nonhuman primate model of PE, after a single dose of human siRNA that silences 3 *sFlt-1* isoforms (hsiRNA^{sFLT-1}-2283/2519). These 3 isoforms are responsible for the placental overexpression of sFlt-1 but do not reduce full-length *Flt-1* mRNA.[147] Data are presented as mean+SEM. The asterisk symbol indicates *P*<.001.

hsiRNA, human short interfering RNA; *mRNA*, messenger RNA; *PE*, preeclampsia; *SEM*, standard error of the mean; *sFlt-1*, soluble fms-like tyrosine kinase-1; *siRNA*, short interfering RNA; *UPI*, uteroplacental ischemia.

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critical tool in this area of study. Although many studies have been performed in animal models, unfortunately limited therapies have reached clinical trials. Nevertheless, investigations in rodent and nonhuman primates continue in the search for a treatment for PE.

Angiogenic imbalance is an early predictor and central player in the development of PE; therefore, sFlt-1, PlGF, and VEGF remain key therapeutic targets. The administration of PlGF in RUPP rats and UPI nonhuman primates have shown reductions in sFlt-1, blood pressure, and proteinuria.[10,56] Another approach that has been studied in nonhuman primates is the administration of short interfering RNAs (siRNAs) that silence the 3 *sFlt-1* mRNA isoforms that are responsible for sFlt-1 overexpression in the placenta. In pregnant baboons, UPI was induced and the human siRNA mixture (siRNA^{sFlt-1}-2283/2519) was administered at gestational day 133.[147] Data were collected at intervals of 4 to 6 weeks and showed significant decreases in circulating sFlt-1 levels and reductions in systolic blood pressure and proteinuria (Figure 3). Thadhani et al[58,59] are currently investigating the clearance of sFlt-1 (via apheresis) to improve angiogenic balance in women with PE. These studies show a transient decrease in mean arterial pressure after apheresis and prolonged gestation by up to 15 days.

In women, PE is associated with increases in the vascular expression of ET-1 and endothelial activation[20] In addition, a number of experimental models of PE are also associated with elevated tissue levels of prepro-ET-1 mRNA. These models have been used

FIGURE 4
## MAP in animal models of PE, with and without the administration of an ET$_A$ receptor antagonist
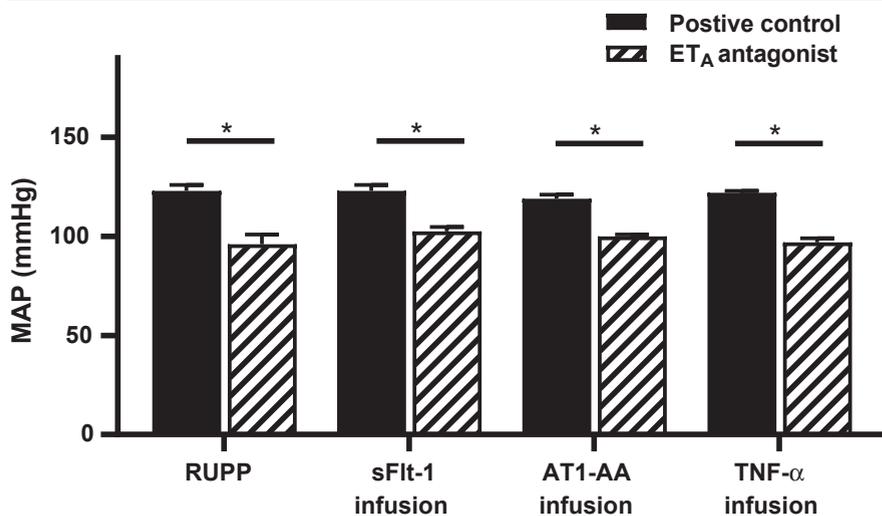
Data are shown for studies in the RUPP,[13] sFlt-1 infusion,[52] AT1-AA infusion,[67] and TNF-α infusion[83] models, suggesting that endothelial activation may be a common final pathway in the PE-related hypertension. Data are presented as mean+SEM. The asterisk symbol indicates *P*<.05.

AT1-AA, angiotensin II type 1 receptor autoantibody; *ET$_A$*, endothelin type A; *MAP*, mean arterial pressure; *PE*, preeclampsia; *RUPP*, reduced uterine perfusion pressure; *SEM*, standard error of the mean; *sFlt-1*, soluble fms-like tyrosine kinase-1; *TNF-α*, tumor necrosis factor-α.

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FIGURE 5
Pathways in the pathogenesis of PE that have been shown and studied in animal models
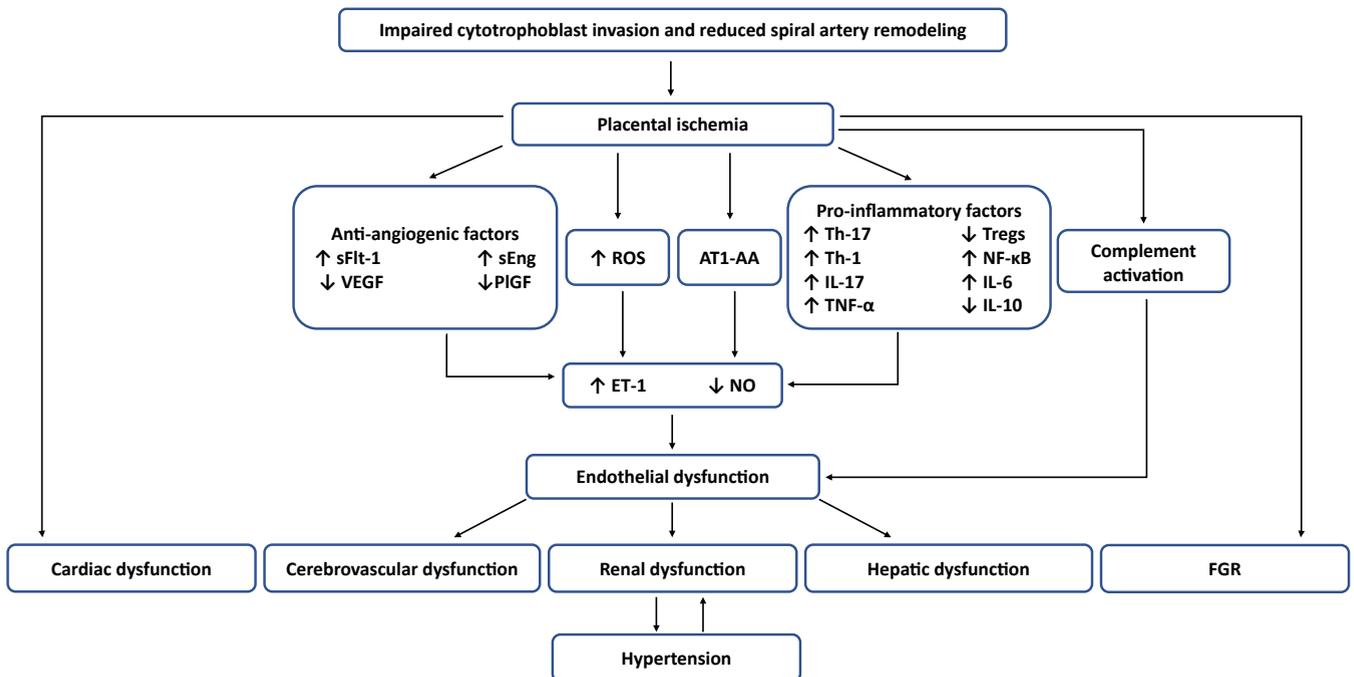
AT1-AA, angiotensin II type 1 receptor autoantibody; ET, endothelin; FGR, fetal growth restriction; IL, interleukin; NF-κB; nuclear factor kappa-light-chain-enhancer of activated B cells; NO, nitric oxide; PlGF, placental-derived growth factor; PE, preeclampsia; ROS, reactive oxygen species; sEng, soluble endoglin; sFlt-1, soluble fms-like tyrosine kinase-1; Th, T helper cell; TNF-α, tumor necrosis factor-α; Tregs, regulatory T cells; VEGF, vascular endothelial growth factor.

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to determine whether blockade of the ET system could improve hypertension. Interestingly, in the RUPP model, sFlt-1 infusion, TNF-α infusion, and AT1-AA infusion, the administration of the ET$_A$ receptor antagonist reduces the mean arterial pressure[13,52,67,83] (Figure 4). Results from these studies suggest that ET-1 may be a final common pathway whereby placental factors act on the maternal vasculature to cause vasoconstriction and hypertension. Another avenue to improve endothelial function is by stimulation of the NO-sGC-cyclic guanosine monophosphate (cGMP) pathway. Compounds that promote NO production or blockade of cGMP degradation to increase activity of this pathway are 2 methods that have been tested in animal models[95,122,125−129] and reached clinical trials.[130−136] In animal models, the administration of a phosphodiesterase type 5 inhibitor (sildenafil) can result in reduced blood pressure, increased fetal weight, decreased uterine artery resistance, and improved

angiogenic balance.[95,122,128] However, clinical studies showed little to no improvement in women with PE.[135,136]

In addition to these studies to directly block culprit pathways in PE, a number of vitamins and drug have been tested in animal models, including vitamin D, vitamin B, and statins. In the RUPP model, vitamin D administration reduced blood pressure, ET-1, sFlt-1, and AT1-AA but did not improve fetal outcomes.[137−139] In the L-NAME model, vitamin D reduced sFlt-1 and TNF-α.[140] The administration of the cholesterol-lowering drug, pravastatin, has been shown to improve placental blood flow and weight and long-term cardiovascular outcomes in the C1q$^{−/−}$ mouse.[141] In the RUPP rat, pravastatin treatment results in reduced blood pressure, improved angiogenic balance, and reduced ROS.[142] Although clinical studies have not shown comparable results, no adverse effects have been reported in women.[143−145] These avenues continue to be researched.

Conclusions

PE is a complex, multiorgan disease associated with pregnancy. The discovery of pathophysiological processes involved in PE has resulted from the interplay between basic research involving animal models and clinical research in humans. A number of animal models have been developed to address the many pathways involved in PE (Figure 5). A major consideration for any model of PE is that the animal is manipulated, whether it be surgically, pharmacologically, or genetically, to express these features. Moreover, some of the animal models discussed earlier focus on a single characteristic or mediator in the development of PE. Although these preclinical models have been crucial in understanding the pathophysiological importance of individual factors, it is important to remember that PE is a multiorgan, multifaceted disease that first develops as a result of impaired spiral artery remodeling and placental development. Some of the models

discussed earlier such as the *ASB4* deletion model and the Dahl S rat could be useful in studying these very early stages of PE. Regardless of the mechanism, each of these animal models has been critical in understanding the following 2 phases of PE: (1) impaired extravillous trophoblast migration and invasion of the endometrium causing placenta ischemia and (2) the release of factors from the ischemic placenta into the maternal circulation leading to endothelial dysfunction and the clinical syndrome. Preclinical studies in animal models have been instrumental not only in understanding the pathophysiology of PE but also in the search for novel therapeutic options for the treatment of this disease. ∎

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