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Targeting the vascular dysfunction: potential treatments for preeclampsia

Running head: Potential vascular treatments for preeclampsia

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Author profile

In 2017, Sarah was awarded her PhD at the University of Melbourne, Australia, and is currently an NHMRC Peter Doherty Research Fellow in the Department of Obstetrics and Gynecology at Monash University, Australia. The focus of her research is to assess the potential of new and old therapeutics to target the vascular dysfunction of preeclampsia, and translating these studies into clinical application.

Abstract

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Preeclampsia is a pregnancy specific disorder, primarily characterized by new-onset hypertension in combination with a variety of other maternal or fetal signs. The pathophysiological mechanisms underlying the disease are still not entirely clear. Systemic maternal vascular dysfunction underlies the clinical features of preeclampsia. It is a result of oxidative stress and the actions of excessive anti-angiogenic factors, such as soluble fms-like tyrosine kinase, soluble endoglin and activin A, released by a dysfunctional placenta. The vascular dysfunction then leads to impaired regulation and secretion of relaxation factors, and an increase in sensitivity/production of constrictors. This results in a more constricted vasculature rather than the relaxed vasodilated state associated with normal pregnancy. Currently, the only effective 'treatment' for preeclampsia is delivery of the placenta and therefore the baby. Often, this means a preterm delivery to save the life of the mother, with all the attendant risks and burdens associated with fetal prematurity. To lessen this burden, there is a pressing need for more effective treatments that target the maternal vascular dysfunction that underlies the hypertension. This review details the vascular effects of key drugs undergoing clinical assessment as potential treatments for women with preeclampsia.

Key words: preeclampsia, vascular dysfunction, drug treatment

Introduction

Preeclampsia is a pregnancy specific disorder clinically characterized by new-onset hypertension (> 20 weeks gestation) with one or more of proteinuria, maternal organ dysfunction such as renal insufficiency, liver dysfunction, neurological complications, fetal growth restriction or uteroplacental dysfunction^{1,2}. Each year preeclampsia is the principle cause of death in over 60,000 women and in more than 500,000 babies globally³. The pathophysiological mechanisms underlying the disease are still not entirely clear. Briefly, impaired invasion of trophoblast cells in early pregnancy leads to compromised maternal uterine spiral artery remodeling. As the placenta grows, this results in progressively compromised blood flow to the placenta (and fetus) causing placental ischemia-reperfusion, excessive inflammation and oxidative stress⁴. Increased systemic markers of inflammation and oxidative stress such as increased circulating levels of tumor necrosis factor- α (TNF- α) and adhesion molecules occur, as does the activation of endothelial cells, contributing to dysfunction⁵. The systemic maternal vascular dysfunction that underlies the clinical features of preeclampsia occurs because of many contributing factors, including oxidative stress and

the actions of excessive anti-angiogenic factors, such as soluble fms-like tyrosine kinase (sFlt-1), soluble endoglin (sEng) and activin A released by the injured placenta^{6,7}. Vascular dysfunction leads to impaired regulation and secretion of relaxation factors, and an increase in the bioavailability of and sensitivity to contracting factors⁸. This results in a more constricted systemic vasculature rather than the relaxed state associated with normal pregnancy. The key to unlocking new therapies is the concept that, irrespective of the underlying placental dysfunction, the maternal vascular dysfunction of preeclampsia can be corrected.

For the past 60 years treatment has focused almost exclusively on controlling the maternal hypertension of preeclampsia. There is no question that this has been beneficial. Controlling maternal hypertension has saved the lives of millions of women and babies worldwide. However, insights into the pathophysiology of the preeclampsia over the past 15 years have revealed that managing the hypertension does not correct the fundamental causative mechanisms but instead only temporarily stabilizes the clinical manifestation, leaving the underlying vascular dysfunction largely untouched. This is particularly important as there are long-term consequences for vascular health in women with a history of preeclampsia⁹. Currently, the only effective ‘treatment’ for preeclampsia is delivery of the placenta, and therefore the baby. For women with severe, early-onset preeclampsia (development prior to 34 weeks gestation) this means a preterm delivery to save the life of the mother, with all the attendant risks and burdens associated with fetal prematurity. To lessen this burden, there is a pressing need for more effective treatments that target the maternal vascular dysfunction that contributes to the hypertension and other symptoms. Despite our expert use of anti-hypertensives, preeclampsia still imposes a significant burden of mortality and morbidity - both maternal and perinatal. The ultimate endpoint for the treatment of preeclampsia should be to control the maternal syndrome sufficient to prolong pregnancy. It is estimated that for every extra day pregnancy is prolonged between the 24th and 32nd week of gestation, fetal survival is increased by 1%¹⁰ with larger attendant reductions in significant morbidity. In this review, we outline different approaches to targeting the dysfunction of the maternal vasculature, discussing the various drugs currently undergoing assessment as novel candidate therapies for preeclampsia. These drugs target three key pathways affecting vascular health; placental-derived anti-angiogenic factors, oxidative stress and endothelial function (Figure 1). For novel treatments undergoing clinical assessment that target the inflammatory system, the reader is directed elsewhere¹¹.

Pregnancy-induced vascular adaptations

In the vasculature, there is a critical balance between endothelium-derived relaxation factors (EDRFs) and endothelium-derived contracting factors (EDCFs) to maintain vascular homeostasis. In a healthy pregnancy, this balance is shifted towards enhanced vasodilation. Production of EDRFs such as nitric oxide (NO) and prostacyclin (PGI₂) are increased, whereas production of EDCFs such as angiotensin II (AngII) and thromboxane A₂ (TxA₂) are reduced⁸. In particular, systemic pressor responses to AngII and norepinephrine are attenuated^{12,13}, further contributing to the vasodilation of pregnancy. These vascular adaptations are essential to maintain pre-pregnancy blood pressure or decrease it, and prevent the development of hypertension throughout pregnancy.

Brief overview of the disruption to vascular homeostasis

Endothelial dysfunction is a complex and multifactorial process, contributing to a large number of cardiovascular related diseases¹⁴. In preeclampsia, the endothelial dysfunction within the maternal vasculature is associated with decreased bioavailability and response of smooth muscle cells to EDRFs, and increased bioavailability and sensitivity of smooth muscle cells to EDCFs. This is coupled with a phenotypic change to a more pro-inflammatory and pro-thrombotic state, leading to enhanced vasoconstriction. The role of the immune and complement systems in vascular dysfunction is covered elsewhere^{15,16}.

Vasodilation

Impaired endothelium-dependent vasodilation has been demonstrated in small arteries taken from subcutaneous fat biopsies and the myometrium from women with preeclampsia. Subcutaneous resistance arteries from preeclamptic women have reduced relaxation to the endothelium-dependent vasodilator acetylcholine (ACh) compared to normotensive pregnant women¹⁷⁻¹⁹. The underlying smooth muscle functions normally, implying that the problem lies with deficits in EDRFs. A similar study reported increases in bradykinin (BK)-mediated relaxation in subcutaneous arteries taken from normotensive women undergoing caesarean relative to non-pregnant women and those with preeclampsia²⁰. The attenuated response to BK is independent of the smooth muscle and likely due to a reduction in a normal pregnancy-induced increases in BK-mediated NO synthase (NOS). Many of the initial studies investigating differences in vascular reactivity of systemic arteries from normotensive and

preeclamptic women did not investigate potential underlying mechanisms of endothelial dysfunction.

Myometrial resistance arteries regulate the blood supply to the fetus and placenta, making them key resistance arteries during human pregnancy, especially as uteroplacental blood flow is reduced in pregnancies complicated by preeclampsia²¹. In general, myometrial arteries from preeclamptic women have reduced or almost abolished endothelium-dependent relaxation to ACh and BK compared to normotensive pregnant women^{17,22-26}. This is thought to contribute to the decreased placental perfusion and fetal growth restriction commonly associated with preeclampsia. The impaired BK-mediated relaxation is attributed to a reduction in NO and a lack of the normal compensatory mechanism of endothelium-dependent hyperpolarization (EDH)²⁷. This reduction in EDH-mediated relaxation in myometrial arteries of preeclamptic women is thought to involve disruption of myoendothelial gap junctions^{28,29}. However, as the full process of EDH has yet to be elucidated, the contribution of EDH to vascular reactivity of systemic arteries during complicated pregnancies is not understood. Chronic exposure to hypoxia prevents the normal pregnancy-induced upregulation of small-conductance Ca^{2+} -sensitive K^+ (K_{Ca}) channels in the uterine artery of pregnant sheep, which are crucial in the regulation of vascular tone and blood pressure³⁰, implying these channels may be relevant to preeclampsia. Dysregulated small and intermediate K_{Ca} channels were identified in the fetal placental circulation on chorionic plate resistance vessels from the placenta of preeclamptic women, coupled with reduced endothelial NOS (eNOS)³¹. These observations were supported by an *in vivo* study that demonstrated suppressed small and large K_{Ca} channels, which were involved in downregulating eNOS, in primary human umbilical endothelial cells (HUVECs) from women with preeclampsia³².

Vasoconstriction

Preeclampsia and gestational hypertension are associated with an increased sensitivity and response to the vasoconstrictor AngII^{12,33,34}. Interestingly, an increased response to AngII occurs in preeclamptic women before the condition is clinically apparent³⁵, and after delivery, may contribute to increased risk of future cardiovascular disease^{36,37}. Although circulating levels of AngII are not increased in preeclamptic women³⁸, augmented AngII signaling is, implying the AngII vasoconstrictor receptor (AT1R) is involved in this increased response³⁹. AT1Rs are increased in preeclamptic women⁴⁰ and can form heterodimers with

the BK type 2 receptor (B2) to amplify AngII stimulated signaling, causing enhanced sensitivity to AngII⁴¹. In preeclamptic women, there is also increased AT1R-B2 heterodimers in platelets, and potentially omental vessels³³. Furthermore, preeclampsia is associated with AT1 autoantibodies (AT1-AA), which are produced by mature B cells⁴². AT1-AA stimulate AT1Rs to cause increased vasoconstriction of rodent arteries⁴³ and endothelial cell injury and dysfunction in HUVECs⁴⁴. AT1-AA may also stimulate the expression of the common anti-angiogenic markers associated with preeclampsia, sFlt-1 and sEng^{45,46}. In addition to AngII, endothelin-1 (ET-1) and TxA₂ contribute to the vascular dysfunction of preeclampsia. Circulating levels of ET-1 and TxA₂ are elevated in women with preeclampsia^{47,48}. ET-1 is also increased in amniotic fluid and blood vessels from preeclamptic women (reviewed⁴⁹). Because increased ET-1 levels do not precede the disease manifestation, ET-1 is unlikely to be involved in the initiation of preeclampsia but rather be a result of the endothelial dysfunction. However, there is a positive correlation between sFlt-1 and ET-1 levels in preeclamptic patients^{50,51}.

Targeting anti-angiogenic factors

Angiogenic growth factors play an important role in the development of the placental and fetal vasculature. Vascular endothelial growth factor (VEGF) and placental growth factor (PlGF) are two important pro-angiogenic factors promoting angiogenesis and vasodilation⁵². A disturbance in the balance of pro- and anti-angiogenic factors has been repeatedly reported in women with preeclampsia. Of the most studied anti-angiogenic factors, sFlt-1 and sEng are two soluble anti-angiogenic factors released after the damaging inflammatory response occurs in the placenta and significantly increased in the circulation of women with preeclampsia⁵³⁻⁵⁸. Soluble Flt-1, a splice variant of Flt-1, is primarily released by the placenta and binds to circulating free VEGF and PlGF⁵⁹. Soluble Eng, a truncated form of endoglin, antagonizes TGF- β by directly binding to it. This in turn prevents the binding of these pro-angiogenic factors to their endothelial cell-surface receptors, thereby contributing to endothelial dysfunction. In animal models, adenovirus overexpression of sFlt-1, sEng or both in pregnant rats results in the manifestation of a preeclampsia-like condition, including hypertension and kidney dysfunction^{53,56}. In an attempt to counteract the effects of sFlt-1/sEng, therapies have focused on reducing circulating levels or blocking the effects of these factors, or increasing circulating VEGF/PlGF.

There has been a small pilot study of extracorporeal apheresis to reduce circulating sFlt-1 levels in women with early onset preeclampsia⁶⁰. Multiple treatments in three women with severe (<32 weeks) preeclampsia transiently normalized blood pressure and reduced proteinuria without any adverse events. The same group recently repeated this study using a more superior plasma-specific apheresis device in eleven more severe (<32 weeks) preeclamptic women, treated once (n=6), twice (n=3) or three (n=1) times with apheresis⁶¹. This treatment was also associated with transient reductions in both blood pressure and proteinuria, and had no adverse effects on either the mother or fetus. As this technique likely removes other factors such as LDL cholesterol, it is uncertain if this is safe for long term treatment in pregnant women. Additionally, the high costs associated with this technique make it a less viable treatment for preeclamptic women in developing countries. However, as a proof-of-principle, this approach is promising and worthy of further development.

Another approach to neutralize sFlt-1 is to overexpress VEGF. This has been shown to reduce blood pressure in animal models of sFlt-1-induced “preeclampsia”, including adenoviral-mediated overexpression of sFlt-1^{62,63} and the AT1-AA mouse model⁶⁴. Similarly, in the reduced uteroplacental perfusion pressure (RUPP) model of preeclampsia, VEGF administration lowers blood pressure, restores renal function and reverses endothelial dysfunction of the carotid artery⁶⁵. Although increasing VEGF appears to be a direct therapy to neutralize excessive sFlt-1, and thereby protect the maternal vasculature from sFlt-1-mediated damage, there are safety concerns with VEGF treatment. Excessive maternal VEGF contributes to cardiac failure in the fetuses of mice, resulting in embryonic lethality⁶⁶. As VEGF is an important promoter of angiogenesis and vascular permeability, overexpression could also promote tumorigenesis⁶⁷ and potentially cause widespread pulmonary edema, making the removal of sFlt-1 by other methods more attractive.

An alternative approach to VEGF as a therapeutic is PlGF. In the adenovirus-induced sFlt-1 mouse model of preeclampsia, administration of PlGF for two days reduced hypertension without affecting proteinuria in late pregnancy⁶⁸. Similarly, treatment of RUPP rats for five days with recombinant human PlGF abolishes placental ischemia-induced hypertension, decreases circulating sFlt-1 and oxidative stress but does not prevent fetal growth restriction⁶⁹. However, in another study, while PlGF administration into RUPP rats had no effect on circulating sFlt-1 levels it did reduce blood pressure and prevented a reduction in fetal size induced by RUPP⁷⁰. Potassium chloride-induced vascular contractions are significantly

reduced in PlGF-treated RUPP rats in the carotid, mesenteric and renal arteries. PlGF was also able to restore ACh-mediated relaxation in these vessels and renal vasculature of both RUPP rats and sFlt-1-treated rats. The RUPP-induced decrease in total eNOS and phosphorylated eNOS was restored in the aorta with PlGF treatment. Similarly, in a nonhuman primate model of uteroplacental ischemia of preeclampsia, recombinant human PlGF administration for 5 days significantly reduced systolic blood pressure without changing circulating levels of sFlt-1⁷¹. Neonates appeared normal on physical examination but teratogenicity was not formally assessed. Transgenic fetuses overexpressing PlGF in T cells often result in embryonic lethality due to angiogenic defects⁷². Further studies are required to assess potential fetal growth abnormalities and teratogenic effects of exogenous PlGF. Although these animal studies suggest PlGF is a potential therapeutic in preeclampsia, there are more promising potential treatments that do not directly target vasoactive molecules but often effect placental production of sFlt-1/sEng, an in turn, VEGF/PlGF.

Another important anti-angiogenic factor associated with preeclampsia is activin A. It is a member of the transforming growth factor- β (TGF- β) superfamily and is involved in many diverse biological actions, including folliculogenesis, angiogenesis and inflammation⁷³. Activin A is primarily produced by the gonads and pituitary gland, but is also expressed by the placenta and the ovary⁷⁴. In women with preeclampsia, activin A is significantly increased in the circulation compared to women with a healthy pregnancy⁷⁵⁻⁷⁷, a difference occurring months prior to clinical onset of disease⁷⁸. Administration of activin A to pregnant mice from day 10 to day 16 of pregnancy causes a preeclampsia-like syndrome, including increased blood pressure, proteinuria and decreased fetal weight⁶. Furthermore, activin A administration increases superoxide production in the maternal aorta and carotid artery, decreases eNOS mRNA expression in the aorta and decreases NO bioavailability in the carotid artery⁶. Oxidative stress induces activin A production from HUVECs and placental explants⁷⁹, while activin A treatment impairs HUVEC endothelial tube formation⁸⁰. These studies provide evidence that high levels of activin A contribute to the endothelial dysfunction of preeclampsia.

Follistatin is a glycoprotein produced by many organs, including pituitary gland, placenta and the ovary⁸¹. It acts as an activin antagonist, offering potential as a therapy to prevent further endothelial damage in women with established preeclampsia. In HUVECs, follistatin co-

treatment prevents endothelial dysfunction induced by activin A, as assessed by a significant reduction in expression of vascular and intracellular cell adhesion molecules (VCAM-1, ICAM-1) and ET-1⁸². Furthermore, follistatin also prevents increases in expression of VCAM-1, ICAM-1 and ET-1 in HUVECs treated with serum from women with preeclampsia. The therapeutic potential for follistatin has been demonstrated in numerous animal models of ischemia-reperfusion injury⁸³. However, the activin A-follistatin system is now recognized as an important regulator of lipid and glucose metabolism, that could influence fetal growth⁸⁴. Further disrupting this balance in a preeclamptic pregnancy could have a negative impact on fetal development. Therefore, the next stage in activin A research is to assess whether follistatin can reverse or protect maternal vasculature from endothelial dysfunction induced by activin A. It is unknown if different doses of follistatin have teratogenic effects in rodents. However, we do know that inhibiting activin A-induced free radical production with an activin receptor-like kinase inhibitor (MKP-1-140A) caused fetal abnormalities in a preeclamptic-like mouse model, suggesting caution with future treatment studies⁸⁵.

Directly targeting the endothelial dysfunction of preeclampsia

Numerous drugs can improve endothelial dysfunction. As expected, many of these are deemed unsuitable for the treatment of preeclampsia, either because they are unsafe in pregnancy or because we simply do not know their safety profile in pregnancy. However, there are a handful of therapeutic agents with good safety profiles that are used for other indications in pregnancy that might also target the maternal vascular dysfunction.

NO pathway

Glyceryl trinitrate

Nitric oxide is synthesized from L-arginine by calcium (Ca^{2+})-dependent NOS enzymes⁸⁶, the most relevant isoform in this context being eNOS. Endothelial NOS is constitutively expressed, calcium-dependent and membrane-bound to endothelial cells⁸⁷. In women with preeclampsia, a key feature of the endothelial dysfunction is reduced bioavailability of NO⁸⁸. The idea of treating preeclamptic women with NO donors is based on their established use in cardiovascular disease and ability to act directly on the smooth muscle to cause relaxation⁸⁹. Glyceryl trinitrate (GTN) is an organic nitrate that has been demonstrated to significantly reduce maternal blood pressure and umbilical artery resistance, without affecting uterine artery resistance in a pilot clinical trial of 12 women with severe preeclampsia⁹⁰. Many other

clinical trials assessing the potential for GTN as an anti-hypertensive treatment for preeclampsia report a reduction in blood pressure⁹¹⁻⁹⁴. These studies also report a side effect of severe headaches from nitrate. Chronic treatment of cardiovascular disease with GTN often results in tolerance, cross tolerance to other nitrovasodilators, and endothelial dysfunction, limiting its potential clinical effect⁹⁵. Furthermore, an outstanding concern is that GTN can cross the placenta to the developing fetus, however, this has been reported to have no effect on fetal arterial perfusion pressure⁹⁶. Therefore, GTN is unlikely to be a suitable treatment.

Pentaerithrityl-tetranitrate

To overcome the adverse effects of headaches, nitrate tolerance and endothelial dysfunction associated with GTN treatment, another NO donor, known as pentaerithrityl-tetranitrate (PETN), has been investigated. Like other organic nitrates, PETN releases NO and also enhances NO bioavailability by reducing eNOS uncoupling^{97,98}. It also has antioxidant effects, including inducing the antioxidants heme oxygenase 1 (HO-1)⁹⁸ and superoxide dismutase 3^{97,99,100}. Animal studies suggest PETN is safe in pregnancy and has no toxicity, even at high doses^{101,102}. Interestingly, treatment of pregnant spontaneously hypertensive rats (SHRs) with PETN does not affect blood pressure, but results in reduced blood pressure of female offspring¹⁰⁰. PETN treatment also increases ACh-mediated relaxation and eNOS protein expression in the aortas of these offspring. Furthermore, in a small randomized, double-blind, controlled study, PETN improved flow mediated vasodilation of the radial artery after ischemia reperfusion injury in 9 male participants¹⁰³. Importantly, 111 women at risk for adverse pregnancy outcomes because of impaired uteroplacental perfusion in mid-gestation were treated with placebo or PETN until week 35 of pregnancy as a part of another pilot randomized, double-blind controlled study¹⁰⁴. This study reported that PETN significantly decreases the risk of having an intrauterine growth restricted baby and perinatal death, but does not affect the risk of developing preeclampsia. Even so, PETN is the most promising organic nitrate that could be employed as an adjuvant treatment for preeclampsia. Larger clinical trials are now needed to establish efficacy in directly targeting the endothelial dysfunction of women with preeclampsia.

Sildenafil

Sildenafil citrate (commonly known as Viagra) is one of numerous phosphodiesterase-5 (PDE-5) inhibitors that acts by preventing the degradation of cyclic guanosine 3',5'-

monophosphate¹⁰⁵, resulting in increased NO production and vascular smooth muscle relaxation. Sildenafil improves uteroplacental blood flow in pre-constricted uterine arteries of pregnant sheep¹⁰⁶, while improving endothelial-dependent relaxation in small myometrial arteries of preeclamptic women when treated with a PDE-5 inhibitor²⁴. In the catechol-O-methyl transferase knockout mouse model of preeclampsia, sildenafil normalizes reduced pup growth and umbilical blood flow velocity, while increasing endothelium-dependent relaxation of the uterine artery¹⁰⁷. Chronic inhibition of NOS results in a preeclamptic-like syndrome in pregnant rat; sildenafil treatment in these rats reduces blood pressure and improves placental perfusion¹⁰⁸, and decreases circulating levels of sFlt-1 and sEng¹⁰⁹.

A small Phase II clinical trial reported no benefits of sildenafil treatment in preeclamptic women [93]. However, it was well tolerated with no increases in maternal or fetal morbidity and mortality¹¹⁰. A larger randomized controlled trial demonstrated that sildenafil prolongs pregnancy by 4 days, reduces maternal blood pressure 24 hours after treatment and improves maternal and fetal blood flow¹¹¹. Recently, it was reported that the treatment of an early onset preeclamptic patient with sildenafil not only prolongs pregnancy, but also stabilizes blood pressure, while significantly reducing circulating levels of sFlt-1 and sEng¹¹².

In early 2018, the UK STRIDER trial (ISRCTN 39133303) for the use of sildenafil as a treatment for severe fetal growth restriction (22 to 30 weeks gestation) reported their results. Neither delivery interval, live births, neonatal deaths or birth weight was different between placebo and sildenafil treatment¹¹³. Sildenafil treatment did not improve pregnancy or fetal outcomes for these women, with no adverse outcomes reported due to sildenafil. Unfortunately, the Dutch STRIDER trial (NCT02277132) was halted recently after 11 babies died due to lung complications¹¹⁴. While it is unlikely the results for this trial will be available anytime soon, the results from these two STRIDER trials are likely to seriously influence any further sildenafil trials for the treatment of preeclampsia¹¹⁵.

L-arginine

Recently, small and intermediate K_{Ca} have been implicated in NO release¹¹⁶, prompting interest in these channels as therapeutic targets to restore endothelial function¹¹⁷. Importantly, it is not known if modulation of small and intermediate K_{Ca} will increase uptake of L-arginine and yield increases in NO bioavailability. As L-arginine concentrations are reduced in women with preeclampsia¹¹⁸, the treatment of these women with the NO

precursor L-arginine is another attractive therapeutic. Numerous clinical trials have demonstrated that women directly supplemented with L-arginine are at reduced risk of developing preeclampsia (reviewed ¹¹⁹). Unfortunately, one study using L-arginine and antioxidant supplementation reported numerous mild adverse effects, including headaches, palpitations, dizziness and dyspepsia ¹²⁰. In a more recent clinical trial, supplementation with L-arginine in women who were at a high risk of developing preeclampsia significantly reduced blood pressure and preterm birth rates, and the number of women developing preeclampsia, while increasing birth weight relative to placebo treated women ¹²¹. The previous adverse effects listed by Vadillo-Ortega et al, were absent in the more recent study, likely due to a reduction in the dose of L-arginine supplementation. However, dyspepsia was reported in the L-arginine supplemented women, but was not severe enough to discontinue treatment ¹²¹. Furthermore, while these results are very promising, these studies are detailed here as they advise as to the safety of L-arginine administration during pregnancy. They have been used in these studies as a preventative, not a treatment. A larger clinical trial is now needed to assess both safety and efficacy on a large scale, and to assess whether L-arginine is a potential treatment for women with established preeclampsia.

Relaxin

The pregnancy hormone relaxin is now recognized as a potential vascular treatment for preeclampsia ¹²². Relaxin is produced by the corpus luteum, placenta and uterus ¹²³. Its receptor, RXFP1, is localized to endothelial and smooth muscle cells of several arteries, including the aorta, femoral, small renal, mesenteric and uterine arteries ¹²⁴⁻¹²⁶. Low circulating levels of relaxin are associated with an increased risk of developing preeclampsia ^{127,128}. Furthermore, relaxin acts as a renal and systemic vasodilator in both rats and humans, increasing renal blood flow and glomerular filtration rate ¹²⁹⁻¹³⁴. Relaxin administration in rats and mice increases global arterial compliance ¹³⁵, decreases myogenic reactivity in rodent renal and mesenteric and human subcutaneous fat arteries, and enhances BK-mediated relaxation in the mesenteric artery primarily via NO or PGI₂ depending on length of exposure ^{124,136-138}. Relaxin also reduces the response of rodent systemic arteries to vasoconstrictors such as ET-1 ¹³⁹ and AngII ^{129,140,141}. Importantly, relaxin induces expression of VEGF and promotes angiogenesis ^{142,143}. Taken together, these results suggest that relaxin could be a candidate drug treatment for women with preeclampsia.

Ex vivo studies have reported numerous vasoprotective properties of relaxin. Relaxin co-incubation for 72 hours prevents the development of high glucose-induced dysfunction in the aorta of male mice by improving ACh-mediated relaxation by ameliorating ROS and PGI₂ levels¹⁴⁴. Relaxin also rapidly reverses TNF- α induced endothelial dysfunction in rat aorta by enhancing responses to ACh via increasing basal eNOS and reducing ET-1 expression¹⁴⁵. Additionally, relaxin and the relaxin mimetic B7-33 protect the mesenteric artery of female mice from endothelial dysfunction induced by trophoblast conditioned media high in sFlt-1^{140,146}. Importantly, relaxin administration *ex vivo* significantly reduces myogenic constriction of human subcutaneous arteries from preeclamptic women¹²².

Recently, relaxin treatment in a rat RUPP model of preeclampsia caused a reduction in blood pressure, uterine artery resistance, plasma TNF- α and sFlt-1 levels and increases plasma nitrate-nitrite levels¹⁴⁷. However, other than uterine artery resistance, vascular function was not directly assessed. It remains to be established if these beneficial effects of relaxin are associated with enhanced vasoconstriction or reduced agonist-dependent relaxation. In an sFlt-1 adenovirus animal model of preeclampsia, sFlt-1 increases myogenic constriction in small renal arteries, while relaxin treatment inhibits this increase in myogenic constriction¹²². The next phase of relaxin research will be further assess relaxin's vascular actions *in vivo*, in preparation for a pilot trial in pregnant women.

Targeting oxidative stress

During pregnancy there is an important balance between reactive oxygen species (ROS) and antioxidants¹⁴⁸. In preeclampsia, defective trophoblast invasion of spiral arteries results in intermittent blood flow that contributes to placental reperfusion injury and a hypoxic environment. This results in an increase in ROS relative to antioxidant defenses, which causes oxidative stress and results in a damaging inflammatory response¹⁴⁹. Re-established blood flow after reperfusion injury results in the release of inflammatory markers and high levels of ROS such as superoxide, triggers placental oxidative stress. This coupled with the ROS produced by the maternal systemic vasculature contributes to the widespread maternal endothelial dysfunction of preeclampsia. In particular, preeclampsia is associated with an increase in substances that increase ROS in the maternal circulation such as cytokines¹⁵⁰, lipid peroxides¹⁵¹ and oxidized low-density lipoprotein¹⁵².

Pravastatin

Statins (3-hydroxy-3-methyl-glutaryl-coenzyme A reductase inhibitors) are a commonly prescribed lipid-lowering medication used to prevent cardiovascular morbidity and mortality. Statins have diverse effects, including endothelial protection, antioxidant and anti-inflammatory properties, and proangiogenic effects¹⁵³. This has made them an appealing treatment for preeclampsia. As cholesterol is essential for fetal development, there are concerns that statins used during pregnancy could have teratogenic effects. A meta-analysis¹⁵⁴ and recent review¹⁵⁵ of statin use during pregnancy confirmed that this is still unclear. Accordingly, statins remain contraindicated during pregnancy, based on the results from animal studies utilizing high doses¹⁵⁶⁻¹⁵⁸. Nonetheless, to date, no human studies have revealed any specific pattern of congenital abnormalities, suggesting statins are safely tolerated during pregnancy at low doses.

While there are many statins available, pravastatin is the most hydrophilic statin and the least potent¹⁵⁹. Furthermore, transfer of pravastatin across the placenta is limited and slow¹⁶⁰. In particular, pravastatin prevents endothelial dysfunction and improves preeclampsia-like symptoms. In animal models of preeclampsia, pravastatin reduces the pronounced and sustained pressor response to AngII, attenuates glomerular injury and increases circulating free VEGF levels by inhibiting sFlt-1 release from macrophages and stimulating VEGF from trophoblast cells^{161,162}. In mice treated with sFlt-1 to induce a preeclamptic-like syndrome, pravastatin treatment improves vascular reactivity in the carotid artery, significantly reduces circulating sFlt-1 levels¹⁶³ and upregulates eNOS expression in the aorta¹⁶⁴. In other sFlt-1 mouse models of preeclampsia, pravastatin treatment decreases circulating sFlt-1 levels by inducing circulating PlGF¹⁶⁵ and increasing placental VEGF and PlGF expression¹⁶⁶.

In vitro studies in human tissues support the animal model studies, and report that pravastatin reduces sFlt-1 secretion from HUVECs and trophoblast cells, while increasing production of sEng from HUVECs^{167,168}. Pravastatin blocks sFlt-1 production but increases sEng production. Pravastatin also decreases markers of endothelial dysfunction including VCAM-1, ICAM-1 and ET-1 expression after exposure of HUVECs to conditioned media, which contains sFlt-1. The same study also reported a pilot study that demonstrates the stabilization or decreases in circulating sFlt-1 in 3 of 4 preeclamptic women treated with pravastatin. These promising results led to a larger pilot randomized clinical trial, which assessed the safety and pharmacokinetics of a low dose of pravastatin administration from recruitment at weeks 12-16 of pregnancy until delivery in women at high risk of developing preeclampsia

¹⁶⁹. There were no adverse events, with 4 women developing preeclampsia in the placebo-treated group and no women developing preeclampsia in the pravastatin group. Furthermore, umbilical cord cholesterol concentrations were unchanged, with the majority of umbilical cord and maternal plasma pravastatin levels below detectable ranges at time of delivery. This study informs in regards to safety but does not answer whether pravastatin is a potential treatment for preeclampsia. However, a double blind, randomized placebo-controlled, multicenter trial of pravastatin to ameliorate early onset preeclampsia (StAmP) was completed some time ago (isrctn.com clinical trial registry: ISRCTN23410175). The eagerly awaited results will hopefully inform us if pravastatin is a potential treatment for preeclampsia.

Melatonin

Melatonin is a hormone primarily produced by the pineal gland in the brain that has antioxidant activities and is produced by the placenta throughout pregnancy. It is commonly prescribed to help regulate sleep (reviewed ¹⁷⁰). It has a well-known safety profile in pregnancy and has been used in a large number of clinical trials as a potential treatment for numerous conditions, including cardiovascular disease ¹⁷¹, rheumatoid arthritis and diabetes ¹⁷². Reduced circulating levels of melatonin have been reported in women with preeclampsia ¹⁷³⁻¹⁷⁵, with placental expression of melatonin and the melatonin receptors also reduced in preeclamptic pregnancies ¹⁷⁶. Because of this, melatonin has been hypothesized as a potential treatment to protect against the oxidative stress generated by the placenta and potentially reducing placental expression of vasoactive substances ¹⁷⁷.

Melatonin reduces sFlt-1 secretion from primary trophoblasts, but does not affect sFlt-1 or sEng excretion from placental explants or HUVECs ¹⁷⁸. This work was supported by another recent study also reporting that melatonin does not affect placental explant production of sFlt-1 or sEng after treatment with the ROS generator xanthine/xanthine oxidase ¹⁷⁹. However, melatonin treatment increases mRNA expression of numerous antioxidant response element genes from placental explants, including thioredoxin, glutamate-cysteine ligase and NAD(P)H quinone acceptor oxidoreductase ¹⁷⁸. Melatonin also reduces oxidative stress and enhances HO-1 in placental explants treated with xanthine/xanthine oxidase ¹⁷⁹. One group reported that melatonin is unable to prevent endothelial dysfunction induced by TNF- α (increased mRNA expression of VCAM-1 and ET-1) from HUVECs, whereas another

recently demonstrated that melatonin prevents TNF- α -induced VCAM-1 expression from HUVECs¹⁷⁹. This may be due to the use of much higher doses of melatonin. Melatonin also improves endothelial dysfunction in animal models of vascular injury, including enhanced aortic ACh-dependent relaxation via NO in rats exposed to chronic intermittent hypoxia¹⁸⁰ and the reduction of VCAM-1 and ET-1 expression in the aorta of rat model of smoke-induced vascular injury¹⁸¹.

Melatonin's vascular effects are very complex. It can promote both constriction and relaxation depending on concentration and the arterial bed. For example, melatonin reduces relaxation in porcine coronary vessels¹⁸², causes vasoconstriction in the same vessel¹⁸³, causes relaxation in porcine pulmonary vessels¹⁸³ and rat mesenteric arteries¹⁸⁴, and induces constriction in rat cerebral arteries¹⁸⁵. These observations are attributed to inhibition of NO-induced smooth muscle relaxation¹⁸², enhanced NO and eNOS¹⁸⁴, inhibition of large K_{Ca} channels¹⁸⁵ and activation of large K_{Ca} channels¹⁸⁴. These effects are primarily mediated via the melatonin receptors, MT1 and MT2, which are differentially localized throughout the vasculature^{186,187}, with smooth muscle MT1 mediating constriction and MT2 mediating relaxation¹⁸⁸. Furthermore, melatonin administration to healthy patients reduces renal blood flow, increases forearm blood flow while unchanging cerebral blood flow¹⁸⁹. These effects are thought to be mediated by the relative distribution of MT1 and MT2.

In animal models of placental ischemia, melatonin administration prevents oxidative placental DNA and mitochondrial damage, and prevents fetal growth restriction^{190,191}. Treatment of SHR with melatonin increases endothelium-dependent relaxation to ACh in the aorta¹⁹². Furthermore, in the L-N^G-Nitroarginine methyl ester (L-NAME)-induced hypertensive rats, melatonin slightly reduces blood pressure, increases aortic NOS activity but is unable to prevent impaired ACh-mediated relaxation of femoral arteries¹⁹³. Furthermore, melatonin treatment reduces mean arterial pressure and increases circulating levels of VEGF in pinealectomised RUPP rats¹⁹⁴. These effects are thought to be due to increases in NO availability.

Recently, the first clinical trial of melatonin in preeclamptic women, an open label single arm trial – PAMPR, was reported¹⁷⁷. Twenty women with early onset preeclampsia were administered melatonin until delivery. Results were compared with a cohort of matched

historical controls from the preceding two years. Compared to controls melatonin significantly prolonged pregnancy by an average of six days¹⁷⁹. Despite a 100-fold increase in maternal and fetal melatonin levels, there were no adverse maternal events, drug reactions or excessive maternal sleepiness. However, there were no improvements in markers of oxidative stress or endothelial dysfunction. Importantly, there was an increase in the incidence of growth restriction at birth with melatonin administration. A similar effect of melatonin was reported in a high altitude ovine model of fetal growth restriction¹⁹⁵. Pregnancy was extended by 7.5% but there were decreases in birth weight of lambs. These studies suggest that melatonin administration, at least at high doses, may be beneficial for maternal outcomes but possibly detrimental for fetal growth. It is clear that there is still much to learn about the vascular effects of melatonin and whether this is a safe treatment.

Sulforaphane

The failure of numerous large scale clinical trials utilizing vitamin C and E as antioxidants for the prevention and treatment of preeclampsia has hindered progression of antioxidant supplements as potential treatments for preeclampsia¹⁹⁶⁻¹⁹⁸. Sulforaphane is a potent inducer of nuclear factor erythroid 2-related factor 2 (Nrf2), an endogenous inducer of cellular antioxidant pathways and a strong anti-inflammatory¹⁹⁹. Sulforaphane is attractive as a clinical therapy in preeclampsia because it has an established clinical safety profile, with proven efficacy in experimental cardiovascular disease by decreasing oxidative stress²⁰⁰. Sulforaphane occurs naturally in broccoli sprout extracts and is readily available as a nutritional supplement. Preclinical studies show that sulforaphane improves vascular function *in vitro* when blood vessels are exposed to sFlt-1 and sEng (personal unpublished data), and reduces inflammatory (TNF- α)-induced expression of VCAM-1 and ICAM-1 in HUVECs (personal unpublished data).

A broccoli sprout extract high in active sulforaphane (BroccoMax®, Jarrow Formulas) has recently begun a Phase III clinical trial for the treatment of women with early-onset preeclampsia (Prolong, anzctr.org.au clinical trial registry: ACTRN12618000216213). Of the secondary outcomes being measured is the vascular reactivity of arteries from subcutaneous fat. To the best of our knowledge, BroccoMax® has not been used before in a clinical trial for pregnant women. However, as this product is composed from broccoli sprouts, the expectation is that this treatment will be well tolerated and safe for use during pregnancy.

Selenium

Another potential approach which focusses on tackling the oxidative stress of preeclampsia is the essential trace mineral and antioxidant selenium. Selenium acts as an anti-inflammatory and an antioxidant by incorporating into selenoproteins, including glutathione peroxidase (GPx) and thioredoxin reductase (TRx)²⁰¹. In an animal model of homocysteine-induced endothelial dysfunction, selenium treatment in rats increases plasma NO concentrations and reduces HUVEC apoptosis²⁰². Furthermore, selenium treatment prevents homocysteine-induced endothelial cell injury and impaired endothelium-dependent relaxation of the thoracic aorta. Selenium supplementation also protects trophoblast cells and trophoblast mitochondria from exogenous oxidative stress via GPx and TRx^{203,204}, while increasing trophoblast viability under conditions of hypoxia²⁰⁵. Rats fed a low selenium diet are more susceptible to ischemia/reperfusion, and have a preeclamptic-like syndrome when pregnant²⁰⁶.

Numerous studies have reported that women with preeclampsia are often deficient in selenium²⁰⁷⁻²¹⁴. A pilot trial in Iran assessed whether or not selenium supplementation from the first trimester could prevent the development of preeclampsia²¹⁵. In the placebo group, 3 out of 64 women developed preeclampsia, whereas none of the 61 selenium supplemented women developed preeclampsia. Another pilot study assessed if selenium supplementation from early pregnancy (12-14 weeks) until delivery could prevent the development of preeclampsia²¹⁶. This study reported that by 35 weeks, circulating sFlt-1 levels were significantly reduced by 30% in participants in the lowest quartile of selenium status at baseline. Of the 214 women who took part in the trial, 8 of the 109 women treated with placebo continued on to develop preeclampsia, compared with 3 of the 105 selenium-treated women. However, this was not measured as a primary or secondary outcome due to the small sample sizes. Importantly, no serious adverse events were reportedly due to selenium supplementation in either trial.

No large scale clinical trial has yet attempted to establish if selenium could be a potential treatment for women with established preeclampsia. As a concern, high selenium concentrations are toxic and induce many different symptoms including hair loss, nail discoloration and fatigue²¹⁷. Furthermore, selenium may not be any more beneficial at combating the oxidative stress of preeclampsia than any other antioxidant. However, recently

a water-soluble selenium-containing sugar (1,4-anhydro-4-seleno-D-talitol; SeTal) that is a potent antioxidant²¹⁸ was demonstrated to accelerate wound closure in diabetic mice by improving vascular perfusion at the wound site (J.C Kwan et al., 2016 unpublished data). Furthermore, SeTal, but not selenium, prevents the onset of vascular dysfunction in isolated mouse aorta under conditions of acute oxidative stress by decreasing superoxide levels and increasing basal NO availability²¹⁹. This modified selenium compound is still in the preclinical research phase, but may be a potential novel future treatment to target the maternal vasculature of women with preeclampsia.

Cell-free hemoglobin (Hb) originating from the placenta is hypothesized to contribute to the development of preeclampsia²²⁰. Hb is a scavenger of NO and contributes to oxidative stress²²¹. By aggravating existing oxidative stress, Hb is believed to damage the placental barrier and leak into the maternal bloodstream causing endothelial damage and vasoconstriction²²². In particular, the HbF subunit has been demonstrated to be upregulated and localized to the vascular lumen of placentas from preeclamptic women²²³. Another study demonstrated that HbF was elevated in the first trimester of women who later went on to develop preeclampsia, as was another protein that could be of therapeutic potential, alpha-1-microglobulin (A1M)²²⁴. A1M has recently emerged as a tissue housekeeping protein that plays a role in cleaning oxidative waste products and antioxidant repair²²⁵. It is primarily produced in the liver and is upregulated in the liver and blood during oxidative stress²²⁶. A1M has been localized to many areas, including the interface between maternal blood and fetal tissues in the placenta²²⁷. It has also been demonstrated to be upregulated in the plasma and placenta of women with preeclampsia²²⁰. *Ex vivo* placental perfusion was performed by perfusing the fetal side with free HbF and demonstrated a rapid increase in blood pressure²²⁸. After 1 hour, these placentas showed considerable damage to the extracellular matrix and the collagen fibrils that maintain the tissue structure, causing leakage. Those placentas also demonstrated damage to nuclei and mitochondria, and the formation of apoptotic vesicles. However, when A1M is perfused simultaneously on the maternal side, HbF leakage from the fetal to the maternal side ceased.

In pregnant rabbits, administering species-specific HbF from mid-gestation until term developed “preeclampsia-like symptoms” in the placenta and kidney²²⁹. These symptoms were characterized by proteinuria, intracellular and extracellular tissue damage of the kidneys and placenta, with no changes in blood pressure. These effects were restored by co-

administration of A1M. In late pregnant ewes, starvation for 36 hours induces hemolysis, and also results in placental and kidney damage²³⁰. These ewes were given two bolus injections of recombinant A1M or placebo within 2 hours. After a further 72 hours of normal feeding, ewes were euthanized. Once again, A1M affected the kidneys by increasing glomerular permeability and glomerular endotheliosis, without signs of proteinuria. It is important to note that these ewes did not have any changes in blood pressure. To the best of our knowledge, A1M has not been administered into a classical animal model of preeclampsia, where a study on the maternal vasculature could be performed. Furthermore, a clinical development program for a human recombinant A1M variant known as RMC-035 is now in preparation as a candidate to become the first pharmacological treatment for preeclampsia according to Gunnarsson et al²³¹.

Miscellaneous agents

Resveratrol

Resveratrol (3,5,4'-trihydroxy-trans-stilbene) is a naturally occurring polyphenol extracted from certain fruits that has shown a beneficial effect in cardiovascular disease²³². Resveratrol is an activator of SIRT1, which promotes expression and activity of eNOS to improve ACh-mediated relaxation in rat aorta²³³. Furthermore, in the isolated aorta of SHRs, resveratrol enhances ACh-mediated relaxation *ex vivo* by increasing eNOS phosphorylation²³⁴. Resveratrol treatment also enhances NO production in primary bovine aortic endothelial cells, while lowering blood pressure in SHR's after 4 weeks administration. Long term resveratrol treatment lowers blood pressure in obese Zucker rats, which is associated with an increase in eNOS protein expression in the aorta²³⁵. Resveratrol also directly induces relaxation of the mesenteric and main uterine arteries of non-pregnant guinea pigs *ex vivo*²³⁶, an effect of endothelium-dependent and endothelium-independent pathways, utilizing both NO and voltage-dependent K⁺ and ATP-sensitive K⁺ channels in the mesenteric artery²³⁷.

In animal models of reduced uterine blood flow, resveratrol increases uterine artery blood flow velocity and fetal weight in the catechol-*O*-methyltransferase knockout mice, but not in the eNOS knockout mouse of chronic hypertension²³⁸. However, resveratrol did not improve impaired methacholine-dependent relaxation in the uterine artery of either genotype. Resveratrol did not modulate blood pressure in these mouse strains, however, neither of these strains had the expected hypertension normally associated with these models. In a rat model

of preeclampsia that utilizes desoxycorticosterone acetate to induce hypertension and proteinuria, resveratrol did not reduce hypertension or increase placental blood flow²³⁹. Resveratrol significantly improved proteinuria in nephritic rats²⁴⁰. In an L-NAME-induced preeclamptic rat model, resveratrol reduces systolic blood pressure and proteinuria, and oxidative stress by increasing the superoxide scavenger superoxide dismutase²⁴¹.

Resveratrol reduces primary trophoblast and HUVEC expression of sFlt-1 and sEng^{242,243}. Treatment of primary HUVECs with resveratrol significantly increases phosphorylated eNOS to total eNOS, implying the potential to cause vasodilation²⁴². The same study used TNF- α to mimic endothelial dysfunction with increases in VCAM-1 expression. Surprisingly, resveratrol further increases VCAM-1 expression, while reducing ET-1 expression. Interestingly, resveratrol has no effect on anti-oxidant mRNA expression in primary trophoblasts, and actually decreases expression of HO-1 at very high doses, an effect not replicated in HUVECs. These studies report conflicting data, suggesting resveratrol's effect on the placenta may be complicated. In pregnant rats fed a low protein diet resveratrol acts as an anti-oxidant and reduces oxidative stress²⁴⁴. However, it acts as both an antioxidant and a pro-oxidant depending on concentration, adding a further complication to its potential use as a therapeutic²⁴⁵.

Acute high doses of resveratrol are well tolerated in humans²⁴⁶, with no evidence of teratogenic effects in rodents²⁴⁷. There are, however, still safety concerns for use in pregnant women. In non-human primates fed a high fat diet (36% fat), chronic resveratrol treatment for 3 months prior to and throughout pregnancy increases uterine artery blood flow and decreases placental inflammation by the third trimester, among other benefits²⁴⁸. However, they reported an increase in fetal pancreatic mass by 42%, the cause of which is unknown. This study recommends caution for the use of resveratrol in pregnancy. Recently, a clinical trial published results of a study that treated severe preeclamptic women with nifedipine, an antihypertensive medication, in combination with resveratrol or placebo²⁴⁹. Time required to effectively control blood pressure is significantly reduced in the resveratrol group compared to the placebo group. Resveratrol also significantly increases the time until a new hypertensive crisis occurring following effective blood pressure control. Resveratrol reduces the amount of nifedipine needed to control blood pressure, with no serious adverse events reported for women or their babies. However, this study only treated women for one day and

therefore other clinical symptoms and biomarkers of preeclampsia were not reported. While there are many positive *in vivo* results from resveratrol administration, it is evident that further work is required to establish whether there are potential deleterious effects of resveratrol during pregnancy before a large scale clinical trial of preeclamptic women should be undertaken.

Metformin

Metformin (dimethylbiguanide hydrochloride) is a commonly prescribed medication for the treatment of type 2 diabetes²⁵⁰. It rapidly transfers to the fetal circulation during pregnancy²⁵¹, but with no teratogenic effects²⁵². Metformin improves pregnancy success²⁵³ and endothelial function²⁵⁴ in women with polycystic ovarian syndrome, and improves endothelial function in diabetic patients²⁵⁵. The use of metformin to treat gestational diabetes mellitus is well known. Metformin also reduces the frequency of gestational hypertension²⁵⁶, and reduces the risk of women developing preeclampsia²⁵⁷.

Metformin reduces secretion of sFlt-1 and sEng from primary trophoblast cells, HUVECs and placental explants in a dose-dependent manner, an effect thought to be regulated via the mitochondria²⁵⁸. Metformin also reduces TNF- α -induced expression of VCAM-1 from HUVECs, suggesting it may be able to decrease endothelial dysfunction. To further explore this concept, omental arteries from term women undergoing elective caesarean section were incubated *ex vivo* in placental explant media high in sFlt-1. These arteries develop endothelial dysfunction after 3 hours as characterized by a significant reduction in relaxation to the endothelium-dependent agonist BK. Metformin co-incubation prevents this dysfunction. Furthermore, metformin rescues sFlt-1-induced inhibition of angiogenic sprouting of omental arteries, further supporting a role for metformin to protect the maternal vasculature from sFlt-1 induced vascular dysfunction. The preclinical evidence for metformin is promising. The use of metformin in animal models of preeclampsia is the next step to establish whether this is a candidate drug for preeclampsia.

Esomeprazole

Esomeprazole is a proton-pump inhibitor commonly prescribed to women for the treatment of gastric reflux during pregnancy. Numerous studies have reported on the safety profile of proton-pump inhibitors in pregnancy, concluding that there are no fetal risks associated with

use²⁵⁹⁻²⁶¹. A preclinical screen of different proton-pump inhibitors identified esomeprazole as a suitable candidate for the treatment of preeclampsia²⁶². Esomeprazole significantly reduces sFlt-1 and sEng production from primary trophoblast cells, HUVECs and placental explants from the placentas of women with preeclampsia. Treatment of primary trophoblasts also increases the mRNA expression of VEGF, while increasing phosphorylated eNOS in HUVECs. Esomeprazole co-treatment with TNF- α significantly reduces ET-1 and VCAM-1 secretion from HUVECs, and increases translocation of Nrf2 to the nucleus in trophoblast cells. Treatment also upregulates the endogenous antioxidant HO-1 in trophoblasts cells, HUVECs, placental explants from a preeclamptic woman and uterine microvascular cells. Furthermore, in omental arteries from normotensive and preeclamptic women, esomeprazole directly induces relaxation of pre-constricted arteries, an effect that is endothelium-dependent. The same study also administered esomeprazole to a pregnant transgenic mouse model that overexpresses human sFlt-1 in the placenta. Interestingly, esomeprazole administration prevents the sFlt-1 induced increase in blood pressure by late pregnancy.

A Phase II double blind, randomized, placebo-controlled trial was recently completed (Preeclampsia Intervention with Esomeprazole [PIE] trial)²⁶³. In this trial 120 women with early-onset preeclamptic (between 26-32 weeks gestation) received either esomeprazole or placebo²⁶⁴. There were no differences in maternal or neonatal outcomes, including no differences in sFlt-1 levels or prolongation of pregnancy. However, a prospective cohort study measured sFlt-1 levels in 40 women with preeclampsia or superimposed preeclampsia collectively on three different protein-pump inhibitors²⁶⁵. This group reported reduced plasma sFlt-1, endoglin and endothelin-1 levels at time of recruitment across a mix of gestations (23 to 41 weeks), with a trend towards pregnancy prolongation. As this study reported the beneficial use of numerous different protein-pump inhibitors with only 6 of these women were on esomeprazole, it is difficult to compare the results with that of the above larger study. In addition, another similar clinical trial design was registered in 2017 to recruit a target of 390 early-onset preeclamptic women to treat with esomeprazole or placebo in Egypt (clinicaltrials.gov clinical trial registry: NCT03213639). It is unknown whether this trial has started recruitment.

Interestingly, a recent study looked at utilizing both esomeprazole and metformin together at lower doses to establish whether they could replicate the known beneficial effects of these

769 drugs²⁶⁶. This study reported that combined, esomeprazole and metformin additively reduce
770 sFlt-1 secretion from primary trophoblast cells and placental explants, while additively
771 increasing mRNA expression of VEGF in primary trophoblast cells. Esomeprazole and
772 metformin also additively reduce mRNA, but not protein expression of VCAM-1 and ET-1 in
773 HUVECs treated with TNF- α . No direct vascular assessment has been made, nor has any *in*
774 *vivo* assessment been completed. However, this study raises a very important strategy, which
775 is the potential of two drugs as a successful treatment for preeclampsia.

776 777 **Conclusion**

778 The maternal syndrome that is preeclampsia is fundamentally a disease of the maternal
779 systemic vasculature. For women with preeclampsia, the treatment options remain limited,
780 with care primarily focused on controlling blood pressure. However, a better understanding
781 of the causes of the vascular dysfunction and the repurposing of drugs known to be safe in
782 pregnancy has opened up many new potential adjuvant therapies that offer great promise. We
783 suggest that correcting the maternal vascular dysfunction associated with preeclampsia will
784 be crucial to prolonging pregnancy and combating the maternal and fetal morbidity and
785 mortality associated with this disease. There are now numerous treatments in clinical trial
786 assessment that either directly act on the maternal vasculature or indirectly act on the
787 placenta to modify expression of vasoactive molecules. While these achievements prompt
788 great excitement in this space, it is very clear that more work is needed to fully elucidate the
789 vascular effects of these drugs in an attempt to better target the vascular dysfunction of
790 preeclampsia. More work on suitable animal models of preeclampsia can be used to first
791 assess potential vascular effects of drugs, focusing directly on key maternal vasculature
792 including the uterine, renal and mesenteric arteries. We suggest that moving forward, the next
793 phase in clinical trial design for preeclampsia should be to assess vascular therapies that
794 target multiple disrupted pathways of preeclampsia, rather than using a treatment that solely
795 focusses on one aspect that needs correcting in this heterogeneous disorder. Further, with the
796 recent safety concerns regarding sildenafil and the failure of esomeprazole to improve either
797 maternal or fetal outcomes, it would appear that therapies targeting oxidative stress pathways,
798 such as melatonin or sulforaphane, are the most promising therapies to be next assessed.

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- Figure legend:**
- Figure 1. The three key pathways currently targeted for clinical assessment as future therapies for women with preeclampsia. Endothelin-1 (ET-1), nitric oxide (NO), prostacyclin (PGI₂), placental growth factor (PlGF), soluble endoglin (sEng), reactive oxygen species (ROS), soluble fms-like tyrosine-1 (sFlt-1) and vascular endothelial growth factor (VEGF).

Current clinical trial targets for the future treatment of preeclampsia

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