Protein Pump Inhibitors Decrease Soluble fms-Like Tyrosine Kinase-1 and Soluble Endoglin Secretion, Decrease Hypertension, and Rescue Endothelial Dysfunction

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Abstract—Preeclampsia is a severe complication of pregnancy. Antiangiogenic factors soluble fms-like tyrosine kinase-1 (sFlt-1) and soluble endoglin are secreted in excess from the placenta, causing hypertension, endothelial dysfunction, and multiorgan injury. Oxidative stress and vascular inflammation exacerbate the endothelial injury. A drug that can block these pathophysiological steps would be an attractive treatment option. Proton pump inhibitors (PPIs) are safe in pregnancy where they are prescribed for gastric reflux. We performed functional studies on primary human tissues and animal models to examine the effects of PPIs on sFlt-1 and soluble endoglin secretion, vessel dilatation, blood pressure, and endothelial dysfunction. PPIs decreased sFlt-1 and soluble endoglin secretion from trophoblast, placental explants from preeclamptic pregnancies, and endothelial cells. They also mitigated tumor necrosis factor-α–induced endothelial dysfunction: PPIs blocked endothelial vascular cell adhesion molecule-1 expression, leukocyte adhesion to endothelium, and disruption of endothelial tube formation. PPIs decreased endothelin-1 secretion and enhanced endothelial cell migration. Interestingly, the PPI esomeprazole vasodilated maternal blood vessels from normal pregnancies and cases of preterm preeclampsia, but its vasodilatory effects were lost when the vessels were denuded of their endothelium. Esomeprazole decreased blood pressure in a transgenic mouse model where human sFlt-1 was overexpressed in placenta. PPIs upregulated endogenous antioxidant defenses and decreased cytokine secretion from placental tissue and endothelial cells. We have found that PPIs decrease sFlt-1 and soluble endoglin secretion and endothelial dysfunction, dilate blood vessels, decrease blood pressure, and have antioxidant and anti-inflammatory properties. They have therapeutic potential for preeclampsia and other diseases where endothelial dysfunction is involved. (Hypertension. 2017;69:457-468. DOI: 10.1161/HYPERTENSIONAHA.116.08408.)

Key Words: hypertension ■ preeclampsia ■ pregnancy ■ proton pump inhibitors ■ therapeutics

Preeclampsia affects 3% to 8% of pregnancies and is a leading cause of mortality during pregnancy. It is globally responsible for 60,000 deaths annually and far greater rates of fetal losses.1 Placental factors are released in excess into the maternal circulation, causing hypertension and endothelial dysfunction. This leads to multisystem organ injury to the maternal kidneys, liver, hematological system, and the brain (causing eclamptic seizures).1

The only treatment for preeclampsia is delivery of the placenta as this removes the source of placental-derived factors responsible for the maternal organ injury.1,2 In preterm preeclampsia, clinicians are often forced to deliver the fetus preterm to save the mother. However, this inflicts iatrogenic prematurity, which can lead to cerebral palsy, chronic disability, or death. A treatment that quenches the severity of the maternal disease could allow these pregnancies to safely advance to a gestation where neonatal outcomes are significantly improved. Severe preeclampsia at any gestation can progress rapidly over hours and become life-threatening. Therefore, a treatment that reduces the
severity of preeclampsia at any gestation could improve clinical outcomes.

Factors released in excess from the placenta in preeclampsia thought to play an important role in causing endothelial dysfunction (and subsequent maternal organ injury) are the antiangiogenic factors soluble fms-like tyrosine kinase-1 (sFlt-1)\(^1\)\(^-\)\(^4\) and soluble endoglin (sENG).\(^1\)\(^-\)\(^6\) sFlt-1 is a splice variant of vascular endothelial growth factor receptor 1 and comprises only the extracellular domain.\(^1\)\(^-\)\(^3\)\(^4\) sFlt-1 binds and sequesters circulating vascular endothelial growth factor, decreasing vascular endothelial growth factor signaling and its ability to promote vascular homeostasis.\(^5\) Furthermore, recent evidence suggests that sFlt-1 may induce hypertension by impairing endothelial nitric oxide synthase (eNOS) phosphorylation and increasing angiotensin II sensitivity.\(^7\) sENG shares sequence homology with the extracellular variant of vascular endothelial growth factor receptor 1 and is thought to play an important role in causing endothelial injury.\(^3\)\(^-\)\(^5\) Circulating levels of sFlt-1 and sENG are significantly elevated in women with preeclampsia and are associated with disease severity.\(^8\) Administering sFlt-1 to rats causes hypertension and proteinuria, hallmarks of preeclampsia.\(^3\) Adenoviral coadministration of both sFlt-1 and sENG to rats recapitulate the full spectrum of multorgan injury seen in preeclampsia, including severe proteinuria, thrombocytopenia, elevated liver enzymes, and fetal growth restriction.\(^9\) Preeclampsia is also associated with placental and systemic oxidative stress\(^2\)\(^-\)\(^10\) and intravascular inflammation,\(^2\) which are thought to exacerbate the endothelial injury.

Therefore, an attractive candidate therapeutic for preeclampsia may be a drug that is considered safe in pregnancy and can do the following: (1) decrease sFlt-1 and sENG secretion, (2) decrease blood pressure, (3) decrease endothelial dysfunction, (4) upregulate endogenous antioxidant defenses, and (5) decrease secretion of inflammatory cytokines. As far as we are aware, no such drug has been reported.

Proton pump inhibitors (PPIs) are widely prescribed during pregnancy for symptomatic gastric reflux. Large epidemiological studies have shown that they are safe in pregnancy, even when administered during the first trimester.\(^11\)\(^-\)\(^12\)

It was reported that heme-oxygenase-1 (HO-1) may negatively regulate sFlt-1 secretion.\(^13\) Noting that it has also been reported that the PPI lansoprazole upregulates HO-1 in gastric mucosa,\(^14\) we hypothesized that PPIs might upregulate HO-1, which should then decrease sFlt-1 and sENG secretion. In this study, we report functional experiments on primary human tissues and mouse models that show PPIs have diverse actions that may mitigate the pathophysiology underlying preeclampsia. Our data identify PPIs as candidate therapeutics for preeclampsia.

Methods

Detailed Methods are available in Methods in the online-only Data Supplement.

Methods Overview

We performed functional experiments where we administered proton pump inhibitors to primary human tissues or cells and assessed their effects on sFlt-1 and sENG secretion, endothelial dysfunction (using in vitro assays), whole vessel dilatation, and secretion of cytokines. To perform these experiments, we examined primary tissues from placenta (isolated cytotrophoblast cells or placental explant tissue from women diagnosed with preeclampsia), endothelial cells (human umbilical vein endothelial cells [HUVECs] and uterine microvascular cells), and whole vessels isolated from the omentum (maternal fatty apron tissue). All experiments were run with technical triplicates, and each experiment was repeated a minimum of 3× (using samples from different patients). We also performed in vivo experiments where we administered esomeprazole to a mouse model of preeclampsia (where sFlt-1 is overexpressed specifically in the placenta) and pregnant eNOS\(^-/-\) mice.

We obtained ethical approval to perform these studies. All women provided written informed consent.

Statistical Analysis

All in vitro experiments were performed with technical triplicates, and all experiments were repeated a minimum of 3×. Data were tested for normal distribution and statistically tested as appropriate. When ≥3 groups were compared a 1-way ANOVA (for parametric data) or Kruskal–Wallis test (for nonparametric data) followed by multiple comparison with Dunnet or Bonferroni ad hoc test where appropriate or post hoc analysis using either the Tukey (parametric) or Dunn test (nonparametric). When 2 groups were analyzed, either an unpaired t test (parametric) or a Mann–Whitney U test (nonparametric) was used. Data are expressed as mean fold change from control ± SEM.

Results

Proton Pump Inhibitors Decrease sFlt-1 Expression and Secretion From Placenta and Endothelium

In preeclampsia, excess secretion of sFlt-1 is thought to cause hypertension, endothelial dysfunction, and subsequent maternal organ injury. Administering PPIs (lansoprazole, rabeprazole, and esomeprazole) to primary trophoblast cells dose dependently reduced sFlt-1 secretion (Figure 1A, this ELISA detects all sFlt-1 variants) and reduced mRNA expression of the 2 major sFlt-1 variants present in placenta: sFlt-1 e15a and sFlt-1 i13 (Figure S1A and S1B in the online-only Data Supplement). They also reduced sFlt-1 secretion from placental explants obtained from normal (Figure S1C) and preeclamptic pregnancies (Figure 1B; see Table S1 for clinical information). PPIs also decreased sFlt-1 e15a protein secretion from preeclamptic placental explants (Figure 1C).

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Microvascular cells that, unlike HUVECs, are a mix of venous and arterial endothelium (Figure S1F). Placental hypoxia increases sFlt-1 secretion, and there is evidence that its release may be regulated by hypoxia inducible factor-1α.17,18 PPIs markedly decreased hypoxia inducible factor-1α protein expression (Figure 1F), raising the possibility that their ability to decrease sFlt-1 secretion may be mediated through hypoxia inducible factor-1α.
Proton Pump Inhibitors Decrease sENG Expression and Secretion From Placenta and Endothelium

sENG is the second major antiangiogenic factor released in excess from the placenta in preeclampsia and may play a role in severe disease.5-9 PPIs reduced sENG secretion from primary trophoblast (Figure 2A), HUVECs (Figure 2B), uterine microvascular cells (Figure S2A), and placental explants from cases of preterm preeclampsia (Figure 2C). When we compared the ability of 5 PPIs to reduce sENG secretion from HUVECs, we identified rabeprazole and esomeprazole were the most potent (Figure 2D).

Interestingly, PPIs also induced proangiogenic vegfa mRNA expression in primary trophoblast (Figure 2E) and HUVECs (Figure S2B), but did not upregulate its family member, placental growth factor (not shown).

At the same doses used in our PPI experiments (up to 100 μmol/L), pravastatin (proposed as a treatment for preeclampsia19,20) did not significantly affect sFlt-1 or sENG secretion.

Figure 2. Proton pump inhibitors (PPIs) decrease soluble endoglin (sENG) secretion from placent and endothelial tissues and increase vascular endothelial growth factor (VEGF) expression. Relative sENG levels in the media after PPI treatment for 24 h in (A) primary trophoblasts, (B) human umbilical vein endothelial cells, and (C) preeclamptic explants. D, IC₃₀ (concentration of drug required to decrease sENG secretion from baseline by 30%) were calculated for 5 PPIs, which demonstrates esomeprazole (Eso) and rabeprazole (Rab) are the most potent at inhibiting sENG secretion. E, VEGFA mRNA expression in primary trophoblast treated with PPIs for 24 h. sENG and VEGFA mRNA expression were measured in the same samples as the experiments shown in Figure 1. Lans indicates lansoprazole; Ome, omeprazole; and Pant, pantoprazole.
from HUVECs (Figure S2C and S2D). We conclude that PPIs reduce secretion of sFlt-1 and sENG from primary placental and endothelial cells and increase both placental and endothelial vega mRNA expression.

**Decrease in sFlt-1 and sENG Secretion Observed With Proton Pump Inhibitors Is Not Explained by Inhibition of V-ATPase**

We next set out to examine possible molecular pathways to explain how PPIs may be inhibiting sFlt-1 and sENG secretion. As a medication for gastric reflux, PPIs were specifically designed to inhibit H⁺/K⁺ ATPase V-ATPase (a proton pump in the gastric epithelium) to decrease gastric acidity. Of note, the V-ATPase enzyme is also present in placenta.21–23 To examine whether the reduction in sFlt-1 and sENG secretion observed with PPI treatment is mediated through V-ATPase inhibition, we examined the effects of blocking V-ATPase by administering bafilomycin, a potent V-ATPase inhibitor.24 Indeed, bafilomycin dose dependently decreased sFlt-1 secretion by human trophoblast (Figure S3A). However, bafilomycin did not decrease intracellular levels of sFlt-1 protein or the mRNA expression of the sFlt-1 variants (sFlt-1 i13 and sFlt-1 e15a; Figure S3B through S3D). In contrast, we confirmed esomeprazole (added as a positive control in the same experiment) significantly decreased sFlt-1 and mRNA expression of the 2 sFlt-1 variants. Furthermore, bafilomycin did not decrease sFlt-1 secretion from HUVECs, and in fact significantly increased sENG secretion from HUVECs (Figure S3E and S3F). Given blocking V-ATPase with bafilomycin did not decrease intracellular sFlt-1 protein or mRNA expression or consistently reduce sFlt-1 and sEng secretion, it is unlikely that PPIs decrease the production of these antiangiogenic factors through V-ATPase.

**Reduced sFlt-1 and sENG Secretion With Proton Pump Inhibitors Is Not Explained by Altered Expression of Cargo Export Proteins**

ARF1 (ADP-ribosylation factor 1) and RAB11 (Ras-related Protein 11) are proteins involved in the secretion and transport of proteins from cells. Given Jung et al25 previously concluded that these proteins facilitate sFlt-1 secretion from cells, we examined whether PPIs may decrease sFlt-1 secretion by inhibiting the expression of these proteins. Treating trophoblast with esomeprazole in fact increased ARF1 expression (Figure S4A and S4B) and did not affect RAB11 expression (Figure S4C and S4D). Thus, it seems unlikely that these molecules are involved in the decrease in sFlt-1 secretion induced by PPIs.

**Proton Pump Inhibitors Vasodilate Whole Human Vessels Ex Vivo and Decrease Blood Pressure in an Animal Model of Preeclampsia**

Vasoconstriction and hypertension are hallmarks of preeclampsia. We next examined whether PPIs have vasoactive properties. We isolated maternal omental arteries obtained at caesarean section from normal and preeclamptic women and performed pressure myography ex vivo. We first induced vasoconstriction by incubating arteries in U46619 (thromboxane agonist [thromboxane is elevated in preeclampsia]26) and then added cumulative doses of esomeprazole. Esomeprazole caused potent dilation of arteries from both preeclamptic and normal pregnancies (Figure 3A and 3B; see Table S2 for clinical characteristics of the preeclampsia cohort). The vasodilatory effects of esomeprazole seemed to be mediated through the endothelium because vessels denuded of the endothelium did not dilate in response to esomeprazole (Figure 3C). To obtain in vivo evidence that PPIs are vasoactive, we added esomeprazole to a transgenic preeclampsia mouse model where human sFlt-1 is overexpressed specifically in the placenta.27 As expected, a significant increase in blood pressure (both systolic and diastolic) was observed in untreated mice from E16.5.27 However, daily administration of 150 μg of esomeprazole (which approximately equates to a 30-mg dose in humans) from E8.5 completely abrogated this increase in blood pressure (Figure 3D). There was a nonsignificant trend toward decreased maternal proteinuria in mice treated with esomeprazole compared with controls, and there were no differences in measurements of various fetal or placental parameters (Figure S5A through S5E). There was also a nonsignificant trend toward a decrease in the blood pressure of nonpregnant female mice administered esomeprazole when compared with mice receiving vehicle (Figure S5F). We confirmed the presence of human sFlt-1 in the mouse serum obtained at the time of sacrifice (Figure S5G). The fact that there was no difference in sFlt-1 levels in this animal model given sFlt-1 is expected given it is autonomously expressed by the lentiviral plasmid.

**Proton Pump Inhibitors May Be Promoting Vasodilation by Modulating eNOS and Endothelin-1 Expression**

eNOS is an enzyme in the endothelium that produces NO, a potent vasodilator that signals to the underlying vascular smooth muscle. Furthermore, it has recently been shown that sFlt-1 may be directly inducing hypertension in preeclampsia by impairing eNOS phosphorylation.7 We examined whether PPIs may be inducing vasorelaxation via the eNOS pathway. When we treated HUVECs with esomeprazole (Figure 4A), there was a significant increase in phosphorylated eNOS (p-eNOS, the active form). Cotreatment of tumor necrosis factor-α (TNF-α; a proinflammatory cytokine that circulates in excess in preeclampsia28 and likely contributor to endothelial dysfunction29) with esomeprazole and rabeprazole at different doses all induced a nonsignificant increase in p-eNOS protein (Figure S6A and S6B). These data suggest the PPIs upregulate p-eNOS expression. To obtain further evidence for this, we next administered esomeprazole to pregnant mice lacking eNOS (eNOS−/−). These mice are chronically hypertensive, which persists while they are pregnant.30 In contrast to the striking decrease in blood pressure seen in wild-type mice where sFlt-1 was overexpressed in placenta (Figure 3D), there was only a modest and nonsignificant decrease in blood pressure with esomeprazole treatment when the eNOS gene is absent (Figure 4B). Our data suggest that esomeprazole has vasodilatory properties and can decrease blood pressure in vivo (animal model) and ex vivo (whole maternal vessels from women with preeclampsia). Furthermore, this may be mediated through the endothelium, where p-eNOS may play a role.
Endothelin-1 is potent vasoconstrictor released from the endothelium into the circulation and is increased in preeclampsia. \textsuperscript{31} Administering PPIs to HUVECs reduced endothelin-1 mRNA expression (Figure 4C) and protein secretion (Figure 4D). PPIs also decreased endothelin-1 mRNA expression in uterine microvascular endothelial cells (Figure S6C).
Proton Pump Inhibitors to Treat Preeclampsia

Proton Pump Inhibitors Rescue Endothelial Dysfunction In Vitro

Given the importance of endothelial dysfunction in preeclampsia, another therapeutic strategy may be to identify drugs that act at the level of the endothelium to reduce dysfunction. PPIs potently blocked TNF-α–induced upregulation of vascular cell adhesion molecule-1 in HUVECs (Figure 5A; Figure S7A) and primary uterine microvascular cells (Figure S7B). PPIs also decreased vascular cell adhesion molecule-1 expression in HUVECs when preeclamptic serum was used instead of TNF-α to induce endothelial dysfunction (Figure S7C).

Given vascular cell adhesion molecule-1 promotes leukocyte adhesion to the endothelium, we performed a monocyte adhesion assay. PPIs dose dependently decreased TNF-α–induced leukocyte adhesion to HUVECs (Figure 5B). We next performed endothelial tube-forming experiments and found that esomeprazole rescued TNF-α–induced tube disruption (Figure 5C). In addition, we examined endothelial cell migration using the xCELLigence assay, which allows cell migration to be continuously monitored in real time. Treating HUVECs with sFlt-1 reduced cell migration, but this was rescued with the addition of either esomeprazole or lansoprazole (Figure 5D). Therefore, we found that PPIs rescued endothelial dysfunction in multiple in vitro assays.

Proton Pump Inhibitors Upregulate Expression of Endogenous Antioxidant Proteins

Excessive oxidative stress and inflammation is likely to exacerbate the placental and endothelial dysfunction that
occurs in preeclampsia. Therefore, reducing oxidative stress and inflammation may help decrease disease severity. Administering PPIs to primary trophoblast cells induced nuclear translocation of the transcription factor, nuclear factor (erythroid-derived 2)-like 2 (Nrf2), to the nucleus (Figure 6A). One of the targets of Nrf2 is HO-1, which has antioxidant and cytoprotective actions. PPIs potently induced HO-1 expression in primary trophoblast (Figure 6B; Figure S8A), placental explants from women with preterm preeclampsia (Figure 6C), HUVECs (Figure 6D; Figure S8B), and uterine microvascular cells (Figure S8C). They also upregulate NAD(P)H (nicotinamide adenine dinucleotide phosphate-oxidase) dehydrogenase.
quinone 1 and thioredoxin in HUVECs and trophoblasts, both antioxidant molecules that are Nrf2 targets (Figure S8D and S8E). These data demonstrate that PPIs upregulate endogenous antioxidant molecules in placenta and endothelium.

Proton Pump Inhibitors Decrease Secretion of Cytokines From Placenta and Endothelium

Adding PPIs to placental explants from preterm preeclamptic pregnancies reduced secretion of cytokines, including interleukin-1β, interleukin-6, interleukin-10, and C-C motif chemokine ligand (CCL), CCL2 and CCL7 (Figure S9A). Furthermore, PPIs reduced secretion of cytokines from endothelial cells treated with either TNF-α or preeclamptic serum to induce endothelial dysfunction (Figure S9B and S9C). These data suggest that PPIs can decrease cytokine secretion from placenta and endothelium.

Discussion

In this study, we have identified PPIs as candidate treatments for preeclampsia. They appear to have diverse biological actions that could be useful to block important pathophysiological processes thought to occur in preeclampsia. PPIs decrease secretion of sFlt-1 and sENG from primary trophoblast, placental explants from normal and preeclamptic pregnancies, and from 2 types of primary endothelial cells. The PPI esomeprazole vasodilates human vessels from normal and preeclamptic pregnancies and reduces blood pressure in a transgenic mouse model where human sFlt-1 is overexpressed in placenta.

The ability of esomeprazole to vasodilate vessels ex vivo and reduce blood pressure in vivo may be mediated through the endothelium, perhaps by increasing p-eNOS expression. Indeed, for our preeclampsia animal model, we note that sFlt-1 is autonomously expressed in the placenta meaning it is unlikely PPIs can directly decrease placental sFlt-1 secretion in that experiment. Furthermore, PPIs seem to block endothelial dysfunction in many in vitro assays. Finally, PPIs upregulate endogenous antioxidant molecules and decrease secretion of cytokines from placental tissues and endothelial cells.

We believe that our study has several strengths. We performed a significant body of functional experiments on 7 types of primary human tissues where we administered many PPIs at various doses. Importantly, we included vessels and placenta from cases of preterm preeclampsia and biological replicates were performed on samples from different patients. Hence we believe that the functional evidence we have generated is strong.

Although we have generated evidence suggesting that PPIs may alter molecular signaling involved in vessel dilatation, we have not uncovered a unifying molecular mechanism to explain how PPIs have such diverse biological effects (ranging from vessel dilatation, decreasing sFlt-1 and sENG secretion...
to decreasing endothelial dysfunction). It is unclear whether PPIs are exerting these effects by acting on one, or multiple molecular targets.

We have explored relevant pathways to determine the molecular mechanism by which PPIs decreased sFlt-1 and sENG. Initially, we embarked on these studies with the hypothesis that PPIs may upregulate HO-1 and may reduce sFlt-1 and sENG secretion given it had previously been proposed that HO-1 was central to preeclampsia and that HO-1 regulates sFlt-1 and sENG production. However, we have since published data demonstrating that HO-1 is not decreased in preeclampsia and does not regulate placental sFlt-1 or sENG secretion. As such, we do not believe that the effects of sFlt-1 and sENG are mediated by the ability of PPIs to upregulate HO-1. Instead, we examined V-ATPase enzyme (given PPIs were designed to inhibit this proton pump) and the ARF1 and RAB11 cargo proteins (given a previous report suggesting they shuttle sFlt-1 out of cells). We were unable to show that they mediate the decrease in sFlt-1 and sENG secretion induced by PPIs. We have ongoing studies to try to determine the molecular mechanisms behind the actions of PPIs, specifically the decrease in antiangiogenic factors, given such a discovery might yield new insights into new drug targets.

Ghebremariam et al. reported that the PPI omeprazole reduced eNOS and p-eNOS (active eNOS) expression and reduced nitric oxide release from isolated veins. They presented a qualitative blot (without densitometric analysis) to suggest that omeprazole decreased eNOS protein expression, and experiments demonstrating omeprazole impaired vascular reactivity. Furthermore, they showed administrating lansoprazole to nonpregnant mice increased circulating asymmetrical dimethylarginine (antagonist of eNOS) although blood pressure was not reported. In contrast, we generated a body of data to show the opposite that PPIs upregulate p-eNOS and induce vasodilation both ex vivo and in vivo. We administered different doses of esomeprazole or rabeprazole with or without TNF-α stimulation, and in all cases, there was a significant increase or a trend toward an increase in p-eNOS protein (quantitative densitometric analysis). Importantly, we also found that esomeprazole potently vasodilated whole maternal blood vessels and reduced blood pressure in an animal model of preeclampsia. Finally, we observed a possible trend toward a decrease in blood pressure when we administered esomeprazole to nonpregnant mice, but there was certainly no evidence that the blood pressure increased. It is difficult to reconcile our findings with Ghebremariam et al. Despite these conflicting findings, we suggest that clinical studies to explore the potential of PPIs to treat preeclampsia are well justified, given our preclinical data suggest that PPIs seem to have other biological effects (besides vasorelaxation) that may also be beneficial in treating preeclampsia and there is strong epidemiological evidence to suggest PPIs are safe when administered during pregnancy (as discussed below).

We note that it is difficult to know how much drug is seen locally at target tissues. However, we would make some observations. First, the surface area of the target tissues (vessel endothelium and placenta) is vast and easily accessible to circulating drugs. Second, we would anticipate that some decrease in sFlt-1 and sENG may be sufficient to affect the disease progression given these factors are present in normal pregnancies. It may not be necessary to block their secretion entirely. Third, we treated our mouse model (where sFlt-1 was overexpressed in the placenta) with esomeprazole at a dose that equates to ≈30 mg daily dosing in humans (using a formula published by Reagan-Shaw et al.14), and this completely abrogated an increase in blood pressure. Finally, we performed multiple dose–response studies using multiple PPIs to show that the various biological responses are present at a range of doses. Importantly, the doses we used are in the micromolar range; these doses were chosen because the PPIs are found in the circulation at these concentrations,35–37 thus we anticipate that this will be the range that is clinically effective. It may be worthwhile undertaking further in vitro studies using drug doses within the nanomolar range as these might shed further clues as to the mechanisms by which PPIs are exerting the diverse biological effects we have observed.

Recent data have emerged combining microarray or ultrasound findings with histopathology defining further subtypes of preeclampsia, highlighting the heterogeneity of the disease. Leavy et al.9 found specific correlations with microarray data (from large cohorts of preeclamptic placentas) with clinical findings and propose subclasses of preeclampsia distinct to the canonical disease (which is driven by antiangiogenic factors). They identified a cluster of patients they coined immunologic preeclampsia where the main drivers of the disease may be immunologic.39 It is therefore possible that PPIs might be less effective for subtypes of preeclampsia where antiangiogenic factors play less of a role. As the field of preeclampsia subtyping matures, it may be feasible to stratify response to PPIs treatment in future clinical trials with preeclampsia subtyping.

There have been few orally available treatment options identified for preeclampsia that have reached clinical trials. In regards to treating preeclampsia, the field has perhaps been most focused on the potential use of pravastatin, a drug designed to inhibit cholesterol synthesis.19 There is preclinical evidence that pravastatin may have been useful to treat preeclampsia.38,39 A recent clinical trial of pravastatin to treat pre-term preeclampsia has ceased recruitment and the results have yet to be published (unique identifier: ISRCTN23410175). A pharmacokinetic study of 20 participants has just been reported (unique identifier: NCT01717586).41 However, a potential drawback in using pravastatin is that it has been assigned category X classification by the Food and Drug Administration. Although we agree that there are valid arguments to support the use of statins to treat pregnancies complicated by preterm preeclampsia, a drug that is thought to be safe in pregnancy will be more clinically acceptable.

In contrast to pravastatin, large epidemiological studies have demonstrated that PPIs are safe in pregnancy, where they are widely prescribed to relieve symptomatic gastric reflux in pregnancy. In 2010, Pasternak and Hviid examined the first trimester exposure, a time in pregnancy where there is significant organogenesis and the fetus is most vulnerable to teratogens. Examining 5082 pregnancies exposed to PPIs and 840968 that were not exposed, they concluded that the first trimester exposure to PPIs was not associated with an increased risk of major birth defects. A systematic review published in 2009 (1530 pregnancies exposed to PPIs and
133,410 nonexposed controls) did not identify an increase in congenital abnormalities. A subsequent large study examining 112,022 pregnancies (of which 11,86 were exposed to PPIs) confirmed no increase in congenital anomalies, fetal growth restriction, or adverse neonatal outcomes (including preterm birth or low Apgar scores). Thus, large cohort studies indicate that PPIs are safe during pregnancy. Given our preclinical data, and the reassuring epidemiological data suggesting that PPIs are safe, it would seem justifiable to proceed to clinical trials to examine whether PPIs could be used to either prevent preeclampsia or treat women diagnosed with preeclampsia. As such, we are currently recruiting women with preterm preeclampsia to a phase II randomized clinical trial, The PIE trial (Preeclampsia Intervention With Esomeprazole). We will examine whether esomeprazole can safely prolong gestation in women diagnosed with preterm preeclampsia and improve fetal, maternal, and neonatal outcomes.

Perspectives
We have shown in preclinical studies that PPIs potently decrease placental release of sFlt-1 and sENG, decrease endothelial dysfunction, are vasodilatory and have antioxidant and anti-inflammatory properties. They may represent a potential therapeutic option for preeclampsia and perhaps other diseases where there is significant endothelial dysfunction. If PPIs are found to be useful to treat or prevent preeclampsia, they may significantly reduce maternal and perinatal mortality globally.

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Disclosures
None.

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