



# High Dose Vardenafil Blunts the Hypertensive Effects of Toll-Like Receptor 3 Activation During Pregnancy

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### Specialty section:

This article was submitted to  
Translational Virology,  
a section of the journal  
Frontiers in Virology

**Received:** 20 September 2021

**Accepted:** 11 November 2021

**Published:** 08 December 2021

### Citation:

Balasubramanian D, Dharani S,  
Tauseef M, Khan MA, Rahman Z and  
Mitchell BM (2021) High Dose  
Vardenafil Blunts the Hypertensive  
Effects of Toll-Like Receptor 3  
Activation During Pregnancy.  
*Front. Virol.* 1:780298.  
doi: 10.3389/fviro.2021.780298

The maternal innate immune system plays a central role in preeclampsia (PE). Toll-like receptors (TLRs) are innate immune system receptors that recognize characteristics of extracellular endogenous ligands or pathogens, and their activation leads to a pro-inflammatory immune response. We and others have reported that excessive activation of TLRs causes pregnancy-dependent hypertension in animals and is associated with PE in women. Activation of TLR3 by poly I:C mimics the innate immune system activation by viruses that women who develop PE encounter during pregnancy. Vardenafil was approved by the FDA for erectile dysfunction but has recently been examined as a potential PE medication due to studies done with a similar drug, sildenafil. Preclinical as well as recent clinical studies demonstrate the potential effectiveness of sildenafil for PE. However, vardenafil is more potent than sildenafil and acts by increasing expression of placental growth factor in addition to increasing cGMP levels. We hypothesized that vardenafil will be more potent and effective in reducing the negative health effects in a mouse model of virus-induced PE. Pregnant mice were injected with the TLR3 agonist poly I:C (PPIC) on gestational days 13, 15, and 17. We treated PPIC mice with a high dose of vardenafil (50 mg human equivalent), a lower dose of vardenafil (20 mg human equivalent), or sildenafil (50 mg human equivalent) on gestational days 15–17 after hypertension was established. Daily i.p. injections of either high dose or low dose vardenafil significantly decreased systolic blood pressure in PPIC mice whereas sildenafil had no effect. There were no differences in body weight between the groups. The splenomegaly induced in PPIC mice was ameliorated in high dose vardenafil-treated PPIC mice, while low dose vardenafil-treated and sildenafil-treated PPIC mice still exhibited splenomegaly. High dose vardenafil-treated PPIC mice also did not exhibit any fetal demise characteristic of PPIC mice, while low dose vardenafil-treated and sildenafil-treated PPIC mice still had significantly increased incidences of fetal demise. These data support the notion that high dose vardenafil may be safe and effective at reducing blood pressure during a virus-associated hypertensive pregnancy.

**Keywords:** vardenafil, immunity, virus, pregnancy, hypertension

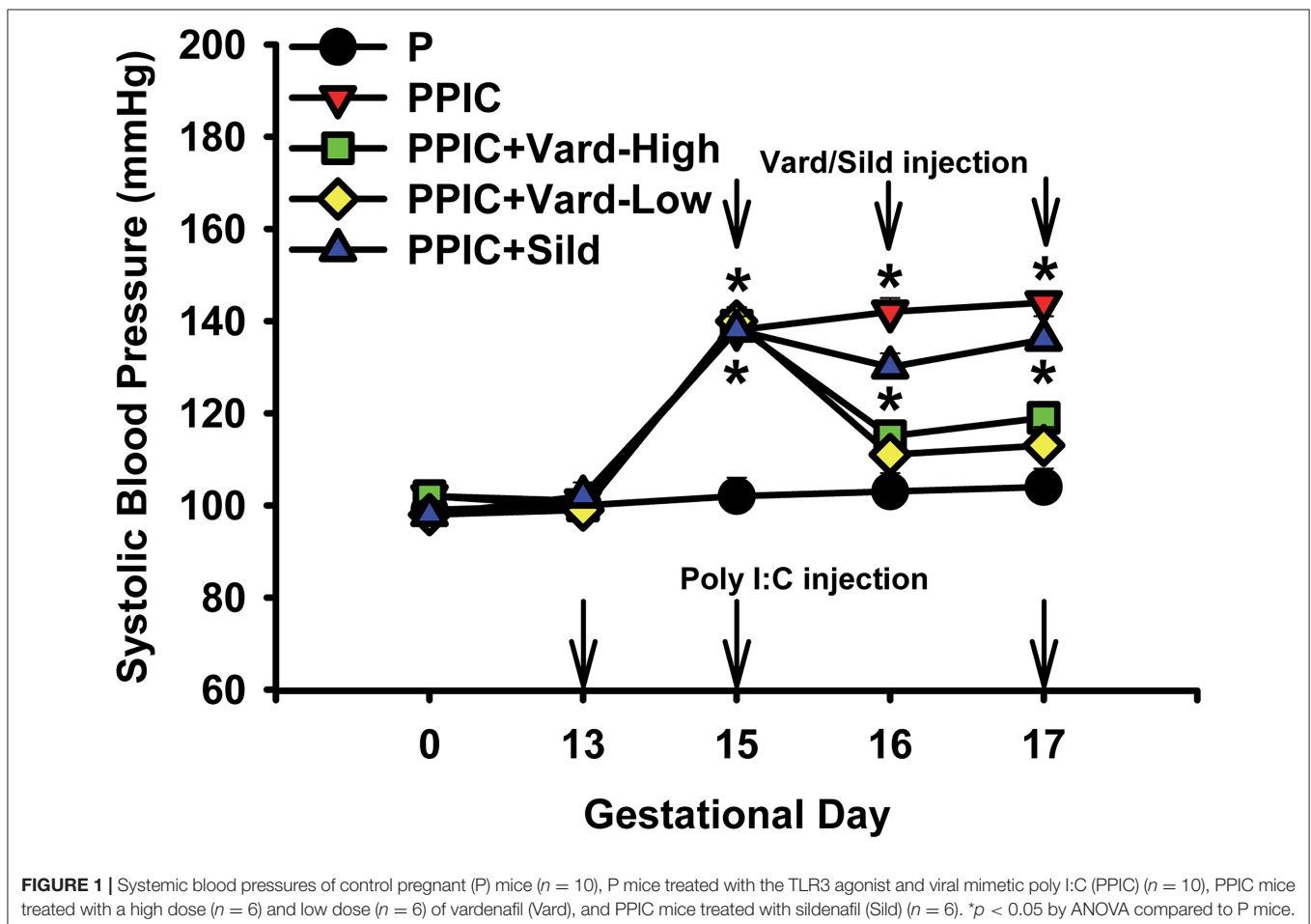
## INTRODUCTION

Hypertensive disorders of pregnancy, including preeclampsia (PE), are high-risk conditions diagnosed in the latter stage of pregnancies (1, 2). Due to the limited availability of effective therapeutic options, PE often leads to a high rate of fetal, neonatal, and maternal morbidity and mortality (2, 3). The underlying pathophysiology of PE is poorly understood and a continued search for novel and effective drugs to manage the condition is needed (4, 5). PE is multifactorial in its pathophysiology, but is widely associated with reduced placental perfusion, immune system activation, and systemic vascular endothelial dysfunction (6). The resultant placental ischemia induces the renin-angiotensin-aldosterone system (RAAS), inflammation, and oxidative stress that may propagate PE. With respect to immune system activation, we and others have reported viral activation of the maternal immune system during pregnancy is associated with PE in women and induces a PE-like syndrome in rodents (7–11). Given the recent rise in viral pandemics, therapies to aid in managing PE associated with a viral infection are needed.

In the clinic a variety of therapeutic agents have been used to acutely lower blood pressure in PE including calcium channel

blockers, methyldopa, diazoxide, hydralazine, prostacyclin, prazosin, and isosorbide (5). In recent years oral extended release nifedipine, oral labetalol, and methyldopa are the generally accepted first-line agents for non-severe hypertension in women with PE. Beta-blockers and diuretics are acceptable, while RAAS inhibitors remain contraindicated (5). However, these agents have their own side effect profile and have been shown to be relatively ineffective (12). Thus, the clinical management of PE is still a challenge and needs effective treatment options (13). One of the quickest ways to develop an effective drug for the treatment of PE is to identify new effects of the drugs that are already approved for other indications and have been found clinically safe (14).

Vardenafil is a phosphodiesterase 5 (PDE5) inhibitor that is closely related in function to sildenafil, a PDE5 inhibitor commonly used to treat male erectile dysfunction and pulmonary vascular diseases (15–17). During pregnancy, nitric oxide (NO) is synthesized in *in utero* placental tissues and endothelial cells and activates the intracellular second messenger cyclic guanosine monophosphate (cGMP). cGMP causes vasodilation and thus maintains low vascular resistance in the uterus and fetoplacental circulations (12, 18). However, cGMP is metabolized by PDE5 and leads to vasoconstriction and may contribute to the development and maintenance of PE. Thus,



inhibition of PDE5 may enhance the vasodilatory effect of NO by preventing cGMP degradation (12). It has been demonstrated that PE is, in part, related to decreased NO-mediated vasodilation of the uterine circulation (18). Earlier studies demonstrated that sildenafil treated PE in mouse models (19–21). However, in clinical studies, it was reported to be relatively ineffective as a treatment for PE, although it was shown that it could be used safely during pregnancy (22). Since vardenafil is a potent PDE5 inhibitor and available clinically as a safe and effective drug (17, 23, 24), we hypothesized that vardenafil will decrease blood pressure and improve fetal outcomes in a mouse model of PE induced by a viral mimetic.

## MATERIALS AND METHODS

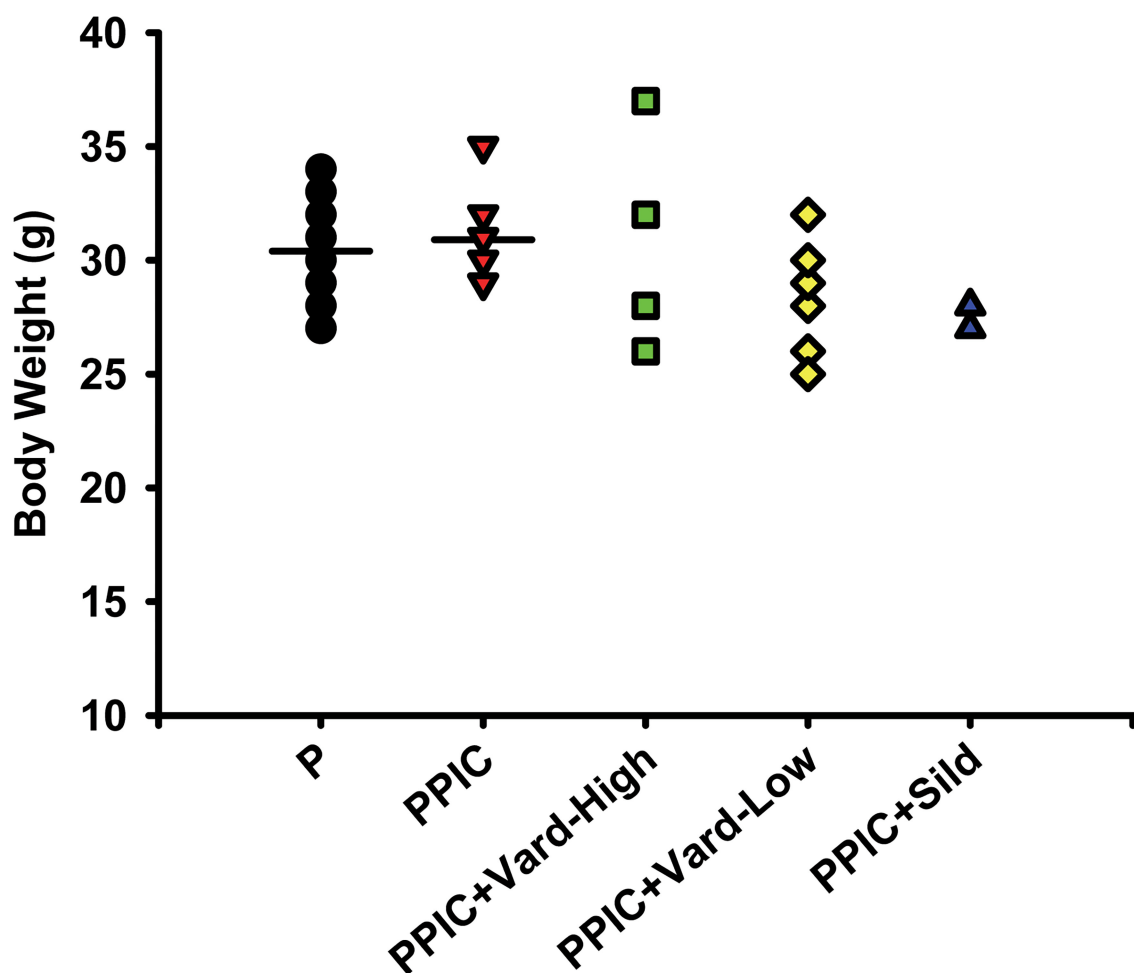
### Mice

Male and female C57BL/6J mice were purchased from Jackson Laboratories (Bar Harbor, ME). All animal use protocols were

approved by the Texas A&M University Institutional Animal Care and Use Committee and were performed in accordance with the NIH Guidelines for the Care and Use of Laboratory Animals.

### Viral Mimetic Induced Hypertension (PPIC), Treatments, and Measures

Pregnant (P) mice were injected i.p. with the TLR3 agonist and viral mimetic poly I:C 20 mg/kg body weight (PPIC) on gestational days 13, 15, and 17 as described previously (8–10, 25–30). These PPIC mice develop hypertension by day 14, which remains when poly I:C is injected every other day until parturition. We treated PPIC mice with a high dose of vardenafil (50 mg human equivalent), a low dose of vardenafil (20 mg human equivalent), or sildenafil (50 mg human equivalent) on gestational days 15–17 after hypertension was established. All PDE5 inhibitors were injected i.p. Mice had free access to normal chow (Teklad 8604, with NaCl content roughly 0.5%) and drinking water. Systolic blood pressure was determined using the tail-cuff method after acclimatization and training



**FIGURE 2** | Body weight measures of control pregnant (P) mice ( $n = 10$ ), P mice treated with the TLR3 agonist and viral mimetic poly I:C (PPIC) ( $n = 10$ ), PPIC mice treated with a high dose ( $n = 6$ ) and low dose ( $n = 6$ ) of vardenafil (Vard), and PPIC mice treated with sildenafil (Sild) ( $n = 6$ ).

as described previously (31). All mice were anesthetized and euthanized on gestational day 18 and body weight, spleen weight, and the number of fetuses and morphology were counted and noted. The P and PPIC groups had 10 mice in each, while the PPIC groups treated with vardenafil or sildenafil had six mice in each group.

## Statistics

A one-way ANOVA followed by a Student–Newman–Keuls *post-hoc* test was used to compare groups and the significance level was set at  $p < 0.05$  compared to P.

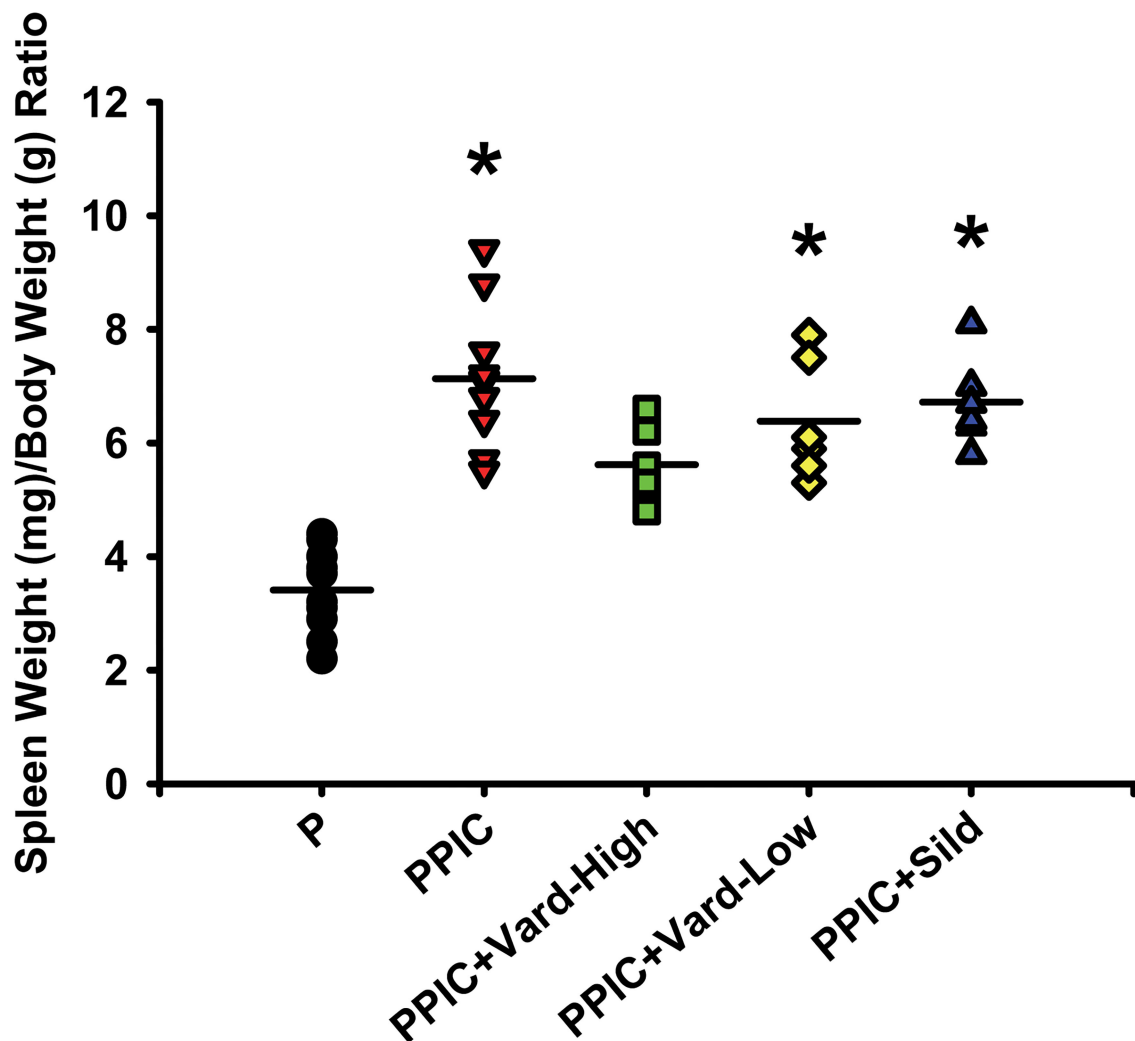
## RESULTS

Daily i.p. injections of either high dose ( $n = 6$ ) or low dose ( $n = 6$ ) vardenafil significantly decreased systemic blood pressure in PPIC ( $n = 10$ ) mice to P ( $n = 10$ ) levels, whereas

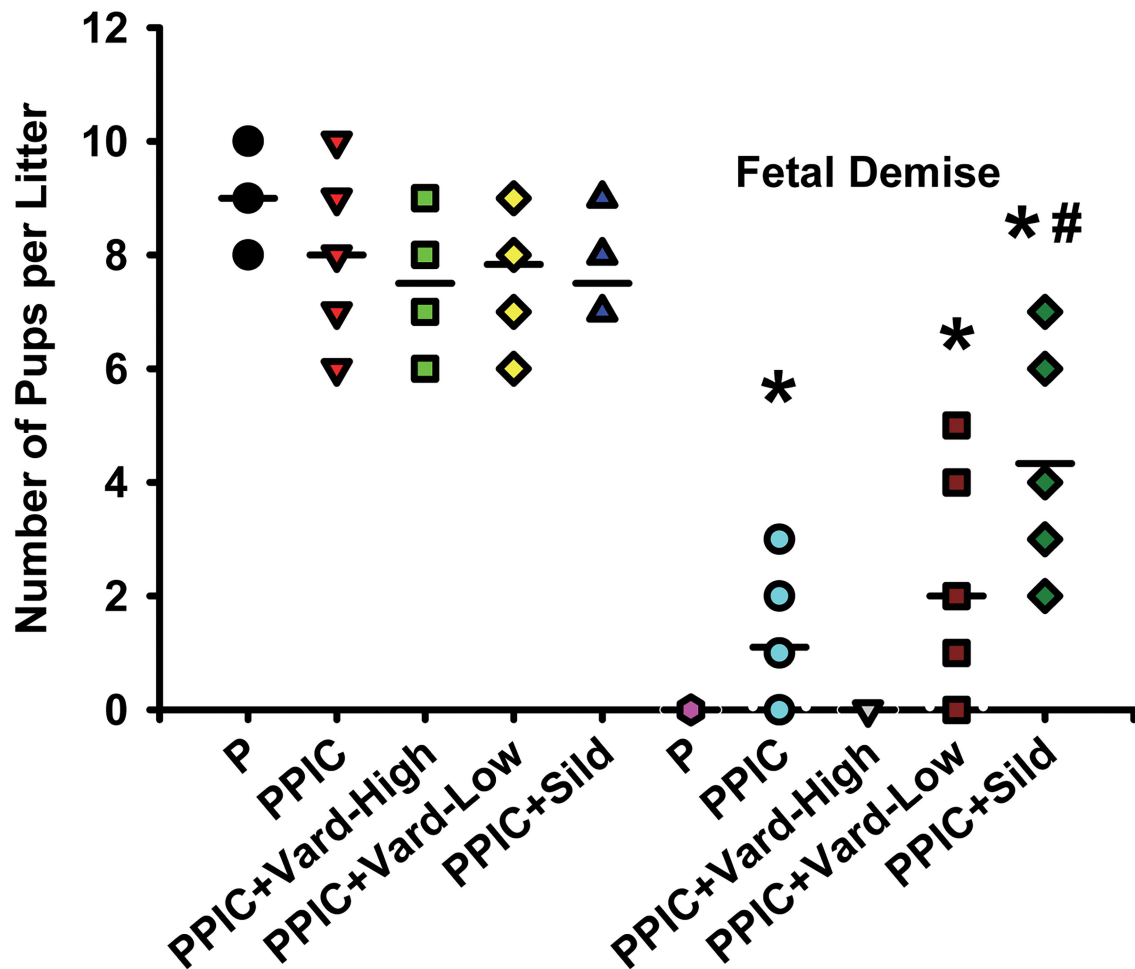
sildenafil ( $n = 6$ ) had no effect (Figure 1). There were no differences in body weight between the groups (Figure 2). The splenomegaly induced in PPIC mice was ameliorated in high dose vardenafil-treated PPIC mice, while low dose vardenafil-treated and sildenafil-treated PPIC mice still exhibited splenomegaly (Figure 3). High dose vardenafil-treated PPIC mice also did not exhibit any fetal demise characteristic of PPIC mice, while low dose vardenafil-treated and sildenafil-treated PPIC mice still had significantly increased incidences of fetal demise (Figure 4). The fetal demise incidence in sildenafil-treated PPIC mice was significantly increased compared to that of PPIC mice (Figure 4).

## DISCUSSION

In the current study, like our previous reports (7–11), we confirm that administration of a viral mimetic during pregnancy induces hypertension, immune system activation as measured



**FIGURE 3** | Spleen weight to body weight ratios of control pregnant (P) mice ( $n = 10$ ), P mice treated with the TLR3 agonist and viral mimetic poly I:C (PPIC) ( $n = 10$ ), PPIC mice treated with a high dose ( $n = 6$ ) and low dose ( $n = 6$ ) of vardenafil (Vard), and PPIC mice treated with sildenafil (Sild) ( $n = 6$ ). \* $p < 0.05$  by ANOVA compared to P mice.



**FIGURE 4** | Number of pups per litter as well as number of pups undergoing fetal demise per litter of control pregnant (P) mice ( $n = 10$ ), P mice treated with the TLR3 agonist and viral mimetic poly I:C (PPIC) ( $n = 10$ ), PPIC mice treated with a high dose ( $n = 6$ ) and low dose ( $n = 6$ ) of vardenafil (Vard), and PPIC mice treated with sildenafil (Sild) ( $n = 6$ ). \* $p < 0.05$  by ANOVA compared to P mice, # $p < 0.05$  by ANOVA compared to PPIC mice.

by splenomegaly, and fetal demise in mice. These results are consistent with PE in women of which there are known associations with various viruses, and these are associated with hypertension, immune system activation, and intrauterine growth restriction. We also report that treatment with a high dose of vardenafil, but not sildenafil, was able to attenuate these negative effects in pregnant mice.

A PubMed search of “vardenafil preeclampsia” only reveals 2 papers. In 2011, Karasu et al. (32) examined vardenafil and sildenafil effects on relaxation responses of human umbilical arteries taken from women with PE and healthy pregnant women. They reported that in all sets of experiments, including the absence and presence of various NO pathway inhibitors, vardenafil induced a maximal relaxation response while sildenafil did not. These results suggest vardenafil acts through NO/cGMP dependent and independent mechanisms. The other paper reported in 2015 by Kakigano and colleagues found that vardenafil markedly increased placental growth factor through

various endothelial cell screens and suggested that vardenafil may be useful to treat PE given its pro-angiogenic and vasodilator properties (1).

A number of previous reports demonstrate beneficial effects of the PDE5 inhibitors sildenafil and tadalafil in isolated vessels from women with PE and animal models of PE (32–35). Others have also reported beneficial blood pressure and/or fetal effects of sildenafil in pregnant hypertensive animals (21, 36–42). However, these promising pre-clinical data have not translated smoothly into clinical trials (19, 43). Earlier studies reported no beneficial effects of sildenafil treatment on pregnancy duration, isolated vessels, or fetal outcomes (22, 44, 45). The more recent STRIDER (Sildenafil TheRapy In Dismal prognosis Early-onset fetal growth Restriction) clinical trial not only reported no beneficial effect of sildenafil on pregnancy duration but that treatment increased the risk of neonatal pulmonary hypertension and mortality and was subsequently ended (46–48). While the negative effect of sildenafil on neonatal pulmonary pressure has not been directly

determined, it is possible that too much NO at certain time points of gestation may be harmful, similar to that found with high doses of antioxidants during pregnancy. Together, these would support the notion that a proper NO-ROS balance is needed for a healthy fetal outcome.

The significantly increased incidence of fetal demise in our sildenafil-treated PPIC mice supports these findings. Additionally, our results in sildenafil-treated PPIC mice showing no beneficial effects on fetal demise, blood pressure, or splenomegaly contrast those in other animal models of PE such as the COMT  $-/-$  mouse, Dahl salt-sensitive rat, L-NAME-treated mice, sFlt-1-treated mice, and others (19–21, 39, 42, 49). This may suggest that viral infection during pregnancy resulting in a PE-like syndrome does not affect the NO pathways that sildenafil targets, but rather that vardenafil improves both NO and placental growth factor and together these contribute to the beneficial effects.

Cellular calcium handling may also play a role in the detrimental effects of a viral infection during a hypertensive pregnancy, as well as the beneficial effects of vardenafil but not sildenafil. Viral infections have been reported to alter calcium dynamics (50). Vardenafil, but not sildenafil, was reported to block calcium channels resulting in vasorelaxation (51, 52). This mechanism may also contribute to the decreased blood pressure and improved fetal development.

Taken together, the beneficial maternal and fetal effects of vardenafil treatment during a virus-induced hypertensive pregnancy in mice may overcome the once promising effects of

sildenafil for treating PE that turned out to be somewhat harmful. In pregnancies complicated by viral infection, vardenafil therapy may be beneficial in decreasing blood pressure, dampening immune system activation, and improving fetal outcomes.

## DATA AVAILABILITY STATEMENT

The original contributions presented in the study are included in the article/supplementary material, further inquiries can be directed to the corresponding author/s.

## ETHICS STATEMENT

The animal study was reviewed and approved by Texas A&M University IACUC.

## AUTHOR CONTRIBUTIONS

MK, ZR, and BM: study concept, design, and obtained funding. DB and SD: acquisition, analysis, and interpretation of data. DB, MT, ZR, and BM: drafting of the manuscript. All authors carried out critical revision of the manuscript for important intellectual content, read, and approved the final manuscript.

## FUNDING

This study was provided by the Texas A&M College of Medicine and the Texas A&M Rangel College of Pharmacy.

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