Effects of sildenafil on maternal hemodynamics and fetal growth in normal rat pregnancy

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Sasser JM, Baylis C. Effects of sildenafil on maternal hemodynamics and fetal growth in normal rat pregnancy. Am J Physiol Regul Integr Comp Physiol 298: R433–R438, 2010. First published December 2, 2009; doi:10.1152/ajpregu.00198.2009.—It has been suggested that the phosphodiesterase-5 (PDE5) inhibitor sildenafil may be useful in the treatment of hypertension during pregnancy. However, we have reported a selective increase in renal inner medullary PDE5 that participates in the sodium retention of pregnancy. Therefore, the purpose of this study was to determine whether oral sildenafil treatment impairs maternal plasma volume expansion and/or fetal growth during rat pregnancy. Rats received sildenafil (10 mg·kg⁻¹·day⁻¹, 50 mg·kg⁻¹·day⁻¹, or 90 mg·kg⁻¹·day⁻¹) or vehicle on days 4–20 of pregnancy. On days 14–19, rats were housed in metabolic cages for collection of urine and measurement of food and water intake. Terminal hemodynamic and fetal measurements were taken on day 20. None of the sildenafil doses lowered blood pressure, and although all doses increased plasma cGMP concentrations, only the highest dose increased aortic and inner medullary cGMP content. Sildenafil had no effect on maternal weight gain; however, the highest dose decreased both plasma volume and renal sodium retention. The pup number and size were similar among the groups. Therefore, these studies suggest that low doses of systemic sildenafil may be safe during pregnancy in the rat, but higher doses may interfere with the physiological sodium retention and volume expansion of pregnancy.

The effects of systemic sildenafil on blood pressure and sodium retention during hypertension in human pregnancy remain to be examined.

APPROXIMATELY 5% OF ALL PREGNANCIES are affected by hypertensive disorders (including chronic hypertension and pregnancy-associated hypertension), and the incidence of these hypertensive disorders is increasing each year (11). Hypertension during pregnancy is a major cause of maternal and neonatal mortality and morbidity. Although maternal mortality has been greatly reduced in developed countries, hypertensive disorders of pregnancy still have a significant effect on the fetus and can result in intrauterine growth restriction (IUGR), preterm birth, low birth weight, or perinatal death (15). Some advancements have been made in the treatment of pregnancy-associated hypertension; however, delivery of the baby is still the only definitive treatment (17). Therefore, antihypertensive agents that are both effective and safe during pregnancy should be found.

It has been hypothesized that the phosphodiesterase-5 (PDE5) inhibitor sildenafil may be useful in the treatment of hypertension during pregnancy and IUGR (6, 20). Sildenafil improves endothelial function of myometrial vessels ex vivo from women whose pregnancies are complicated by IUGR (21) and induces vasodilation of chorionic plate arteries from women with normal pregnancies (10). In the pregnant rat, sildenafil treatment improved endothelial function in the suramin rat preeclampsia model (19), but sildenafil worsened fetal outcome in an ovine model of IUGR (12). Additionally, PDE5 inhibition has also been proposed as therapy for the treatment of pulmonary hypertension during pregnancy due to its vasodilatory effects in the pulmonary circulation (10). However, data on the safety of sildenafil in women and pregnant women, in particular, are scant, although a recent clinical trial suggests that sildenafil is well tolerated with no maternal or fetal morbidity in women with preeclampsia (16).

Studies from our laboratory indicate that increased renal inner medullary PDE5 is involved in the sodium retention of pregnancy (9, 13). Sodium retention and volume expansion are required to meet the demands of optimal fetal development. Therefore, the purpose of this study was to determine the effects of oral sildenafil treatment on maternal plasma volume, sodium balance, and fetal growth during rat pregnancy.

METHODS

All experiments were performed using 39 female Sprague-Dawley rats (3–5 mo old; Harlan Laboratories, Indianapolis, IN) in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals and approved and monitored by the University of Florida Institutional Animal Care and Use Committee.

Females chosen to become pregnant were housed with males. Daily vaginal smears were taken, and the presence of sperm was taken as day 1 of pregnancy. Pregnancy was confirmed by the presence of fetuses in utero at the time of acute study. On day 4 of pregnancy, the rats were randomly divided into one of four groups: Control (CON), gel diet and sildenafil in gel diet at 10 mg·kg⁻¹·day⁻¹ (SILD10), sildenafil at 50 mg·kg⁻¹·day⁻¹ (SILD50), and sildenafil at 90 mg·kg⁻¹·day⁻¹ (SILD90). The gel diet contained all of the required nutrients and was made by dissolving 242 g of powdered Custom AIN 76C low-nitrate diet (MP Biomedicals, Solon, OH) and 6 g of agar in 275 ml of water. All rats received water ad libitum in addition to the water in the gel diet.

On day 14, rats were placed into Nalgene metabolic cages for 5 days and nights. Urine was collected daily, weighed to determine volume, and pooled and frozen, and food and water intake were measured throughout the 5-day period. On day 19 of pregnancy, the rat was returned to a conventional cage. On day 20, each rat was weighed, and an acute experiment was performed to assess blood pressure, hematocrit, and plasma volume. Rats were anesthetized with isoflurane, shaved, and placed on a heated table to maintain body temperature at 37 ± 1°C. The left femoral artery and vein were cannulated with polyethylene (PE)-50 tubing filled with heparinized saline. Arterial blood pressure was measured, and an arterial blood sample was collected for hematocrit and a “blank” for Evans blue measurement. Then, exactly 250 μl of Evans blue solution (0.3 mg/ml) was injected into the venous line. After 5 and 10 min, blood samples were collected for Evans blue measurement. Plasma samples were taken at the end of the experiment, and the left kidney was weighed. The kidneys, mesenteric arterial bed, and aorta were snap
frozen in liquid nitrogen. The number of pups was counted, and the average pup weight and length were measured.

A separate group of control rats and SILD90 rats were implanted with telemetry transmitters (Data Sciences, St. Paul, MN) for the measurement of blood pressure in conscious, freely moving rats. Rats were anesthetized with isoflurane (IsoFlo; Abbott Laboratories, North Chicago, IL), and telemetry transmitters were implanted in the left femoral artery with the transmitter housed in the abdomen and attached to the abdominal wall. Rats were allowed to recover from surgery and returned to individual housing for 7 to 10 days before initiation of data acquisition. Arterial pressure waveforms were recorded continuously for 5 min every 30 min. Baseline recordings were made for 4 days, and then rats were mated and randomly assigned to receive the control or SILD90 diet. Recordings were then made on days 3–5, 10–12, and 17–19 of pregnancy.

Plasma osmolarity was measured using a VAPRO Vapor Pressure Osmometer (Wescor, Logan, UT). The concentration of Evans blue in the plasma was measured on a Tecan Safire optical system (Tecan, MA) at 620 nm, and the plasma volume was calculated from the quantity of dye injected: concentration of dye in plasma. Sodium and potassium concentrations were measured on a flame photometer using cesium as the internal standard (1:100 dilution of sample in 1.5 mmol/l CsCl solution; Instrumentation Laboratory, Bedford, MA).

For creatinine measurements, plasma and urine were prepared using the method of Tsikas et al. (18) with a few modifications. Plasma (80 μl or greater) was precipitated in acetonitrile at 4 times its volume and then centrifuged at 15,000 g for 15 min and dried under nitrogen at 45°C. The dried sample was dissolved in glass-distilled water at half of its original sample volume and then centrifuged for 10 min at 15,000 g. Pooled urine samples from days 14–19 of pregnancy were diluted 1:200. Creatinine was measured by HPLC using the chromatographic method of George et al. (7). Creatinine was eluted on a 3.9 × 150 mm Waters AccQ-Tag C18 column in a 20 mM potassium dihydrogen phosphate (pH 7.4) isocratic mobile phase, followed by a 60/40 buffer/acetoniitrile 12-min column wash out and then a 5-min reequilibration in 100% Buffer (20 mM potassium dihydrogen phosphate buffer, pH 7.4). Creatinine was then measured with a Perkin Elmer series 200 HPLC with series 200 UV detector.

Plasma, aortic, mesenteric arterial, and renal inner medullary cGMP concentrations were measured by EIA (Cayman Chemical, Ann Arbor, MI), and tissue levels were normalized to total protein concentration as determined by Bradford assay (Bio-Rad Laboratories, Hercules, CA) using BSA as the standard.

Results are presented as mean ± SE. Data were analyzed by ANOVA with Newman-Keuls post hoc analysis or repeated-measures ANOVA (telemetric blood pressure data) using Prism 4 software (Graph Pad Software, San Diego, CA). Normality was confirmed with the D’Agostino and Pearson omnibus normality test. P < 0.05 was considered statistically significant.

RESULTS

As shown in Fig. 1, all three doses of sildenafil increased plasma concentrations of cGMP; however, only the highest dose, 90 mg·kg⁻¹·day⁻¹, increased aortic and renal inner medullary cGMP concentrations. The treatments did not alter cGMP content in the mesenteric arterial bed, although there was a trend for an increase in arteries from the SILD90 group. This suggests that only the highest dose was effective in inhibiting the action of PDE5 in the tissue.

As shown in Fig. 2, sildenafil treatment had no effect on MAP in anesthetized rats on day 20 of pregnancy. Mean arterial pressure measured by telemetry in separate groups of conscious rats treated with vehicle or SILD90 was also determined (Fig. 3). There was a small fall in pressure upon beginning treatment with sildenafil early in pregnancy that occurred prior to when blood pressure fell in the vehicle-treated group. By midterm, the normal gestational fall in blood pressure was seen in vehicle-treated rats, and there were no differences in blood pressure between groups during mid or late pregnancy. Sildenafil treatment had no effect at any dose on hematocrit or plasma osmolarity in normal pregnant rats (Fig. 2). Plasma volume was significantly reduced by treatment with 90 mg·kg⁻¹·day⁻¹ of sildenafil. Fig. 4 illustrates that significant sodium retention occurred over days 14–19 in control pregnancy (at 0.91 ± 0.03 meq/day) and that this was slightly attenuated with 10 mg·kg⁻¹·day⁻¹ sildenafil treatment and more markedly reduced with the 90 mg·kg⁻¹·day⁻¹ dose. Food intake (and thus sodium intake) was unaffected by

Fig. 1. Effect of sildenafil treatment (10 mg/kg/day, 50 mg/kg/day, or 90 mg/kg/day) on plasma, aortic, renal inner medullary, and mesenteric arterial concentrations of cGMP. *P < 0.05 vs. control; n = 5–10.
sildenafil treatment; therefore, the reduction in sodium retention must reflect increased renal sodium excretion. Water intake, creatinine clearance, and urine volume were all increased by the highest dose of sildenafil. Sildenafil treatment had no effect on the number of pups per pregnancy or on the weight or length of the pups (Table 1). No fetal abnormalities were observed.

DISCUSSION

The main finding of this study is that three doses of sildenafil increased circulating cGMP levels when given to normal pregnant rats on days 4–20 of pregnancy with only an early small drop in blood pressure during pregnancy with SILD90 but by middle and late pregnancy, blood pressure was similar to vehicle-treated pregnant rats. The two lower doses (10 and 50 mg·kg\(^{-1}\)·day\(^{-1}\)) had no effect on tissue levels of cGMP and did not alter plasma volume expansion. However, the highest dose (90 mg·kg\(^{-1}\)·day\(^{-1}\)) increased aortic and renal cGMP levels substantially. This highest dose also attenuated the sodium retention and plasma volume expansion of pregnancy. None of the treatment regimens had an effect on the number, weight, or length of the pups.

Hypertension occurs in about one of every 20 pregnancies (11) and can cause major complications for the mother and baby. Medical advances have improved maternal outcomes; however, hypertension during pregnancy often results in intrauterine growth restriction, preterm birth, low birth weight, or perinatal death (15). Despite these serious consequences, there is still no good option for managing blood pressure during pregnancy that is effective and safe for the developing fetus.

Recently, PDE5 inhibitors, such as sildenafil, have been investigated for roles beyond their effects on erectile function. These agents inhibit the breakdown of cGMP and thereby preserve vasodilatory nitric oxide-cGMP signaling pathways (2, 5). Although PDE5 was originally a target for cardiovascular drug development, initial studies showed little cardiovascular impact, and the focus shifted to erectile dysfunction. However, now PDE5 inhibitors are also approved for the treatment of primary pulmonary hypertension (8), and there is increasing interest in the potential use of these agents in cardiovascular diseases.

In 2004, Downing et al. (6) published the hypothesis that selective PDE5 inhibition may be an option for the treatment of preeclampsia. Sildenafil improves endothelial function of myometrial vessels ex vivo from women whose pregnancies are complicated by IUGR (21) and produces a cGMP-dependent vasodilation in second-order chorionic plate arteries obtained from patients after normal pregnancy (10). In a rat model of preeclampsia, sildenafil treatment decreased blood pressure
In contrast, our current study does not show a sustained effect of sildenafil on blood pressure, although these rats were not hypertensive. We did not see any change in fetal number or development, while Turgut et al. (18) showed increased fetal growth and improved aortic endothelial function in rats treated with suramin. Another group has reported that administration of sildenafil to normal pregnant rats results in a decrease in fetal size, but, in this same study, when pregnant rats were exposed to hypoxia, sildenafil increased fetal size (14). A study by Miller et al. (11), used a model of single umbilical artery ligation to induce placental insufficiency in sheep. In these sheep, administration of sildenafil reduced uteroplacental perfusion and fetal body weight compared with controls, possibly due to systemic vasodilation, highlighting the possibility of deleterious effects if sildenafil is used during pregnancy. Recently, a small clinical trial (35 patients) was conducted to assess the efficacy of sildenafil in prolonging pregnancy in women with preeclampsia. Sildenafil was found to have no effect on maternal or fetal morbidity or mortality in this study; however, sildenafil was not effective in delaying delivery in these women (16).

Our rationale for performing the current study is that we have previously shown that there is a selective increase in PDE5, an enzyme that specifically degrades cGMP, in the inner medulla of pregnant rats (13). In inner medullary collecting duct cells isolated from pregnant rats, cGMP accumulation in response to atrial natriuretic peptide is reduced, and cGMP hydrolysis is increased compared with cells from virgin controls, and this is normalized by inhibition of PDE5. Furthermore, PDE5 inhibition in vivo reversed the blunted natriuresis to an infusion of ANP in pregnant rats (9). Because these studies suggest that PDE5 may be essential to volume expansion.

Table 1. Effect of sildenafil treatment during pregnancy on fetal parameters

<table>
<thead>
<tr>
<th></th>
<th>Number of Pups</th>
<th>Weight of Pups, g</th>
<th>Length of Pups, mm</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>13 ± 0.4</td>
<td>2.5 ± 0.1</td>
<td>33 ± 0.5</td>
</tr>
<tr>
<td>SILD10</td>
<td>13 ± 1.4</td>
<td>2.7 ± 0.3</td>
<td>34 ± 1.5</td>
</tr>
<tr>
<td>SILD50</td>
<td>13 ± 0.2</td>
<td>2.5 ± 0.1</td>
<td>33 ± 0.4</td>
</tr>
<tr>
<td>SILD90</td>
<td>14 ± 0.6</td>
<td>2.5 ± 0.1</td>
<td>34 ± 0.6</td>
</tr>
</tbody>
</table>

Data are presented as means ± SE; n = 5–10. SILD10, 10 mg·kg⁻¹·day⁻¹; SILD50, 50 mg·kg⁻¹·day⁻¹; or SILD90, 90 mg·kg⁻¹·day⁻¹. Weights and lengths are representative of 5 pups per dam.
sion and maintenance of a healthy pregnancy, it was important to directly examine the effects of sildenafil on plasma volume and maternal hemodynamics during pregnancy. The initial preclinical safety study for sildenafil done by Pfizer, showed “slight or minimal maternal toxicity” at doses up to 200 mg/kg. This study also showed that treatment of female rats with 60 mg·kg⁻¹·day⁻¹ of sildenafil from day 6 of pregnancy to the end of lactation “manifested as a decrease in the number of viable pups and lower pup body weight at birth” (1).

In the present study, we found that plasma volume expansion was not compromised by either 10 or 50 mg·kg⁻¹·day⁻¹ of sildenafil. These two doses did result in increased circulating cGMP levels, although neither lowered blood pressure, and they were not sufficient to increase tissue (aortic and renal inner medullary) levels of cGMP. Since cGMP functions exclusively as an intracellular 2nd messenger, this explains the lack of systemic hemodynamic actions of these lower doses of sildenafil. Treatment with 90 mg·kg⁻¹·day⁻¹ of sildenafil did significantly elevate cGMP levels in both the aorta and the renal inner medulla, indicating a local effect on PDE5 activity. This treatment sufficiently attenuated sodium retention (by increasing urine sodium excretion) to inhibit the plasma volume expansion. This finding supports our earlier studies, suggesting that increased inner medullary PDE5 in pregnancy mediates the regulation of sodium retention and consequent volume expansion.

We also found that the highest sildenafil dose led to a marked rise in glomerular filtration rate. This probably reflects a sildenafil-mediated renal vasodilation due to cGMP accumulation in the renal circulation. The highest dose also resulted in increased water intake. This is likely secondary to increased water excretion due to stimulation of sodium excretion by increased cGMP accumulation in the renal inner medulla in rats treated with the high dose of sildenafil.

The conclusions of this study and others using animal models are limited in that findings in animal models do not always translate to human patients. For example, there are differences observed in nitric oxide metabolite measurements between rats and humans during pregnancy. Conrad et al. (3) showed an increase in both cGMP and NO₂ in both plasma and urine and increased NO-hemoglobin during rat pregnancy; however, although CGMP increased in human pregnancy, NO₂ levels did not rise and NO-hemoglobin adducts were absent in pregnant women (4). Therefore, our findings that sildenafil does not induce fetal changes and that high doses of sildenafil interfere with plasma volume expansion in rats should be interpreted with caution. Definitive human trials are needed before clinical safety and efficacy of PDE5 inhibitors can be determined.

Despite these changes in maternal sodium handling, fetal development was unaffected. Therefore, at least at the doses used here, sildenafil has no obvious damaging effects on the fetus during rat pregnancy. Even with the 90 mg·kg⁻¹·day⁻¹ dose of sildenafil, there was still significant plasma volume expansion that presumably was adequate to maintain fetoplacental perfusion without compromise of fetal development in normal rat pregnancy.

Perspectives and Significance

Our studies found no adverse fetal effects when a PDE5 inhibitor was administered during rat pregnancy and therefore provide no indication that these agents should not be tested in human pregnancy if used at lower doses that do not interfere with renal inner medullary PDE5 activity. Whether these drugs are an option for the treatment of hypertension during pregnancy remains unknown since it may be impossible to establish a dose that is antihypertensive without also inhibiting renal sodium retention and plasma volume expansion. Because of the possible effects on sodium handling by the maternal kidney, plasma volume and sodium balance should always be assessed when evaluating the safety and efficacy of these agents in the treatment of hypertensive disorders of pregnancy.

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GRANTS

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DISCLOSURES

No conflicts of interest are declared by the authors.

REFERENCES