Cytochrome P-450 Inhibition Attenuates Hypertension Induced by Reductions in Uterine Perfusion Pressure in Pregnant Rats

Maria T. Llinás, Barbara T. Alexander, Maria F. Capparelli, Mairead A. Carroll, Joey P. Granger

Abstract—The present study tested the hypothesis that cytochrome P-450 (CYP) metabolites of arachidonic acid (AA) are involved in mediating hypertension and renal vasoconstriction during chronic reductions in uterine perfusion pressure (RUPP) in pregnant rats. 1-aminobenzotriazole (ABT), a CYP enzyme inhibitor (25 mg/kg per day), or vehicle (saline 0.9%) was administered for 7 days to normal pregnant (NP) rats and to pregnant rats with chronic RUPP. RUPP rats infused with vehicle showed significantly (P<0.01) higher mean arterial pressure (MAP) (130±2 versus 106±1 mm Hg), renal vascular resistance (RVR) (22.6±1.8 versus 16.3±1.1 mm Hg/mL per minute) and lower (P<0.05) glomerular filtration rate (GFR) (1.6±0.1 versus 2.3±0.1 mL/min) than NP rats. ABT decreased (P<0.01) MAP in RUPP rats (111±1 mm Hg), whereas it had no effect in NP rats (108±2 mm Hg). CYP inhibition also attenuated the differences in renal hemodynamics observed between NP and RUPP rats. After treatment with ABT, RVR and GFR were similar in RUPP rats (19.3±1.5 mm Hg/mL per minute and 2.0±0.2 mL/min, respectively) and NP rats (16.3±2.4 mm Hg/mL per minute and 2.4±0.2 mL/min). The effects of CYP enzymes inhibitor in RUPP rats were associated with a reduction (P<0.05) of 20-HETE formation (32%) and a decreased (P<0.05) expression (33%) of CYP4A protein in renal cortex. In contrast, renal epoxygenase activity did not change in these animals. These results suggest that 20-HETE contributes to hypertension and renal vasoconstriction induced by chronic RUPP in pregnant rats.

Key Words: preeclampsia ■ CYP-450 ■ hypertension

Preeclampsia is a specific syndrome of pregnancy that is generally characterized by hypertension, proteinuria, and edema. Although the cause of preeclampsia remains undefined, a reduced uteroplacental perfusion has been postulated to be the initial event in this disorder.1–3 Numerous studies in pregnant women and experimental animal models have suggested that placental ischemia may produce the release of placental factors that alter endothelial and vascular smooth muscle cell function, leading to widespread vasoconstriction, renal hypoperfusion, and elevated arterial pressure.4

Renal cytochrome P450 (CYP)-derived metabolites have been implicated in the genesis of hypertension in different genetic and experimental animal models.5–6 20-HETE is a potent vasoconstrictor of renal arteries,7 and it has been reported to be an essential component of renal autoregulation and tubuloglomerular feedback.8,9 In contrast, epoxyeicosatrienoic acids (EETs) are generally considered vasodilators in renal vasculature.10–12 20-HETE and EETs may also contribute to the control of salt and water balance by inhibiting sodium reabsorption in different segments of the nephron.13,14 These results indicate that abnormalities in the production of these metabolites may result in renal vasoconstriction and sodium retention, leading to the development of hypertension.

A recent study in rats has shown that renal synthesis of 20-HETE and expression of CYP4A isoforms, which preferentially catalyze its formation in rat kidney, are altered during gestation.15 That study indicates that this vasoconstrictor may be implicated in the control of renal function and blood pressure during pregnancy. However, a significant increase in urinary excretion of EETs and their respective diols, dehydroxyeicosatrienoic acids (DHETs), have been found in women with pregnancy-induced hypertension, as compared with healthy pregnant women.16 These findings, together with the demonstrated importance of these metabolites in regulating renal function, support the hypothesis that CYP-derived eicosanoids might contribute to the increased renal vascular tone and elevated blood pressure associated with PE.

Previous studies in our laboratory have reported that chronic reductions in uterine perfusion pressure (RUPP) in pregnant rats lead to a hypertensive state that closely resembles preeclampsia in women. In addition to hypertension,
these animals show endothelial dysfunction, reduced renal plasma flow and glomerular filtration rate (GFR), proteinuria, and intrauterine growth restriction.17–19 The importance of CYP metabolites in mediating the renal and cardiovascular abnormalities in the RUPP model is unknown. Therefore, the goal of this study was to determine whether CYP metabolites of AA contribute to hypertension and renal vasoconstriction induced by chronic RUPP in pregnant rats. To achieve this goal, the effects of a CYP enzyme inhibitor, 1-aminobenzotriazol (ABT), on blood pressure and renal hemodynamics were studied in normal pregnant (NP) rats and in pregnant rats with chronic RUPP.

Methods

All studies were performed in 250- to 300-g female Sprague-Dawley rats purchased from Harlan Sprague Dawley (Indianapolis, Ind). Female rats were placed with a fertile male, and day 1 of pregnancy was determined by the presence of sperm in the vaginal smear. All experimental procedures in this study were executed in accordance with the Guide for the Care and Use of Laboratory Animals (NIH publication 93-23, revised 1985), and the Animal Care and Use Committee at the University of Mississippi Medical Center approved all protocols.

Chronic RUPP in Pregnant Rats

Chronic reductions in uteroplacental perfusion in rats were achieved by a method previously reported by our laboratory.17–20 In brief, all rats undergoing surgical procedures were anesthetized with 2% isoflurane. Pregnant rats entering the RUPP group underwent the following clipping procedure at day 14 of gestation. After a midline incision was made, the lower abdominal aorta was isolated and a silver clip (0.230-mm internal diameter) was placed around the aorta above the iliac bifurcation. Because compensation of blood flow to the placenta occurs in pregnant rats through an adaptive increase in ovarian blood flow, branches of the right and left ovarian arteries were also clipped using a silver clip (0.100-mm internal diameter). Sham operations were performed in control rats.

Measurement of Arterial Pressure and Renal Hemodynamics in Conscious Rats

To determine the role of CYP metabolites in mediating hypertension and renal vasoconstriction in pregnant rats with chronic RUPP, the effects of systemic administration of a CYP enzyme inhibitor, ABT, on mean arterial pressure (MAP) and renal hemodynamics were compared in NP and in pregnant rats with chronic RUPP. ABT was infused at a rate of 25 mg/kg per day for 7 days. ABT is a mechanism-based CYP inhibitor, which blocks the formation of prosthetic heme group of the enzyme through catalytic formation of a carbamoyl metabolite. The CYP enzyme inhibitor ablates the prosthetic heme group of the enzyme through catastrophic formation of benzene.21

Studies were conducted in 4 groups of rats: NP treated with vehicle (n=8) or ABT (n=8) and pregnant rats with chronic RUPP treated with vehicle (n=8) or ABT (n=8). Alzet mini-osmotic pumps (model 2ML1; Direct Corporation) containing saline (0.9%) or ABT were implanted on day 14 of gestation into the jugular vein and inserted subcutaneously in the back of each animal. Animals were also instrumented with catheters in the femoral vein (PE-50 tubing) and bladder (flare-tipped PE-90 tubing) for intravenous infusions and collection of urine samples, respectively. On day 18 of pregnancy, the carotid artery was cannulated (PE-50 tubing) for blood sampling and blood pressure monitoring. All catheters were exteriorized at the back of the neck. The bladder catheter was plugged to allow the rats to void normally through the urethra.

Renal hemodynamics and arterial pressure were determined in conscious animals on day 20 of gestation. Rats were placed in a restraining cage, the bladder pin was removed, and a collection tube was attached to the catheter. The femoral vein catheter was connected to an infusion pump that delivered isotonic saline containing sodium isothalamate (Glotrol, [131I], 0.05 mCi/kg per minute, Cyprus) and para-aminohippurate (24 mg/mL; Sigma Chemical) at a fixed rate of 3 mL/h. Arterial pressure was monitored in conscious rats with a pressure transducer connected to a Grass model 7B chart recorder (Grass Instrument) for continuous recording. After a 60-minute stabilization period, two 20-minute urine collections were obtained, followed by collection of blood samples. At the end of the experiment, animals were euthanized and all fetuses were weighed.

expression of CYP4A Protein in Renal Cortex

Proteins were separated by electrophoresis on a 7.5% SDS polyacrylamide gel for 1.5 hours at 150 V and transferred to a nitrocellulose membrane using a Criterion System (Bio-Rad). Nonspecific binding was blocked by incubating the membranes for 1 hour at room temperature with 5% nonfat dry milk in Tris-buffered saline-T containing 10 mmol/L Tris-HCl, 0.1% Tween 20, and 150 mmol/L NaCl and then washed 3 times with Tris-buffered saline-T buffer. The membranes were incubated overnight at 4°C with anti-CYP4A1 polyclonal antibody (1:3000; Gentest, Woburn, Mass) that cross-reacts with CYP4A1, CYP4A2, CYP4A3, and CYP4A8 isoforms. The membrane was then washed 3 times with Tris-buffered saline-T buffer and incubated with 1:4000 dilution of horseradish peroxidase conjugated second antibody (Sigma; St Louis, Mo) for 1 hour. Immunoblots were developed with an enhanced chemiluminescence kit (ECL plus kit; Amersham). Actin (actin antibody; Amersham) was used as an internal control, and CYP4A expression was normalized relative to actin.

Statistical Analysis

All data are expressed as mean±SEM. Comparisons of control pregnant rats with RUPP rats, both treated and untreated, were analyzed by use of factorial ANOVA, followed by the Fisher test. A value of P<0.05 was considered statistically significant.
Results

Effect of ABT on MAP and Renal Hemodynamics in Pregnant Rats With Chronic RUPP

Chronic RUPP resulted in hypertension in pregnant rats. As is shown in Figure 1, MAP was elevated ($P<0.01$) in pregnant rats with chronic RUPP (130±2 mm Hg) compared with control pregnant rats (106±1 mm Hg) on day 20 of pregnancy. Administration of the CYP enzyme inhibitor, ABT, from day 14 to 20 of gestation markedly reduced ($P<0.01$) arterial pressure in the group with chronic RUPP (111±1 mm Hg), whereas it did not significantly alter MAP in control pregnant rats (108±2 mm Hg) (Figure 1).

The development of hypertension in RUPP rats was accompanied by a significant renal vasoconstriction. Figure 2 illustrates the renal hemodynamic changes observed in the 4 groups of rats studied on day 20 of gestation. Compared with control group, RUPP rats demonstrated marked increases in renal vascular resistance (16.3±1.1 mm Hg/mL per minute versus 22.6±1.8 mm Hg/mL per minute; $P<0.01$) (Figure 2A) and significant decreases in effective renal plasma flow (7.2±0.4 mL/min versus 5.7±0.6 mL/min; $P<0.05$) and GFR (figure 2B) (2.3±0.1 mL/min versus 1.6±0.1 mL/min; $P<0.05$). Inhibition of CYP enzymes markedly reduced (Figure 2A, B) the differences in renal hemodynamics observed between NP and RUPP rats. After the treatment with ABT, renal vascular resistance, effective renal plasma flow, and GFR were similar in NP rats (16.3±2.4 mm Hg/mL per minute, 7.5±0.8 mL/min, and 2.4±0.2 mL/min, respectively) and RUPP rats (19.3±1.5 mm Hg/mL per minute, 6.7±0.5 mL/min, and 2.0±0.2 mL/min, respectively).

RUPP pregnant rats attenuated ($P<0.05$) more increased body weight from day 14 to 20 of gestation (from 291±3 g to 294±4 g) than did NP rats (from 298±3 g to 318±5 g). ABT augmented ($P<0.05$) maternal weight gain in pregnant rats with chronic RUPP at day 20 of gestation (from 296±2 g to 307±3 g) but did not significantly alter the increase in body weight observed in NP rats (from 294±3 g to 319±2 g). Pup weight did not change significantly with the treatment.

Metabolism of AA in Renal Cortex

The effects of ABT on the renal metabolism of AA in NP rats and in pregnant rats with chronic RUPP are presented in

![Figure 1. Measurements of mean arterial pressure (MAP) in response to chronic RUPP in pregnant rats with and without treatment with ABT. $*P<0.01$ versus NP control group. †$P<0.01$ versus RUPP group.](image1)

![Figure 2. Renal hemodynamics in response to chronic RUPP in pregnant rats with and without treatment with ABT. A, Renal vascular resistance in NP and RUPP rats treated with vehicle or ABT. *$P<0.01$ versus NP control group. B, Glomerular filtration rate (GFR) in NP and RUPP rats treated with vehicle or ABT. †$P<0.05$ versus NP control group.](image2)

![Figure 3. The formation of 20-HETE and EETs by renal cortex was not significantly different between NP rats ($n=5$) (50±11 and 25±4 ng/mg protein per 30 minutes, respectively) and RUPP rats ($n=7$) (59±6 and 21±4 ng/mg protein per 30 minutes, respectively) treated with vehicle. Intravenous infusion of ABT during 7 days reduced ($P<0.05$) 20-HETE production in NP rats ($n=5$) (24±3 ng/mg protein per 30 minutes) and RUPP rats ($n=7$) (40±4 ng/mg protein per 30 minutes) (Figure 3). In contrast, epoxygenase activity (EETs + DHETs) was inhibited ($P<0.05$) by ABT in renal cortex of NP rats (14±1 ng/mg protein per 30 minutes), whereas it was not affected significantly in RUPP rats (16±5 ng/mg protein per 30 minutes) (Figure 3).](image3)

Renal CYP4A Expression

A representative Western blot of renal CYP4A expression in the 4 experimental groups ($n=5$) is shown in Figure 4A. Densitometry analysis (Figure 4B) revealed that cortical expression of CYP4A protein was decreased by 27% ($P<0.05$) on day 20 of gestation in RUPP rats relative to NP
rats. In addition, the treatment with ABT significantly reduced the levels of CYP4A protein by 47% and 33% in NP rats and RUPP rats, respectively. The loss of CYP4A protein after treatment with ABT has been reported in previous studies and may reflect the increased turnover of this enzyme after the irreversible binding of the inhibitor.

Discussion
Several lines of evidence support the idea that uteroplacental hypoperfusion is a crucial factor in the pathogenesis of PE. Reduced placental perfusion has been hypothesized to cause endothelial dysfunction and the subsequent manifestations of the disease. Although many markers of endothelial activation have been documented in PE, the precise mechanisms linking endothelial dysfunction with the symptoms of the disease are unknown. In this regard, abnormal production of endothelial-derived factors has been associated with the compromised renal circulation and hypertension characteristic of PE. However the role of these endothelial factors in mediating the renal vasoconstriction and elevated blood pressure observed in this disorder remains unclear.

Previous studies in our laboratory have demonstrated that chronic RUPP in pregnant rats lead to a hypertensive state that closely resembles preeclampsia in women. In these animals, hypertension is associated with impaired endothelium-dependent relaxation, enhanced systemic vascular reactivity, renal vasoconstriction, proteinuria, and intrauterine growth restriction. Furthermore, we have shown that alterations in the synthesis of endothelial-derived factors such as nitric oxide and endothelin contribute to renal vasoconstriction and hypertension induced by chronic RUPP in pregnant rats.

In the present study we demonstrated that CYP metabolites of AA also contribute to the hypertension and renal vasoconstriction observed during chronic RUPP in pregnant rats. To study the role of CYP-derived eicosanoids in mediating these alterations, we chronically treated RUPP pregnant rats with a mechanism-based CYP inhibitor, ABT. The results confirmed our previous findings that chronic RUPP in pregnant rats resulted in reduced effective renal plasma flow and GFR and significant elevations in renal vascular resistance and blood pressure compared with control pregnant rats during late pregnancy. Administration of ABT for 7 days markedly attenuated the development of hypertension in these animals.

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Chronic treatment with ABT also reduced the differences in renal hemodynamics observed between NP rats and RUPP rats, thus suggesting that CYP-derived metabolites are involved in renal alterations induced by chronic RUPP. Although ABT reduced ω-hydroxylase and epoxygenase activity in renal cortex of NP rats, the inhibitor only affected
ω-hydroxylation in renal cortex of RUPP pregnant rats. These data further support the idea that a vasoconstrictor metabolite derived from renal CYP ω-hydroxylase activity, likely 20-HETE, is involved in mediating renal vasoconstriction and hypertension during chronic RUPP in pregnant rats.

However, a diminished formation of 20-HETE in the systemic vasculature may also contribute to the decrease in blood pressure observed in RUPP animals treated with ABT. In this regard, it has been shown that mesenteric arterial vessels express CYP4A enzymes and manufacture 20-HETE. Moreover, they also demonstrated in this study that renal nitric oxide binds to the heme moiety of CYP4A1 and CYP4A3, the major isoforms expressed in NP rats is inactive. Endogenous nitric oxide, which is elevated during pregnancy, could modulate 20-HETE synthesis in the kidney. In this regard, Wang et al have recently shown that nitric oxide binds to the heme moiety of CYP4A1 and CYP4A3, the major isoforms expressed in the female rats, and inhibits their catalytic activity.

Given that CYP4A enzymes are considered to be the major AA ω-hydroxylases in the rat kidney and thereby the primary contributors of 20-HETE synthesis, we also examined whether the expression of CYP4A protein was altered in renal cortex of RUPP rats compared with NP rats. We found that CYP4A expression was significantly lower in pregnant rats with chronic RUPP than in control pregnant rats. Because the rate of 20-HETE formation was similar in the 2 experimental groups, these results suggest that the cortical activity of CYP4A enzymes was lower in NP rats than in pregnant rats with chronic RUPP. One interpretation of these findings is that CYP4A enzymes may be differentially regulated in NP and RUPP rats. One possibility is that some of the CYP4A protein expressed in NP rats is inactive. Endogenous nitric oxide, which is elevated during pregnancy, could modulate 20-HETE synthesis in the kidney. In this regard, Wang et al have recently shown that nitric oxide binds to the heme moiety of CYP4A1 and CYP4A3, the major isoforms expressed in the female rats, and inhibits their catalytic activity.

Moreover, they also demonstrated in this study that renal microvessel production of 20-HETE is increased in pregnant rats treated with L-NAME during late gestation, further supporting the idea that nitric oxide may regulate renal 20-HETE synthesis during pregnancy. In this regard, Hercules et al have reported recently that CYB4A1, CYB4A2y, and CYB4A3 contribute to the renal hemodynamic effect of L-NAME. It is also possible that there may be differences in the levels of cofactors such as CYP reductase and cytochrome b₅ between both groups of animals, which are essential for the catalytic activity of CYP proteins. We also found that ABT reduced the levels of CYP4A protein in NP and RUPP rats. The loss of CYP4A protein after treatment with ABT has been reported in previous studies and may reflect the increased turnover of this enzyme after the irreversible binding of the inhibitor.

The mechanism by which the reduced formation of 20-HETE may attenuate renal vasoconstriction and the development of hypertension in pregnant rats with chronic RUPP is unclear in this study. Because there was not significant difference in the production rate of 20-HETE in the cortex of NP and RUPP rats, an increased responsiveness to vasoconstrictor actions of 20-HETE in renal vasculature may partly explain renal vasoconstriction and hypertension observed in pregnant rats with chronic RUPP. In this regard, the decrease in renal nitric oxide production as well as enhanced levels of endothelin found in RUPP rats relative to NP rats may account for the increased renal vascular sensitivity to 20-HETE observed during chronic RUPP in pregnant rats. Indeed, it has been shown that 20-HETE requires elevated basal tone to exert its vasoconstrictor action on the afferent arteriole, a crucial vascular segment in the control of renal vascular resistance. Alternatively, it has been demonstrated that endothelin may activate phospholipases, which could alter the production of 20-HETE by elevating substrate availability. In addition, recent studies have shown that endothelin increases the formation of 20-HETE in the kidney and contributes to the vasoconstrictor actions of this peptide. Taken together, these studies suggest that endothelin would also account for an enhanced production of 20-HETE independent of any differences in CYP activity observed in this study.

In addition to the effects of ABT on 20-HETE formation, the antihypertensive effects of the CYP enzyme inhibitor during chronic RUPP in pregnant rats also may be partly mediated by the actions of the epoxygenase-derived metabolites. Although ABT reduced renal EETs production in NP rats, this CYP enzyme inhibitor had no effect in the formation of these metabolites during chronic RUPP in pregnant rats. Given that EETs are mainly considered vasodilators in the kidney, it is possible that the antihypertensive properties of these metabolites may become quantitatively more important after the reduction of 20-HETE formation in RUPP rats. The reason for the differences in the production of epoxygenase metabolites between NP and RUPP rats treated with ABT is unclear. One possible explanation would be that different isoforms of CYP epoxygenase are involved in the synthesis of EETs in both groups of animals. Chronic RUPP in pregnant rats may result in the expression of CYP epoxygenases more resistant to inhibition by ABT than the isoforms of CYP epoxygenase expressed in NP rats. These results are consistent with the findings of a previous study in SHR rats, in which treatment with ABT did not inhibit epoxygenase activity at doses lower than 100 mg/kg. Furthermore, enhanced expression of epoxygenase enzymes has been associated with hypertension, thus suggesting that it might represent a compensatory response to the elevated blood pressure. However, additional studies will be needed to determine the exact contribution of EETs to the antihypertensive effects of ABT during chronic RUPP in pregnant rats.

In summary, we found that the CYP enzymes inhibitor, ABT, markedly attenuated the development of hypertension induced by chronic RUPP in pregnant rats. The administration of this inhibitor also eliminated the differences on renal hemodynamics observed between NP rats and pregnant rats with chronic RUPP. The renal effects of the CYP enzyme inhibitor in RUPP pregnant rats were associated with a reduction of 20-HETE formation without any changes in EETs production. These results suggest that 20-HETE may contribute to the enhanced renal vascular tone and hypertension during chronic RUPP in pregnant rats. Moreover, because the formation rate of 20-HETE was similar in the renal cortex of RUPP and NP rats, an enhanced responsiveness of the renal vasculature to this vasoconstrictor in the hypertensive pregnant rats may account partly for the differences in...
renal vascular tone and blood pressure observed between these groups of animals.

**Perspectives**

Alteration of renal hemodynamics is one of the most important complications in the pathogenesis of preeclampsia. However, information regarding the mechanisms ultimately responsible for the compromise of the renal circulation and the elevated blood pressure in preeclampsia has been limited because of the difficulty in performing studies in pregnant women. In the present study, we used an experimental model induced by chronic uteroplacental ischemia in pregnant rats that closely resembles preeclampsia in women. The data obtained suggest that 20-HETE may be involved in the renal vasoconstriction and hypertension associated with preeclampsia. The present study also suggests that the vascular tone may be more sensitive to the actions of 20-HETE in hypertensive states associated with endothelial dysfunction in women. In the present study, we used an experimental model induced by chronic uteroplacental ischemia in pregnant rats where the renal circulation and the vascular tone and blood pressure observed between these groups of animals.

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