Pregnancy and IL-6

Hypertension Produced by Reductions in Uterine Perfusion in the Pregnant Rat
Role of Interleukin 6

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Abstract—The purpose of this study was to determine the role of interleukin (IL) 6 in mediating the increase in arterial pressure (AP) in response to chronic reductions in uterine perfusion pressure (RUPP) in pregnant rats. AP was higher in RUPP rats (138±1 mm Hg) than in normal pregnant (NP) rats (104±1 mm Hg). Serum IL-6 levels in the RUPP rats were 104.5±28.6 pg/mL as compared with 36.6±7.4 pg/mL in NP rats. To determine the long-term effects of a 2- to 3-fold elevation in plasma IL-6 on renal function and AP in pregnant rats, we infused IL-6 for 5 days (2.5 ng/day) in NP rats starting at day 14 of gestation. Five days later, serum IL-6 levels were 55.5±6.5 pg/mL in the control NP rats and 157.0±36.1 pg/mL in the IL-6–treated NP rats. AP was higher in the IL-6–treated NP rats (115±3 mm Hg) as compared with NP controls (101±1 mm Hg) at day 19 of gestation. Renal plasma flow and GFR were lower in the IL-6–treated NP rats than in the NP group. IL-6 increased plasma renin activity but did not affect endothelin in IL-6–treated NP rats. In contrast to the NP rats, IL-6 had no effect on AP or renal hemodynamics in virgin rats. In summary, these data indicate that plasma IL-6 is elevated in response to chronic reductions in uterine perfusion in pregnant rats and that a comparable elevation in plasma IL-6 increases AP and reduces renal function in pregnant rats. (Hypertension. 2006;48:711-716.)

Key Words: kidney ■ cytokines ■ hypertension ■ endothelin

Inflammatory cytokines such as interleukin (IL) 6 and tumor necrosis factor (TNF)-α are thought to be important links between placental ischemia and cardiovascular and renal dysfunction.1–4 Supporting a potential role of cytokines in preeclampsia are findings that plasma levels of TNF-α and IL-6 are elevated in women with preeclampsia.1–6 Important blood pressure regulatory systems, such as the renin–angiotensin system, sympathetic nervous system, and endothelial factors, interact with proinflammatory cytokines, such as IL-6 and TNF-α.7–12 Angiotensin II not only enhances the synthesis of TNF-α and IL-6, it also seems that IL-6 may play an important role in mediating the hypertension produced by angiotensin II in mice.9

Proinflammatory cytokines also affect vascular function and endothelium-derived factors involved in blood pressure regulation.12–16 TNF-α and IL-6 have both been shown to induce structural, as well as functional, alterations in endothelial cells.1,18 These cytokines alter the production of a number of endothelial cell substances and reduce acetylcholine-induced vasodilatation.1,12–17 Thus, endothelial dysfunction associated with many forms of hypertension may, in part, be mediated by proinflammatory cytokines.

Although inflammatory cytokines, such as IL-6 and TNF-α, have been reported to be elevated in preeclamptic women, the importance of these cytokines in mediating the cardiovascular and renal dysfunction in response to placental ischemia during pregnancy has yet to be fully elucidated. We reported previously that chronic reductions in uterine perfusion pressure (RUPP) in pregnant rats increases arterial pressure (AP) and reduces renal plasma flow (RPF) and glomerular filtration rate (GFR).18–24 Moreover, we reported recently that serum levels of TNF-α are elevated in RUPP rats, and chronic infusion of TNF-α into pregnant rats increases AP and decreases RPF and GFR.25 Although IL-6, which can be activated by TNF-α, is elevated in women with preeclampsia, it is unclear whether the hypertension associated with chronic RUPP in pregnant rats is associated with elevated plasma levels of IL-6. In addition, it is also uncertain whether IL-6 contributes to the hypertension associated with chronic reductions in uterine perfusion in pregnant rats. Therefore, the purpose of this study was to determine the role of IL-6 in mediating the cardiovascular and renal changes observed during chronic RUPP in pregnant rats. To achieve this goal, we first determined whether the hypertension...
associated with chronic RUPP in pregnant rats is associated with elevated serum levels of IL-6. The second objective was to determine the AP and renal hemodynamic effects of infusing IL-6 into chronically instrumented pregnant rats and virgin rats at a rate that mimics plasma levels observed in the RUPP rats. Because important blood pressure regulatory systems, such as the renin–angiotensin and endothelin systems, interact with proinflammatory cytokines, we also assessed the effects of IL-6 on endothelin production and plasma renin activity.

Methods

All of the studies were performed in age-matched, timed pregnant and virgin Sprague–Dawley rats purchased from Harlan Sprague Dawley Inc (Indianapolis, Ind.). Animals were housed in a temperature-controlled room (23°C) with a 12:12-hour light/dark cycle. All of the experimental procedures executed in this study were in accordance with National Institutes of Health guidelines for use and care of animals, and the Institutional Animal Care and Use Committee at the University of Mississippi Medical Center approved all of the protocols.

Effect of Chronic Reductions in Uterine Perfusion Pressure on Arterial Pressure and Serum Levels of IL-6

Experiments were performed in the following groups of rats: normal pregnant (NP) controls (n=16) and RUPP pregnant rats (n=10). All of the pregnant and virgin rats undergoing surgical procedures were anesthetized with 2% isoflurane (W.A. Butler Co) delivered by an anesthesia apparatus (Vaporizer for Forane Anesthetic, Ohio Medical Products). Pregnant rats entering the RUPP group underwent the clipping procedure at day 14 of gestation. After a midline incision, the lower abdominal aorta was isolated, and a silver clip (0.203-mm ID) was placed around the aorta above the iliac bifurcation. Branches of both the right and left ovarian arteries were clipped using a silver clip (0.100-mm ID) as described previously.19,21–23 Rats were also surgically instrumented with a carotid catheter for subsequent AP measurements. Arterial pressure measurements and blood samples were obtained on day 19 of gestation.

Effect of IL-6 on Arterial Pressure and Renal Hemodynamics in Conscious Chronically Instrumented Rats

Experiments were performed in the following groups of rats: pregnant controls (n=9), and IL-6–treated NP rats (2.5 ng/day; n=9). IL-6 (Recombinant Rat IL-6, R&D Systems) was infused for 5 days into NP rats during days 14 to 19 of gestation. Preliminary studies in our laboratory indicated that an IL-6 infusion rate of 2.5 ng/day was sufficient to achieve plasma levels comparable to those measured in RUPP rats. Additional experiments were performed in virgin control rats (n=9) and virgin rats treated with IL-6 (2.5 ng/day for 5 days; n=9) to examine the blood pressure and renal effects of IL-6 in virgin rats. During isoflurane anesthesia, rats at day 14 of pregnancy were surgically instrumented with catheters (PE 50 tubing) in the femoral vein and carotid artery for blood sampling and blood pressure monitoring. Virgin rats were also surgically instrumented with catheters in the femoral vein and carotid artery for blood sampling and pressure monitoring. Virgin and pregnant rats were instrumented with jugular vein catheters connected to miniosmotic infusion pumps (Model 2002, Alzet Scientific Corporation). A midline abdominal incision was made, and the bladder was cannulated with flare-tipped PE 90 tubing for urine collection. All of the catheters were tunneled to the back of the neck and exteriorized. Renal hemodynamics and APs were determined in control pregnant rats and pregnant rats treated with IL-6 at day 19 of gestation. The femoral vein catheter was connected to an infusion pump that delivered isotonic saline containing [125I] iothalamate (Isotex Diagnostics; 0.05 mCi kg−1 min−1) and para-aminohippurate (24 mg/mL, Sigma Chemical Co) at a fixed rate of 3 mL/h. Arterial pressure was monitored with a pressure transducer connected to an AP recording device. After a 1-hour equilibration period, 2- to 20-minute clearances were obtained in each rat. Urine volume was determined gravimetrically, GFR and RPF were calculated from concentrations of [125I] and PAH, respectively, in plasma and urine.23 Renal vascular resistance (RVR) was calculated by dividing the AP by the renal blood flow. After the second clearance, the rats were placed under anesthesia (2% isoflurane), and a midline abdominal incision was performed to access the aorta for blood collection and storage in silicone-coated glasses (Sherwood Medical Co) using 19G 1⁄4 needles (Becton Dickinson Vacutainer Systems). Serum was collected and kept at −80°C. Other samples were drawn into tubes containing EDTA, centrifuged for plasma separation, and stored at −20°C. Kidneys were removed for measurement of preproendothelin.

Determination of Serum IL-6 Levels

The colorimetric ELISA (R&D Systems) method was used for quantification of serum IL-6 levels. Interassay and intra-assay precision was 10.0% and 8.8%, respectively. This assay displayed a sensitivity level of <10 pg/mL.

Measurement of Plasma Endothelin Concentration

Plasma endothelin levels were determined using a human endothelin ELISA kit supplied by R&D Systems. Endothelin was isolated from 1.0 mL of plasma in 1.5 mL of extraction solvent (40:1, acetonitrile:1 n HCl:water). The extraction procedure was carried out following the protocol outlined in the sample preparation of the manufacturer instructions. The interassay and intra-assay precision was 4.5% and 6.5%, respectively. The minimal detectable level of endothelin was <1.0 pg/mL.

Determination of Kidney Preproendothelin Levels

To determine the effect of IL-6 on renal production of endothelin, we also examined preproendothelin mRNA levels in the kidney. The cortex and medulla of the kidneys from 6 NP and 8 IL-6–treated pregnant rats were separated immediately after harvesting and quickly frozen in liquid nitrogen and stored at −80°C.25 Total RNA was extracted using the totally RNA kit supplied by Ambion after the cortex and medulla were crushed in liquid nitrogen with a mortar and pestle. Isolation procedure was then performed as outlined in the instructions provided by the manufacturer.

Genomic DNA was digested with DNase1 following instructions outlined by Ambion. RNA was quantified spectrophotometrically using an Eppendorf BioPhotometer. cDNA was synthesized from 5 μg of RNA with Invitrogen’s Superscript II reverse transcriptase using the following primers: preproendothelin forward 1: ctagtctaaggactcttg and preproendothelin reverse 1: cttgtgtcttgcc, supplied with custom primers from Life Technologies.

Real-time PCR was performed with the BioRad Sybtre Green supermix and iCycler using a nested forward primer, preproendothelin forward 2: cttgtcttgcc, and the reverse primer outlined above. β-Actin was used as the housekeeping gene.

Measurement of Plasma Renin Activity

Renin activity in plasma was measured by radioimmunoassay using a modification of the method of Haber et al31 (AI standards, tracer, and antibody, National Bureau of Standards, New England Nuclear, and Arnel, respectively).

Statistical Analysis

All of the data are expressed as mean±SEM. Comparisons for multigroup and multifactorial analysis were performed by 2-way ANOVA and by using the unpaired t test for comparison between control and experimental groups. The criterion for significant differences between groups of study was P<0.05.
Results

Mean AP and Serum IL-6 Levels in Response to Reductions in Uterine Perfusion Pressure in Pregnant Rats

Mean AP was significantly higher in RUPP rats than in control pregnant rats (Figure 1). Associated with the elevation in AP in RUPP rats were increases in serum levels of IL-6.

Serum IL-6 Levels and APs in Control and IL-6–Treated Pregnant Rats

Figure 2 illustrates that serum levels of IL-6 in control pregnant rats and in pregnant rats infused with IL-6 for 5 days at a rate of 2.5 ng/day had an \( \approx \) 3-fold elevation in serum levels of IL-6, which is comparable to the serum levels observed in RUPP rats. Figure 2 also illustrates mean APs in control pregnant rats and in pregnant rats infused with IL-6 for 5 days at a rate of 2.5 ng/day. Mean AP averaged 101 \( \pm \) 1 mm Hg in control pregnant rats. AP in pregnant rats infused with IL-6 at a rate of 2.5 ng/day averaged 115 \( \pm \) 3 mm Hg, which was \( \approx \) 15% above control rats.

Renal Hemodynamics in Control and IL-6–Treated Pregnant Rats

The differences in renal hemodynamics observed in control pregnant rats and in pregnant rats infused with IL-6 for 5 days at a rate of 2.5 ng/day are shown in Figure 3. RVR was significantly increased in pregnant rats infused with IL-6 at a dose of 2.5 ng/day as compared with control pregnant rats. RPF and GFR were decreased in pregnant rats infused with IL-6 at a dose of 2.5 ng/day as compared with control rats.

Effect of IL-6 on AP and Renal Hemodynamics in Virgin Rats

In contrast to the pregnant rats, increasing IL-6 serum levels in virgin rats had no effect on mean AP (Figure 4). Moreover, there was no significant difference in RVR (10.7 \( \pm \) 1.2 versus 11.2 \( \pm \) 0.6 mm Hg/mL per minute), GFR (2.2 \( \pm \) 0.1 versus 2.1 \( \pm \) 0.2 mL/min), and effective renal plasma flow (ERPF) (9.2 \( \pm \) 1 versus 8.9 \( \pm \) 0.7 mL/min) between the IL-6–treated group as compared with control virgin group, respectively.

Effect of IL-6 on Plasma Endothelin, Renal Preproendothelin, and Plasma Renin Activity in Pregnant Rats

There were no significant differences in the plasma endothelin levels between NP rats and IL-6–treated pregnant rats. In addition, the renal preproendothelin levels were not significantly different between the IL-6–treated pregnant rats and the pregnant control rats. In contrast, IL-6–treated pregnant rats had significantly higher levels of plasma renin activity (Figure 5) when compared with control pregnant animals.
We report in this study that the elevation in AP in RUPP rats is associated with significant increases in serum levels of IL-6. Moreover, we report that chronic elevation in serum levels of IL-6, comparable to levels observed in RUPP rats, results in significant increases in AP in pregnant rats. The increase in AP is associated with increases in RVR and reductions in RPF and GFR. Furthermore, the increase in AP was associated with an activation of the renin–angiotensin system as indicated by an enhanced plasma renin activity in IL-6–treated pregnant rats. These results indicate that IL-6 may play a role in mediating the increase in AP and reduction in renal hemodynamics observed in reductions in uterine perfusion in pregnant rats.

In this study, we infused IL-6 into pregnant rats to mimic levels of serum IL-6 observed in RUPP rats. Rat IL-6 was infused at a rate of 2.5 ng/day for 5 days, and plasma levels of rat IL-6 were measured by ELISA. The 2.5 ng/day dose increased serum concentrations of IL-6 to levels that were slightly above those observed in RUPP rats. The elevation in serum IL-6 resulted in significant increases in AP (≈15%) as compared with untreated pregnant rats at day 19 of gestation. The increase in AP in the IL-6–treated rats was associated with a 30% to 40% reduction in RPF and GFR. The decrease in renal hemodynamics in response to IL-6 is similar to the decrease in renal hemodynamics that we reported previously to occur in RUPP rats. However, because the serum concentration of IL-6 in the IL-6–treated rats was slightly above those observed in RUPP rats, we may be overestimating the importance of IL-6 in mediating the reduction in renal function in RUPP rats.

The exact mechanisms whereby increases in plasma IL-6 elevate AP during pregnancy are not known. IL-6 causes endothelial dysfunction and enhanced vascular reactivity in vessels of pregnant rats. We reported recently that the increase in AP produced by chronic elevations in plasma levels of TNF-α in pregnant rats is associated with significant increases in local production of endothelin in the kidney. In addition, we reported that the increase in AP in response to TNF-α was completely abolished in pregnant rats treated with the endothelin receptor A antagonist. We also reported previously that the hypertension in response to chronic reductions in uteroplacental perfusion pressure in the pregnant rat is associated with significant increases in renal expression of preproendothelin and that selective blockade of the ETₐ receptor virtually abolished the hypertension in response to chronic reductions in uteroplacental perfusion. Collectively, these findings suggest that endothelin may play an important role in mediating cytokine-induced increases in AP in pregnant rats. Previous studies have reported an effect of high concentrations of IL-6 to stimulate endothelin synthesis by airway epithelial cells, human amnion cells, human breast cancer cells, and endothelial cells. However, in the present study, we did not observe significant changes in plasma endothelin levels in pregnant rats infused with IL-6 at a rate to mimic serum levels observed in the RUPP rats. Considering that plasma levels of endothelin may not reflect local synthesis of endothelin, we also investigated renal expression of preproendothelin mRNA. In contrast to our findings in the TNF-α study, chronic increases in plasma IL-6 in pregnant rats did not increase renal expression of preproendothelin mRNA in kidneys. These findings suggest that the increase in AP and reduction in renal function in response to IL-6 in pregnant rats may not be related to the renal endothelin system.

Other important blood pressure regulatory systems interact with proinflammatory cytokines, such as IL-6 and TNF-α. Although there is experimental evidence that proinflammatory cytokines may activate the sympathetic nervous system, it is not clear whether cytokines such as IL-6 activate the renin–angiotensin system. We reported previously that plasma renin activity is not elevated in RUPP rats. However, in the present study, we report that IL-6–treated pregnant rats had significantly higher levels of plasma renin activity when compared with control pregnant animals. Although this effect of IL-6 on plasma renin activity could potentially lead to enhanced vasoconstriction, reduced pressure natriuresis, and hypertension, the quantitative importance of the renin–angiotensin system in mediating the in vivo effects of IL-6 during pregnancy is unknown and remains to be an important area of investigation.

Interestingly, the renal and blood pressure response to IL-6 was unique to the pregnant rat. Comparable infusions of IL-6
in virgin rats had no renal or hypertensive effect. Enhanced susceptibility to the renal and cardiovascular effects of lipopolysaccharide and TNF-α during pregnancy has also been reported. Faas et al. demonstrated previously that a single low-dose infusion of lipopolysaccharide elevated blood pressure and albumin excretion in pregnant rats but not virgin rats. LaMarca et al. also reported recently that chronic elevations in plasma TNF-α increased AP and reduced renal function in pregnant rats while having no effects in virgin rats. Although the exact mechanism for the enhanced sensitivity to inflammatory cytokines during pregnancy is unknown, it may be related to circulatory levels of sex steroids. Because progesterone and 17-β estradiol are markedly elevated during gestation in rats, these steroids are potential mediators. Consistent with this suggestion are the findings of Cid et al., demonstrating the ability of progesterone and mediators. Consistent with this suggestion are the findings of Cid et al., demonstrating the ability of progesterone and 17-β estradiol to markedly enhance TNF-α–induced leukocyte stimulation of endothelial cell expression of adhesion molecules. Although these findings support a potential role for sex steroids in enhancing the endothelial response to inflammatory cytokines, the importance of estrogen and/or progesterone in enhancing endothelial activation and the hypertensive response to cytokines is unknown.

Most, but not all, investigators have reported significant elevations in plasma levels of IL-6 in women with preeclampsia. The source of IL-6 in preeclampsia or in response to placental ischemia is uncertain. Previous in vitro studies have implicated placental cytotrophoblasts as potential sites of cytokine synthesis in preeclampsia. The fact that reductions in uterine perfusion in pregnant rats increase serum levels of IL-6 also suggests that the placenta may be a source for IL-6 production. However, recent studies in humans suggest that sources other than the placenta contribute to the elevated concentrations of cytokines found in the circulation of preeclamptic women. Protein and mRNA levels of IL-6 are comparable in placentas delivered at term or preterm with or without preeclampsia. Moreover, IL-6 production by villous placental tissue from preeclamptic women maintained in culture is decreased compared with that by villous explants from normal term placenta. Thus, although IL-6 levels are increased in the circulation from preeclamptic women, the source of this IL-6 may not be the placenta but sources outside the placenta. Potential sources of IL-6 could be activated leukocytes or the maternal endothelium, which produce IL-6 on exposure to stimuli, such as TNF-α. Although TNFα has been reported to be elevated in preeclamptic women and in RUPP rats, the relative importance of TNF-α in stimulating the increase in IL-6 in response to decreases in uterine perfusion, however, remains to be determined.

Perspectives
Despite being one of the leading causes of maternal and perinatal morbidity and mortality, the pathophysiological mechanisms underlying the hypertension during preeclampsia are unknown. Increases in circulating factors, such as inflammatory cytokines, including IL-6, may serve as an important mediator of maternal endothelial activation and/or dysfunction in preeclampsia. In this study, we report that the elevation in AP in RUPP rats is also associated with significant increases in serum levels of IL-6. Moreover, we found that chronic elevation in serum levels of IL-6, comparable to levels observed in RUPP rats, results in significant increases in pregnant rats. The increase in AP is associated with increases in RVR and reductions in RPF and GFR. These results indicate that IL-6 may play a role in mediating the hypertension and reduction in renal hemodynamics observed in reductions in uterine perfusion in pregnant rats.

Although we have shown that IL-6 may play an important role in mediating the elevation in AP in response to reductions in uterine perfusion, the importance of IL-6 and other cytokines, such as TNF-α, in mediating the cardiovascular and renal alterations during preeclampsia in humans remain unclear. Furthermore, it is unknown whether drugs that inhibit the actions of inflammatory cytokines may be of benefit to women at high risk of developing preeclampsia. These important questions will not be answered until well-controlled clinical studies using specific inhibitors of cytokines are performed in women with preeclampsia.

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Disclosures
None.

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