L-arginine Attenuates Hypertension in Pregnant Rats With Reduced Uterine Perfusion Pressure

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Abstract—A chronic reduction in uterine perfusion pressure in the pregnant rat is associated with significant elevations in mean arterial pressure, proteinuria, and reductions in kidney function as is chronic nitric oxide blockade, suggesting that nitric oxide deficiency may contribute to the clinical manifestations of preeclampsia. The purpose of this study was to determine whether supplementation with L-arginine, the precursor for nitric oxide, attenuates the hypertension produced in response to a chronic reduction in uterine perfusion pressure in the pregnant rat. Reduced uterine perfusion was initiated at day 14 of gestation with arterial pressure determined at day 19 of gestation in conscious, chronically instrumented rats. Arterial pressure was significantly elevated in pregnant rats with chronic reductions in uterine perfusion as compared with pregnant control rats (132±2 mm Hg, \( P < 0.01 \), respectively). Treatment with L-arginine (2%) in the drinking water was initiated at day 10 of gestation. L-arginine supplementation resulted in a significant decrease in arterial pressure in both pregnant rats with reduced uterine perfusion pressure (113±2 mm Hg treated, \( P < 0.01 \) versus untreated pregnant) and pregnant control (97±3 mm Hg treated, \( P < 0.01 \) versus untreated pregnant) rats. However, supplementation with L-arginine decreased blood pressure by 19 mm Hg in pregnant with reduced uterine perfusion pressure (untreated versus treated) as compared with 12 mm Hg in pregnant (untreated versus treated) rats. Thus, these results suggest that L-arginine supplementation may be beneficial in attenuating the hypertension in preeclampsia. (Hypertension. 2004;43:1-5.)

Key Words: arginine ■ arterial pressure ■ hypertension, pregnancy ■ nitric oxide ■ preeclampsia

Preeclampsia, a major cause of maternal and fetal morbidity and mortality, is estimated to affect 5% to 10% of all pregnancies in the United States. 1, 2 Symptoms generally associated with preeclampsia include increased responsiveness to vasoconstrictors, elevations in arterial pressure, proteinuria, reduced glomerular filtration rate (GFR), and intrauterine growth restriction. 1, 2 The initiating event in preeclampsia is suggested to involve reduced placental perfusion leading to maternal endothelial cell dysfunction. 1, 5 A role for endothelial dysfunction in the pathophysiology of preeclampsia is implicated; as this pregnancy-specific disorder is associated with reduced synthesis of vasodilators and increased production of vasoconstrictors. 3-5 Numerous studies indicate that nitric oxide (NO) production is elevated in normal pregnancy. 6, 10 In the rat, plasma and urinary levels of cGMP, a second messenger of NO, and levels of urinary nitrite/nitrate, metabolites of NO and indicators of whole body NO production, are increased during pregnancy. 10-12 In addition, renal protein expression of inducible and neuronal nitric oxide synthase (iNOS and nNOS, respectively) are increased by 31% and 25%, respectively, in the rat at mid-gestation. 11 Further studies have demonstrated that NO plays an important role in mediating systemic hemodynamics and renal vasodilation during pregnancy. 5, 10-15

Chronic NO synthesis inhibition in the pregnant rat is associated with marked elevations in arterial pressure, reductions in GFR, proteinuria, and intrauterine growth restriction, many of the features observed in women with preeclampsia. 11, 16-18 Furthermore, these NO blockade-mediated effects are reversible by administration of L-arginine. 16, 19 We have previously reported that a chronic reduction in uterine perfusion pressure (RUPP) in the pregnant rat results in similar responses, as mid-gestational increases in renal vasodilation and late gestational reductions in blood pressure are attenuated and are associated with variable proteinuria and intrauterine growth restriction. 20 In the RUPP pregnant rat, a significant reduction in renal nNOS protein expression is observed in late gestation as compared with the normal pregnant late-gestational rat. 20 In addition, activity of the endothelium-dependent NO-cGMP pathway is reduced in the RUPP-hypertensive pregnant rat. 21 Thus, as NO plays an important role in mediating systemic and renal hemodynamic changes observed during normal pregnancy, NO deficiency...
may play an important role in preeclampsia and in the rat model of reduced uterine perfusion pressure.

l-arginine supplementation has been utilized for examination of the role for NO in mediating hypertension in both human and animal models of hypertension. For example, essential hypertension in humans is associated with a transient decrease in arterial pressure during infusion with l-arginine. In response to salt load, a greater reduction in arterial pressure is observed in the presence of l-arginine supplementation in salt-sensitive individuals as compared with salt-resistant individuals. In the Dahl salt-sensitive animal model of hypertension, l-arginine supplementation also attenuates sodium chloride loading increases in arterial pressure. In other animal models of hypertension, oral administration of l-arginine also prevents the rise in blood pressure in adrenocorticotropic- or mineralocorticoid-salt-induced hypertension. Thus, numerous reports indicate that l-arginine supplementation lowers blood pressure in animal models of hypertension associated with NO deficiency. Although there is evidence for NO deficiency in RUPP hypertensive rats, the effect of l-arginine supplementation in this rat model of preeclampsia is unknown. Therefore, the overall goal of this study was to determine if supplementation with l-arginine attenuates the hypertension produced in response to a chronic reduction in uterine perfusion pressure in the pregnant rat.

Methods

All studies were performed in timed pregnant Sprague Dawley rats purchased from Harlan Sprague Dawley Inc. (Indianapolis, Ind). Animals were housed 1 to a cage in a temperature-controlled room (23°C) with a 12:12 hour light/dark cycle. All experimental procedures executed in this study were in accordance with National Institutes of Health guidelines for use and care of animals, and the Animal Care and Use Committee at the University of Mississippi Medical Center approved all protocols.

Reduced Uterine Perfusion in the Pregnant Rat

A modification of the method by Eder and McDonald as previously described was used to reduce uterine perfusion by 35% to 45%. Briefly, rats were anesthetized with 2% isoflurane (W.A. Butler Co., Memphis, Tenn) delivered by an anesthesia apparatus (Vaporizer for Forane Anesthetic, Ohio Medical Products, Madison, Wis). At day 14 of gestation, a silver clip (0.203-mm ID) was placed around the lower abdominal aorta above the iliac bifurcation. Because compensation of blood flow to the placenta occurs in the pregnant rat through an adaptive increase in uterine blood flow, both right and left uterine arteries were clipped (0.100-mm ID). When the clipping procedure resulted in total reabsorption of the fetuses, rats were excluded from data analyses. Control pregnant rats were sham operated.

Measurement of Arterial Pressure in Conscious Chronically Instrumented Rats

Mean arterial pressure (MAP) was determined at day 19 of gestation in conscious, chronically instrumented rats as previously described.

Measure of Urinary Nitrite/Nitrate Excretion

Urinary nitrite/nitrate excretion rates were determined in animals as previously described.

Determination of Serum l-arginine Levels

Serum was collected and stored at −20°C for the measurement of serum l-arginine levels as previously described.

Results

Effect of l-arginine Supplementation on Mean Arterial Pressure in Pregnant Rats With a Chronic Reduction in Uterine Perfusion Pressure

A chronic RUPP in the pregnant rat resulted in a marked increase in arterial pressure. Specifically, at day 19 of gestation, MAP averaged 132±2 mm Hg in RUPP as compared with 109±2 mm Hg observed in pregnant (P<0.01, RUPP untreated versus pregnant untreated) rats (Figure 1). Supplementation with l-arginine during gestation resulted in a significant decrease in MAP in both RUPP (113±2 mm Hg, RUPP+l-ARG, P<0.01 versus RUPP untreated) and pregnant (97±3 mm Hg, pregnant+l-ARG, P<0.01 versus pregnant untreated) groups (Figure 1). Although MAP was lower in pregnant rats than in RUPP rats after supplementation with l-arginine (P<0.01, RUPP+l-ARG versus pregnant+l-ARG), a larger decrease in blood pressure was observed in
Effect of L-arginine Supplementation on Urinary Nitrite/Nitrate Excretion in Both Pregnant Control Rats and Pregnant Rats With Chronic Reductions in Uterine Perfusion Pressure

In late gestation, excretion of nitrite/nitrate in the RUPP (9.5 ±1.7 μmol/24 h) did not differ significantly as compared with pregnant (14.3 ±2.2 μmol/24 h) group (Figure 3). However, L-arginine supplementation greatly increased urinary nitrite/nitrate excretion in both pregnant (22.8 ±3.3 μmol/24 h, pregnant+L-ARG, P<0.05 versus pregnant) and RUPP (21.7 ±1.5 μmol/24 h, RUPP+L-ARG, P<0.01 versus RUPP) groups (Figure 3).

Changes in Serum L-arginine Levels During Pregnancy

Serum L-arginine did not differ significantly at day 19 of gestation in comparison of RUPP with pregnant (142 ±22 versus 191 ±29 nmol/mL, RUPP versus pregnant, respectively) rats (Figure 4). L-arginine supplementation did result in a marked increase in serum levels of L-arginine in RUPP (262 ±51 nmol/mL, RUPP+L-ARG, P<0.05 versus RUPP). However, serum L-arginine levels were not significantly increased in pregnant with L-arginine supplementation (281 ±38 nmol/mL) as compared with pregnant untreated rats.

Discussion

The etiology of preeclampsia remains unknown, but one of the leading hypotheses suggests that reduced uterine perfusion in late gestation leads to placental ischemia, the release of placental factors, and subsequent maternal endothelial dysfunction.1–3 Impaired endothelium-dependent responses have been reported in vessels isolated from women with preeclampsia suggesting that a deficiency of NO may contribute to the pathophysiology of preeclampsia.2–4 Numerous studies have shown that inhibition of NO production by inhibitors specific for nitric oxide synthase (NOS) during pregnancy in the rat results in marked elevations in arterial blood pressure. Consequently, the improvement in maternal blood pressure seen after L-arginine supplementation suggests benefit to the fetus.5,6

Figure 2. Measure of fetal and placental weights at day 19 of gestation in a rat model of reduced uterine perfusion pressure. Fetal and placental weights were determined from pregnant control (pregnant), pregnant with a chronic reduction in uterine perfusion pressure (RUPP), pregnant treated with 2% L-arginine administered in the drinking water (pregnant+L-ARG), and RUPP treated with 2% L-arginine (RUPP+L-ARG) litters. *P<0.05 vs pregnant untreated, †P<0.01 vs RUPP untreated. All data are expressed as mean±SEM.

Figure 3. Measure of 24-hour nitrite/nitrate excretion at day 18 of gestation in a rat model of reduced uterine perfusion pressure. Urinary nitrite/nitrate was determined in pregnant control (pregnant), pregnant with a chronic reduction in uterine perfusion pressure (RUPP), pregnant treated with 2% L-arginine administered in the drinking water (pregnant+L-ARG), and RUPP treated with 2% L-arginine (RUPP+L-ARG). *P<0.05 vs pregnant untreated. †P<0.01 vs RUPP untreated. All data are expressed as mean±SEM.

Figure 4. Measure of serum L-arginine at day 19 of gestation in a rat model of reduced uterine perfusion pressure. Serum L-arginine was determined in pregnant (pregnant), pregnant with a chronic reduction in uterine perfusion pressure (RUPP), pregnant treated with 2% L-arginine administered in the drinking water (Pregnant+L-ARG), and RUPP treated with 2% L-arginine (RUPP+L-ARG). *P<0.05 vs RUPP. All data are expressed as mean±SEM.
pressure, proteinuria, intrauterine growth restriction, and attenuation of the mid-gestational increases in renal vaso-
dilation, all features characteristic of women with preeclampsia.14,17,18 In addition, reversal of NO-blockade mediated
effects in the pregnant rat occur in treatment with L-arginine, the precursor for NO.16,19 Thus, impairment of the NO
pathway may play a role in mediating the pathophysiology of preeclampsia.

In the pregnant rat, a reduction in uterine perfusion pressure initiated at day 14 of gestation attenuates late-gestational reduc-
tions in arterial pressure as MAP is elevated by an average of 20 mm Hg at day 19 of gestation as determined in the conscious,
chronically instrumented rat.20 RUPP-induced hypertension in the pregnant rat is also associated with variable proteinuria,
intrauterine growth restriction, and reductions in renal func-
tion.20 Furthermore, endothelial dysfunction in the RUPP hyper-
tensive pregnant rats is evidenced because the endothelium-
dependent NO-cGMP pathway is impaired.21 Thus, the purpose
of this study was to determine if L-arginine supplementation
would attenuate the hypertension produced in the pregnant rat in response to reduced uterine perfusion pressure.
L-arginine supplementation resulted in a marked decrease in
MAP in both pregnant and RUPP rats relative to their untreated
counterparts. The decrease in MAP in pregnant rats supple-
mented with L-arginine was not unexpected, as blockade of NO
formation with L-arginine in RUPP rats led to a significant
increase in serum L-arginine levels associated with marked
reductions in arterial pressure as MAP is elevated by an average of
12 mm Hg difference in arterial pressure observed between
RUPP treated versus RUPP untreated rats as compared with a
19 mm Hg difference in arterial pressure observed between
pregnant treated versus pregnant untreated groups.

Hypertension in women with preeclampsia is associated with
markedly reduced levels of plasma L-arginine compared with
normotensive pregnant women,35 suggesting a role for impair-
ment of NO formation. In this study the significant decrease in
arterial pressure in the RUPP rats on supplementation with
L-arginine was associated with a significant change in serum
L-arginine levels. Specifically, serum L-arginine levels, which
did not differ on comparison of untreated RUPP and untreated
pregnant rats, was significantly increased on supplementation
with L-arginine in RUPP, but not pregnant rats. Thus, supple-
mentation with L-arginine in RUPP rats led to a significant
increase in serum L-arginine levels associated with marked
reductions in MAP.

Assessment of whole body NO production in women with
preeclampsia has yielded variable results on comparison to
normotensive pregnant women.36–39 Evaluation of NO forma-
tion in preeclampsia by supplementation with L-arginine has also
generated inconsistent results. For example, acute infusion of
L-arginine in women during weeks 31 to 32 of gestation led to a
reduction in diastolic blood pressure in both normal pregnant
and preeclamptic women, with the percent change greater in
women with preeclampsia.40 Yet in women with preeclampsia,
the L-arginine–induced reduction in blood pressure was not
associated with increased whole body NO production as noted
by serum nitrite and total L-citrulline.40 However, another study
reported that acute infusion of L-arginine between 36 to 37
weeks of gestation in women with preeclampsia was associated
with increased whole body production of NO as indicated by
increased levels of plasma cGMP, yet arterial pressure was not altered.41 We have previously shown that measurement of whole
body production of NO is not significantly altered in the RUPP
hypertensive pregnant rats relative to control pregnant rats.20
However, determination of whole body NO may not reflect
possible abnormalities in NO production as we have previously
shown that basal and stimulated release of NO from isolated
vascular strips is significantly reduced in RUPP as compared with
pregnant rats.21 Supplementation with L-arginine did lead to a
significant increase in whole body NO production in both
RUPP and pregnant rats as determined by urinary nitrite/nitrate
excretion. However, supplementation with L-arginine resulted in
a more than 2-fold increase in whole body NO production in the
RUPP-treated rats as compared with RUPP untreated rats,
whereas pregnant treated rats exhibited an increase of approxi-
mately 1.5 fold as compared with pregnant untreated. Thus,
supplementation with L-arginine may result in excess L-arginine
and increased NO synthesis, alterations that may occur in
specific vascular beds in a manner differential on comparison of
RUPP and pregnant groups.

Supplementation with L-arginine did result in a differential
effect on placental weight between RUPP and pregnant rats.
Placental weight, which did not differ between untreated RUPP
and untreated pregnant rats, was increased significantly on
supplementation with L-arginine in RUPP rats, but not in preg-
nant rats. Intrauterine growth restriction, a known risk factor
in preeclampsia,2–5 was present in RUPP as compared with control.
However, supplementation with L-arginine did not
significantly improve fetal weights in either group. Thus,
L-arginine supplementation significantly increased placental
weights but not fetal weights in the RUPP, suggesting a
differential effect in response to L-arginine supplementation on
vascular beds of the uteroplacental unit in the presence of
chronic RUPP.

Supplementation with L-arginine in both pregnant and
RUPP rats resulted in a reduction in arterial pressure associ-
ated with an increase in whole body production of NO. The
difference in the MAP reduction was greater in RUPP as
compared with control group as was the increase in whole
body NO. Whether these differences are significant and
indicative of enhanced sensitivity to L-arginine by RUPP is
unknown. However, a deficiency in NO production or im-
pairment in the NO pathway is suggested, as elevations in
serum L-arginine were significant in response to excess
L-arginine in the RUPP.

Perspectives

NO deficiency is thought to play a role in the pathophysiol-
y of preeclampsia. The present study examines the impor-
tance of NO in the rat model of reduced uterine perfusion
pressure. Treatment with L-arginine (2%) resulted in a sig-
nificant decrease in arterial pressure (Δ19 mm Hg) in RUPP
pregnant and normal pregnant rats (Δ12 mm Hg). In addition,
whole body production of NO was increased in both RUPP
and pregnant rats on supplementation with L-arginine. However, significant increases in serum L-arginine levels were observed only in RUPP-treated rats, suggesting de novo synthesis of NO may be stimulated in this rat model by the presence of excess L-arginine. Thus, L-arginine supplementation may be beneficial in attenuating the hypertension in preeclampsia as impairment of the NO pathway may play an important role in mediating the pathophysiology of preeclampsia.

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