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Original Article

Effect of nitric oxide deficiency on tissue-type plasminogen activator expression in the umbilical cord in a pregnancy-induced hypertension rat model

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ABSTRACT

Objectives: Our study investigates the effects of nitric oxide (NO) deficiency during pregnancy on coagulation and fibrinolysis balance in fetal circulation.
Main outcome measures: Pregnant rats were treated with or without oral N^G-nitro-L-arginine methyl ester (L-NAME). Systolic blood pressure (SBP) and urinary protein were measured. On gestational day 20, mRNA levels of tissue-type plasminogen activator (tPA), tissue factor (TF), and TF pathway inhibitor (TFPI) in the umbilical cord, placenta, and maternal aorta were evaluated. Immunohistochemical staining of the placenta for PA inhibitor-1 (PAI-1) was performed.
Results: L-NAME treatment in pregnant rats caused significant SBP elevation and severe proteinuria. In the L-NAME-treated group, weights of fetuses and placentae were diminished. tPA mRNA expression decreased in the umbilical cord and placenta, whereas TF and TFPI mRNA levels did not change. Intense PAI-1 immunoreactivity was observed in a part of degenerated placenta. In the aorta, tPA mRNA expression increased in the L-NAME-treated group, while TFPI mRNA levels were lower than in controls.
Conclusions: NO deficiency during pregnancy decreased tPA mRNA expression in the umbilical cord and placenta but not in the maternal aorta. Imbalance between coagulation and fibrinolysis in fetal and maternal circulations may, at least in part, contribute to fetal growth restriction.

Introduction

Pregnancy-induced hypertension (PIH), related to preeclampsia, is considered one of the most important disorders in the perinatal period that potentially threatens maternal and fetal survival [1]. This disease is clinically defined as arterial hypertension in association with proteinuria during pregnancy and is one of the leading causes of fetal growth restriction and low birth weight [2,3].

It is well known that endothelial dysfunction contributes to PIH pathogenesis. Nitric oxide (NO) plays a role in modulating vascular reactivity and vasodilatation during pregnancy [4,5]. Studies have shown that treatment using N^G-nitro-L-arginine methyl ester (L-NAME), an inhibitor of NO synthase, induces endothelial dysfunction in pregnant rats, causing a PIH-like disorder [5,6].

Hypercoagulation and endothelial dysfunction are partially involved in the pathogenesis of PIH [7,8]. The sensitive balance of the coagulation and fibrinolysis system

Abbreviations: NO, nitric oxide; PIH, pregnancy-induced hypertension; tPA, tissue-type plasminogen activator; PAI-1, plasminogen activator inhibitor-1; TF, tissue factor; TFPI, tissue factor pathway inhibitor; L-NAME, N^G-nitro-L-arginine methyl ester; SBP, systolic blood pressure.

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plays an important role in maintenance of maternal–placental blood flow, which continuously provides nutrition and oxygen to the developing fetus [1,9]. Many kinds of cytokines are known to be involved in the hemostatic system and function in activator–inhibitor pairs. For example, tissue factor (TF), a typical coagulation factor, initiates the external blood coagulation cascade. On the other hand, TF pathway inhibitor (TFPI) can regulate TF-evoked thrombogenesis by inhibiting the TF–VIIa complex and, subsequently, activated factor Xa. As a fibrinolysis factor, tissue- and urokinase-type plasminogen activator (tPA and uPA) are expressed mainly by vascular endothelial cells and trophoblasts. These cytokines convert inactive plasminogen into active plasmin, which selectively degrades insoluble fibrins. PA inhibitor-1 (PAI-1) is also generated by endothelial cells and trophoblasts as a protective mechanism against excess plasmin production to limit fibrinolysis reactions within local sites. Studies in animal models of hypertension demonstrated that chronic NO deficiency induces the alteration of TF, TFPI, PAI-1, and tPA secretion and mRNA expression in the aorta and renal arteriole [10–12].

During normal pregnancy, the blood coagulation and fibrinolysis systems activate physiologically in preparation for obstetric hemorrhage [13]. Clinical studies have reported a significant upregulation of the intravascular coagulation system [14] and a significant increase in plasma tPA levels [15] in the patients with PIH. In vitro studies showed imbalanced synthesis of cytokines related to the coagulation and fibrinolysis systems in cultured placental trophoblasts from patients with preeclampsia [8] and in cultured umbilical vein endothelial cells stimulated by L-NAME [16]. Thus, imbalance between coagulation and fibrinolysis is exerted in the maternal circulation of patients with PIH. However, little is known about the effects of NO deficiency during pregnancy on hemostatic balance in the fetal circulation.

This study was designed to examine whether NO deficiency would alter the expression of tPA, PAI-1, TF, or TFPI in the umbilical cord, placenta, and maternal aorta in L-NAME-treated pregnant rats. In addition, the effect of hemostatic imbalance on fetal growth was discussed.

Materials and methods

Animals and experimental procedure

All experimental procedures were performed in accordance with the Guidelines for Animal Experimentation provided by Aomori University of Health and Welfare. Virgin female Wistar rats aged 10–11 weeks and weighing 151–210 g were obtained from Charles River Laboratories Japan Inc. (Yokohama, Japan). They were maintained at a temperature of 23 ± 1 °C under a 12-h light–dark cycle with ad libitum access to food and tap water. A vaginal impedance reader (Model MK-10C; Muromachi Kikai Co. Ltd., Osaka, Japan) was used to determine whether the female rats were in the appropriate stage of the estrus cycle for mating. This procedure was routinely performed in the afternoon, and a reading of >3 kΩ indicated that the females were in proestrus and presumably in estrus. One appropriate female was mated overnight with one male. The next morning, the presence of a vaginal plug indicated successful mating and was documented as day 0 of gestation. Pregnant rats were randomly divided into two groups: untreated (control, $n = 7$) or treated with L-NAME (Sigma–Aldrich Inc., St. Louis, MO, USA) in the drinking water (0.5 g/L, L-NAME group, $n = 8$) from day 0 throughout pregnancy. On gestational days 6, 13, and 19, systolic blood pressure (SBP) was measured by the tail-cuff plethysmography method (model MK-1100; Muromachi Kikai Co. Ltd., Tokyo, Japan). On gestational day 19, the rats were placed in individual metabolic cages and fasted and 24-h urine samples were collected. On gestational day 20, blood samples were collected under ether anesthesia and plasma was collected after centrifugation (800*g*, 10 min, 4 °C). After sacrifice, the number of fetuses was counted and the aortas, placentae, and umbilical cords were immediately removed, frozen in liquid nitrogen, and stored at −80 °C for evaluation of mRNA expression. Multiple placentae and umbilical cords from each litter were pooled.

Measurement of NO_x and protein content

The NO_x contents of the plasma and 24-h urine samples were measured using the Griess method with an NO_2/NO_3 Assay Kit-C II (Dojindo Laboratories, Kumamoto, Japan). The protein content of the 24-h urine samples was determined using the turbidity assay method with a Rat Urinary Protein Assay Kit (Chondrex Inc., Redmond, WA, USA).

Real-time polymerase chain reaction (PCR)

Total RNA in the thoracic aorta, placenta, and umbilical cord was extracted using the SV Total RNA Isolation System (Promega Corp., Madison, WI, USA) according to manufacturer's instructions, and cDNA was synthesized using the High Capacity cDNA Reverse Transcription Kit (Applied Biosystems, Foster City, CA, USA). The mRNA levels of tPA, TF, and TFPI were, respectively, analyzed using the TaqMan gene expression assays Rn00565767_m1, Rn00564925_m1, and Rn00567935_m1 inventoried primers (Applied Biosystems). GAPDH mRNA was also analyzed using the TaqMan Rodent GAPDH Control Reagent Kit (Applied Biosystems) as an endogenous control. Real-time PCR was performed using the Universal PCR Master Mix (Applied Biosystems) on an Applied Biosystems 7000 Sequence Detection System according to manufacturer's instructions. Gene expression levels were expressed relative to the GAPDH intensity of each co-amplified sample.

Histopathology and immunohistochemistry

For the histological examination, paraformaldehyde-fixed placentae were embedded in paraffin and sections were stained with hematoxylin–eosin (HE). For detection of PAI-1, the avidin–biotin complex method (LSAB2 Kit; Dako, Carpinteria, CA, USA) with rabbit anti-PAI-1 (Santa Cruz Biotechnology, Santa Cruz, CA, USA) diluted 1:50 was used according to manufacturer's instructions.