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Perspective

Effects of low-dose aspirin on maternal blood pressure and vascular function in an experimental model of gestational hypertension

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ABSTRACT

Daily intake of low-dose aspirin after 12 weeks of gestation is currently recommended as a preventative intervention in pregnancies in high risk of developing preeclampsia. This recommendation is based on epidemiological evidence, whereas experimental studies investigating the exact mechanisms of aspirin action during pregnancy are lacking. We previously showed that treating pregnant rats with a synthetic mimetic of unmethylated CpG DNA (bacterial DNA) caused preeclampsia-like characteristics such as maternal hypertension and increased cyclooxygenase (COX) expression and activity. In this study, we tested the hypothesis that daily maternal treatment with low-dose aspirin would prevent the development of maternal hypertension, reduce COX activity and thromboxane A_2 (TxA_2) production, and improve maternal vascular function in pregnant rats exposed to CpG ODN during gestation. Pregnant rats were treated with ODN2395 (synthetic CpG DNA) or saline (vehicle) on gestational days (GD) 14, 16, 18. Daily low-dose aspirin treatment (1.5 mg/kgBW) started on GD10 and continued throughout gestation. Pregnant rats treated with ODN2395 had greater systolic blood pressure compared to controls (120 ± 4 mmHg vs. 100 ± 5 mmHg, $p = 0.03$) and aspirin did not prevent this increase ($p = 0.86$). Aspirin prevented ODN2395-induced increases of TxB_2 (TxA_2 metabolite) in serum and mesenteric arteries. ODN2395 increased expression of COX-1 and COX-2 in mesenteric and uterine arteries and aspirin abolished these effects. Aspirin reduced contractile responses to phenylephrine and U46619 (TxA_2 mimetic) in mesenteric arteries from control rats but not from ODN2395-treated rats. In conclusion, treatment with low-dose aspirin reduced systemic and vascular COX expression and activity but did not prevent the development of maternal hypertension induced by exposure to unmethylated CpG DNA (bacterial DNA).

1. Introduction

Pregnancy-associated hypertensive disorders affect 10–12% of pregnancies worldwide and include, but not limited to gestational hypertension, preeclampsia, and chronic hypertension with superimposed preeclampsia [1]. Preeclampsia is a multisystemic disorder and one of the leading causes of maternal and infant mortality and morbidity [1]. Diagnostic criteria include de novo hypertension with proteinuria, or hypertension without proteinuria but combined with hematological complications, renal insufficiency, impaired liver function, neurological symptoms, or uteroplacental dysfunction [1,2]. The cardiovascular system is severely affected in pregnancies with preeclampsia, with systemic maternal vascular dysfunction being one of the main features of this pregnancy syndrome [3–5]. Pregnant women with preeclampsia and animal models with preeclampsia-like symptoms exhibit vascular oxidative stress [6,7], vascular inflammation [8], excess vasoconstriction [9–11], and endothelial dysfunction [12]. Importantly, women that have experienced preeclampsia during gestation are in high risk of adverse cardiovascular events later in life [13].

Currently, there is no cure and no effective preventative strategy for preeclampsia. Randomized control trials and meta-analyses have shown that daily intake of low-dose aspirin can reduce the overall risk of developing preeclampsia in women with various risk factors for the syndrome [14,15]. The American College of Obstetricians and Gynecologists recommends low-dose aspirin for pregnant women with a history of preeclampsia who require deliv-

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ery before 34 weeks of gestation and women who had preeclampsia in more than one prior pregnancies [2]. On the other hand, the U.S. Preventive Services Task Force recommends that all women at high risk for preeclampsia should take low-dose aspirin [16]. Experimental studies investigating the mechanisms of action of low-dose aspirin during pregnancy are lacking.

Aspirin is a non-steroidal anti-inflammatory drug that inhibits the cyclooxygenase (COX) pathway of arachidonic acid metabolism by acetylating Ser530 of COX-1 and COX-2 leading to their inactivation [17,18]. In low doses (75–150 mg daily), aspirin down-regulates pathways associated with the mammalian innate immune-mediated responses, including the generation of thromboxane A_2 (TxA_2) [19,20], which is a potent vasoconstrictor with pro-thrombotic properties. Interestingly, levels of thromboxane B_2 (TxB_2), a stable product of TxA_2 metabolism, are elevated in women with preeclampsia [21,22].

The innate immune system has been implicated in the pathogenesis of hypertensive disorders of pregnancy [23–26]. The Toll-like receptors (TLRs) are pattern-recognition receptors of the immune system that have the ability to recognize pathogen-associated molecular patterns (i.e., in the presence of an infection) as well as damage-associated molecular patterns (i.e., in the presence of cell damage) [27]. Upon binding to their respective ligands, TLRs initiate the activation of signaling cascades that lead to the production of inflammatory cytokines and expression of inflammatory molecules such as COX and inducible nitric oxide synthase (NOS) [27]. Experimental studies showed that activation of innate immunity pathways through the TLR 3, 4, 7, and 8 induced maternal hypertension, proteinuria, and systemic inflammation in pregnant rodents [24,25,28]. We recently showed that exposure of pregnant rats to a synthetic ligand of TLR9 (CpG oligodeoxynucleotides, CpG ODN) induced preeclampsia-like symptoms (maternal hypertension, excess vasoconstriction, increased expression and activity of COX enzymes) [29].

In the present study, we aimed to investigate whether low-dose aspirin treatment starting at the beginning of the second trimester of pregnancy would ameliorate preeclampsia-like symptoms induced by the activation of TLR9 with CpG DNA (synthetic CpG ODN). We hypothesized that daily maternal treatment with low-dose aspirin would prevent the development of maternal hypertension, reduce COX activity and TxA_2 production, and improve maternal vascular function in pregnant rats exposed to CpG ODN during gestation (animal model with preeclampsia-like symptoms).

2. Methods

2.1. Chemicals

ODN2395 was obtained from Invivogen (cat#: tlrl-2395-5, San Diego, CA, USA). Lyophilized ODN2395 was re-suspended in endotoxin free water (500 mM stock) and administered to animals in sterile saline. U46619 (cat#: 56985-40-1) and thromboxane B_2 (TxB_2, cat#: 501020) ELISA kit were purchased from Cayman (Ann Arbor, MI, USA). Phenylephrine (PE, cat#: P6126), acetylcholine (ACh, cat#: A6625), sodium nitroprusside (SNP, cat#: 228710), N_ω-Nitro-L-arginine methyl ester hydrochloride (L-NAME, cat#: N5751), sodium orthovanadate (NO_3VO_4, cat#: 450243), phenylmethylsulfonyl fluoride (PMSF, cat#: P7626) were obtained from Sigma-Aldrich (Sigma-Aldrich, Saint Louis, MO, USA). Stock solution of U46619 (10 mM) was prepared in dimethylsulfoxide. Stock solutions of PE (100 mM), ACh (100 mM), SNP (10 mM) and subsequent dilutions of U46619 were prepared in distilled water. For all vascular reactivity concentration-responses curves, all subsequent dilutions were prepared fresh before the experiments. T-PER tissue protein extraction reagent (cat#: 78510) and protease and phosphatase inhibitor mini tablets (cat#: 88668) were purchased from ThermoFisher Scientific (Waltham, MA, USA).

2.2. Animals

All protocols were approved by the Institutional Animal Care and Use Committee of the University of North Texas Health Science Center in accordance with the Guide for the Care and Use of Laboratory Animals of the National Institutes of Health.

Male and female Sprague-Dawley rats (Envigo: Indianapolis, IN and Houston, TX, USA) were single-housed under 12 h:12 h light/dark cycles in a temperature and humidity controlled environment. Following one week of acclimatization, we determined a normal estrous cycle using vaginal cytology and familiarized all female rats with the tail cuff method of blood pressure measurements. Rats had free access to tap water and standard laboratory rodent chow throughout the study. Final experiments were performed when rats were 16–20 weeks old.

2.3. Experimental design and procedures

2.3.1. Group assignment, mating procedures, and treatments

After baseline blood pressure (BP) measurements, female rats were assigned to four BP-matched groups. Immediately following baseline BP measurements, females on the estrous stage of their cycle were placed with fertile males (pair breeding) overnight. The presence of spermatozoa in vaginal smears was used as an index of successful mating and the morning on which spermatozoa were found was considered day 1 of gestation (GD1; term = 21–22 days).

In order to induce maternal hypertension, we treated pregnant rats with a synthetic CpG ODN (ODN2395, 100 μg/rat each i.p.) via three intraperitoneal (i.p.) injections on GD14, GD16, and GD18. ODN2395 contains unmethylated CpG sequences (5′-tcgtcgttttcggcgc:gcgccg-3′, palindrome is underlined) and is resistant to nucleases due to its phosphorothioate-modified backbone. ODN2395 was administered to the animals in sterile saline (final volume: 300 μl). The dose of ODN2395 was chosen based on our previous studies, which showed that this dose is sufficient to induce maternal hypertension, changes in vascular function, and an increase expression of proteins associated with TLR signaling [29,30]. A second group of rats was treated only with saline (i.p., final volume: 300 μl, frequency: GD14, GD16, GD18) and served as a control. In order to assess the preventative effects of aspirin on maternal hypertension during pregnancy, we administered aspirin orally starting on GD10 and continued daily throughout gestation (GD10-GD21). Currently, the American College of Obstetrics and Gynecology recommends the initiation of low-dose aspirin treatment (80–100 mg daily) in pregnancies with high risk of developing preeclampsia starting at the beginning of the second trimester [2]. We estimated that GD10 corresponds to the beginning of the second trimester of rat pregnancy. Aspirin (1.5 mg/kgBW/day) was daily admixed with rodent dough (1 g; Transgenic Dough Diet Bacon Flavor, Bio-Serv, Flemington, NJ, USA) and administered to both ODN2395 and saline-treated groups. This dose is approximately equal to 100 mg/day, which is within the range of the current recommended dose prescribed in pregnant women. Some rats treated with ODN2395 and saline received only rodent dough (1 g daily) from GD10-GD21. Summarizing, the effects of aspirin alone and in combination with ODN2395 were examined using the following four experimental groups: 1) Control (received saline and rodent dough), 2) ODN2395 (received ODN2395 and rodent dough), 3) Aspirin (received aspirin and saline), 4) ODN2395+Aspirin (received ODN2395 and aspirin).