Preeclampsia is associated with a deficiency of lipoxin A4, an endogenous anti-inflammatory mediator

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Objective: To test whether lipoxin A4 (LXA4) deficiency results in preeclampsia.

Design: Prospective experimental study.

Setting: Patient and animal research facilities.

Animal(s): Sprague-Dawley rats.

Intervention(s): We measured LXA4 and its biosynthetic enzymes, blocked the LXA4 signaling pathway, treated experimental rats with preeclampsia with LXA4, and detected inflammatory factors, FPR2/ALX, and 11β-HSD2 to systematically test whether lack of LXA4 results in preeclampsia.

Main Outcome Measure(s): We measured serum levels of LXA4 and inflammatory factors using enzyme-linked immunosorbent assay; detected LXA4 biosynthetic enzymes, inflammatory factors, FPR2/ALX, and 11β-HSD2 mRNA expression using reverse transcriptase–polymerase chain reaction (RT-PCR) and real-time RT-PCR; and localized protein expression using immunohistochemistry.

Result(s): FPR2/ALX and LXA4 and its biosynthetic enzymes were found to be decreased in women with preeclampsia. Replenishing LXA4 improved the symptoms of lipopolysaccharide-induced rats with preeclampsia, while blocking LXA4 signaling resulted in preeclampsia. LXA4 significantly reduced interleukin-6 (IL-6), tumor necrosis factor-α, and IFN-γ but increased IL-10, LXA4 up-regulated 11β-HSD2.

Conclusion(s): A deficiency of LXA4 may result in preeclampsia, which might be ascribed to a reduction in inflammation response, oxidative stress, and regulation of 11β-HSD2. (Fertil Steril® 2014; –: – –. ©2014 by American Society for Reproductive Medicine.)

Key Words: Preeclampsia, lipoxin A4, inflammation, 11β-HSD1, 11β-HSD2

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Preeclampsia (PE) is a specific complication of pregnancy, characterized by de novo hypertension, proteinuria, and sometimes edema; it can occur after the 20th week of gestation (1–3). PE is one of the leading causes of maternal, fetal, and neonatal mortality worldwide. PE is thought to be caused by a shallowly implanted placenta that becomes hypoxic, thus leading to an immune reaction characterized by secretion of up-regulated inflammatory mediators from the placenta that act on the vascular endothelium (2). However, at the core of PE is activation of the inflammatory response. Given that the embryo actually is a semiallograft and allograft rejection is mediated through an inflammatory process, it is reasonable to hypothesize that inflammation triggers PE. In fact,
overreaction of the immune system through the abnormal up-expression of proinflammatory cytokines or down-expression of immune inhibitors in the placenta may be a pathogenetic factor for PE. Many studies have examined the relationship between inflammation and PE. For example, the protective role of interleukin-10 (IL-10) in PE has been researched [4, 5], as has the contribution of inflammatory pattern recognition receptors that contribute to the pathogenesis of PE [6]. Yet, despite various speculations, the relationship between inflammation and PE remains enigmatic.

The continual clearance of placental debris from the maternal circulation causes a systemic inflammatory response that is present in all pregnant women, but PE occurs when the systemic inflammatory response decompensates [7]. Inhibiting or quenching inflammation defects may be a real pathogenesis of PE. Lipoxins are recently discovered types of endogenous anti-inflammatory lipid-based autacoids, which are generated from arachidonic acid (AA) via lipoxygenase-mediated transcellular biosynthesis [8–10]. Among them, lipoxin A4 (LXA4) and its analogues are thought to be the “braking signal” of inflammation. By binding its high-affinity G protein–coupled receptor–N-formyl peptide receptor 2 (FPFR2/ALX) [11], the main receptor responsible for the in vivo anti-inflammatory actions of LXA4–LXA4 acts as a down-regulatory signal at sites of inflammation, mediates inhibition of neutrophil and eosinophil chemotaxis [12] and antagonism toward peptide-leukotrienes [13], stimulates the phagocytosis of apoptotic cells by macrophages [8], and decreases the inflammatory infiltrates and edema in vivo [14].

This anti-inflammatory effect suggests important roles for LXA4 in the regulation of inflammation and a possible role in the inflammation of PE. Our group provided the first evidence for the anti-angiogenic role of LXA4 on hypoxic human umbilical vein endothelial cells (HUVECs), and we also proved that LXA4 could prevent the endothelial hyperpermeability induced by lipopolysaccharide (LPS) in HUVECs [15, 16]. Our group has proved that systolic blood pressure (SBP), 24-hour urinary albumin excretion, serum tumor necrosis factor-α (TNF-α) and IL-8 levels, and morphologic damage of placenta and kidney caused by LPS were all effectively alleviated by 5(S) and 6(R)-7-trihydroxymethyl heptanoate (BML-111, synthetic analogue of LXA4) [17].

This paper reports that the BML-111 could alleviate the symptoms of PE in low-dose endotoxin-exposed pregnant rats. BML-111 is a C-7 truncated lipoxin analogue; thus, whether LXA4 is equiactive with BML-111 is unknown, and the relationship between LXA4 and PE and the regulation of PE inflammation by LXA4 remain unclear. The present study, therefore, was undertaken to explore PE and its association with LXA4 deficiency and to discover its underlying mechanisms.

MATERIALS AND METHODS

Patient Samples

Blood samples were obtained from 30 women: 15 women with PE defined by hypertension (systolic and diastolic blood pressures higher than 140/90 mmHg and proteinuria 0.3 g/day) [18] were recruited from the First Affiliated Hospital, Wenzhou Medical College in Wenzhou, Zhejiang, China. As a comparative group, 15 pregnant women were originally selected with characteristics similar to those presented by the preeclamptic patients—including body mass index, gestational age, eliminated high blood pressure, kidney disease, diabetes, and so on. Blood and placenta samples were obtained from diagnosed patients after informed consent and approval by the Ethics Committee of the Medical Faculty of Wenzhou Medical College in accordance with the Declaration of Helsinki. Institutional Review Board approval was obtained for this study (ChiCTR-CCC-13003940).

The patients had not taken any medications before specimen collection. Blood extracted from the pregnant women stood for 20 minutes at room temperature. The serum supernatants were transferred to a 1.5-mL Eppendorf tube and mixed quickly. After centrifugation, serum was collected and stored at −80°C until use.

Animals

Female Sprague-Dawley rats (10–12 weeks old and 220–250 g in weight) were purchased from the Experimental Animal Center for studies approved by the Animal Care and Use Committee of Wenzhou Medical College. The animals were housed individually under pathogen-free conditions in a controlled environment with a temperature at 20°C–25°C and 12-hour cycles of light and dark. They were fed a standard laboratory diet and water ad libitum. The female rats were mated overnight with fertile male rats. A positive vaginal smear for sperm defined day 1 of pregnancy.

Experimental Protocol

Rats were randomly divided into a control group, an LPS group, and an LPS + LXA4 group (n = 10, each group). Experimental PE rats were induced by infusion of LPS (1 μg/kg body weight) in 2 mL of sterile saline through an infusion pump into the tail vein (infusion rate, 2 mL/hour) on day 14 of pregnancy [19]. Normal pregnant control rats were infused with 2 mL of saline alone. LXA4 (5 μg/kg, Cayman Chemical Company) was administrated IP 2 hours after LPS administration from day 14 of pregnancy and at the rate of once per day for 7 days.

Measurement of SBP

The SBPs were measured in conscious, restrained pregnant rats from day 12 through day 20 of pregnancy and once every 2 days in the morning. An automated system with a photoelectric sensor linked to a dual-channel recorder (BP-98A, Softron), tail cuff, and sphygmomanometer was used to obtain indirect blood pressure measurements [20]. On day 20 of pregnancy, after the rats were anesthetized, fetal pups were removed and weighed and blood specimens were stored at −80°C for further assessment.
Urinary Protein Concentration

On day 12 and day 18 of pregnancy, the rats were placed in metabolic cages, and 24-hour urine was collected. The rats were allowed free access to water but were restricted from food to avoid contaminating the collected urine. To avoid the adverse effects of fasting, rats were fed in other cages for 30 minutes every 6 hours. Urine samples were centrifuged at 2,000 rpm for 20 minutes at room temperature, and the top layer was stored at −80°C for later analysis of protein. Urinary protein concentrations were determined with a BCA protein assay kit, using bovine serum albumin as the standard.

Specimen Collection

To illustrate the mechanism of LXA4, 24 hours after LXA4 treatment and after the rats were anesthetized, blood specimens of 2 mL were drawn by heart puncture and the placenta was removed. Serum and placenta were stored at −80°C for further assessment. The placenta was fixed with 10% neutral buffered formalin for histological evaluation.

Reverse Transcriptase–Polymerase Chain Reaction (RT-PCR) and Real-time RT-PCR

Blood and tissues were lysed or homogenized with TRIzol reagent (Invitrogen), and the total RNA was extracted according to the manufacturer’s instructions. An RT-PCR procedure was used to determine the expression level of mRNA (One-Step RT-PCR kit; Qiagen). The sequences of primers were as follows: human GAPDH, forward 5’-CCCTTCACTTACCCACTAC-3’ and reverse 5’-GTTGATGGGATTTCATATTG-3’; human 15-LO, forward 5’-CCATTCAAGCAACACCGAC-3’ and reverse 5’-TCAGGAAGCTGTGACCAAAC-3’; human 12-LO, forward 5’-GACGTG-3’ and reverse 5’-CTTCAAACAGGGAACCATTC-3’; human 11-LO, forward 5’-GGAAAGAGAAGGAAAGTTG-3’ and reverse 5’-GCTACAGAGAATGACCTG-3’; human 11β-HSD1, forward 5’-CTCTAACACATCACCAACAC-3’ and reverse 5’-GGAGAGAGAGGAAGTTG-3’; human 11β-HSD2, forward 5’-CCACCTACCATCACTTCCAC-3’ and reverse 5’-CAGTGCTGTGTGGTCTGCA-3’; rat IL-6, forward 5’-AAGCTGAAGACCCTCTGGATACA-3’ and reverse 5’-CACGTGCTGTGTGGTCTGCA-3’; rat IFN-γ, forward 5’-TTGGATGGTCTTGGTCCTTAGCC-3’ and reverse 5’-GACGTG-3’; rat TNF-α, forward 5’-TCTGATTGCTTCCTTTTATTCTCACA-3’ and reverse 5’-GATGTTACCTGTTTATTTTATTCACA-3’; rat IL-10, forward 5’-GAAGGAGAAGGAAGGTGACTGATGAAGAAG-3’ and reverse 5’-GGAAAGAGAAGGAAAGTTG-3’; rat HSD2, forward 5’-AATTCCATGATCCTCCTTCC-3’ and reverse 5’-TCTGATTGCTTCCTTTTATTCTCACA-3’; rat GAPDH, forward 5’-GTCACCACCATCTCTTACTT-3’ and reverse 5’-GTCACCACCATCTCTTACTT-3’.

For real-time RT-PCR assays, the cDNA sequences of all of the detected genes were retrieved from the National Center for Biotechnology Information database. The primers were designed with the Oligo Primer Analysis 4.0 software (Molecular Biology Insights), and the sequences were subjected to BLAST analysis. The total RNA (100 ng) was used for reverse transcription using Superscript II RNase H reverse transcriptase (Invitrogen) in a volume of 25 µL. Next, 2 µL cDNA was amplified with SYBR Green Universal PCR Mastermix (Bio-Rad) in duplicate. The resulting data were analyzed with the comparative cycle threshold (Ct) method for relative gene expression quantification against GAPDH.

Enzyme-linked Immunosorbent Assay (ELISA)

To assess LXA4, the serum and placental tissue were assessed by ELISA kits (Neogen). IL-10, TNF-α, IL-6, and IFN-γ protein levels in rat serum were assessed by ELISA kits (R&D Systems), and 11β-HSD2 incubated supernatant was assessed by ELISA kits (Westang) according to the manufacturer’s instructions.

Enzyme Chemiluminescence Immunoassay

The concentration of free cortisol in serum was detected by enzyme chemiluminescence immunoassay (automatic biochemical analyzer, Beckman Coulter, Inc.).

Histology and Immunohistochemistry

Placenta tissues were fixed in a 10% neutral buffered formaldehyde solution. After dehydration procedures, the samples were blocked in paraffin, and 4-µm sections were cut by a microtome. The serial sections of the placenta tissues were immunohistochemically stained for 5-LO, 12-LO, 15-LO, FPR2/ALX, 11β-HSD1, and 11β-HSD2. The tissue sections were incubated overnight with primary antibodies (anti-5-LO, 12-LO, and 15-LO antibodies, Santa Cruz, Biotechnology, Inc.; anti-11β-HSD1 and anti-11β-HSD2 antibodies, Biosynthesis Biotechnology Company; anti-FPR2/ALX antibodies, Biorbyt Company,) at 4°C followed by secondary antibodies and peroxidase-labeled avidin for 30 minutes. The sections were subsequently incubated with 3,3′-diaminobenzidine and counterstained with methyl green. The negative controls without primary antibodies were included. The mounted slides were examined under a light microscope. The pathologist assessing the treatment effects was blinded to the treatment groups.

Positive staining was judged if the cell plasma, cell membranes, or nuclei had turned brown. The analysis of immunohistochemical staining was performed by Image-Pro Plus (IPP 6.0, Media Cybernetics) according to the measurement parameter mean density. The mean density was calculated by the formula mean density = integrate optical density/area sum. Intensity was averaged from 10 fields of view. The magnification for the immunohistochemistry study was 400 times.

Cell Culture

The human first-trimester extravillous trophoblast (EVT) cell line TEV-1 was a gift from Dr. S.W. Tsao S.W. (University of Hong Kong, China) (21). Cells were plated in 6-well plates for RT-PCR and ELISA. Cells were cultured at 37°C in a humidified atmosphere with 5% CO₂ in Dulbecco’s modified
Eagle medium (DMEM)/F-12 medium supplemented with 10% heat-inactivated FBS, 25 mmol/L HEPES, 100 U/mL penicillin G, and 100 U/mL streptomycin for 3 days. The serum-free DMEM/F-12 then was used to replace the culture medium. After 12 hours of starvation, cells were divided into four groups according to different treatment times (0, 2, 4, and 8 hours) of LXA4 (200 nM).

Statistics
All statistical analyses were done using SPSS 13.0 software. Numerical results were expressed as mean ± SEM of multiple experiments. The means of the different groups were compared by repeated-measure analysis of variance and Student’s t-test. P < .05 was considered statistically significant.

RESULTS
Serum and Placental Levels of LXA4 Decreased in PE Women
We measured the serum and placental levels of LXA4 in the women to confirm the relationship between LXA4 and PE. The serum and placenta were collected from women in normal pregnancy and women with PE at about 36 weeks of pregnancy, respectively. As shown in Supplemental Figure 1A, the serum LXA4 levels of women with PE were two-fold decreased, compared with women with normal pregnancies. In line with this, the placenta LXA4 levels of women with PE were also significantly lower than those in women with normal pregnancies (Supplemental Fig. 1B). These clinical data suggest that low levels of LXA4 are correlated with women with PE.

Down-regulation of Serum LXA4-synthesizing Enzymes in PE Women
The biosynthesis of LXA4 is catalyzed by different lipoxygenases (LO) and recognized as a transcellular process through cell-cell interactions, including the following: [1] leukocytes use 5-LO to catalyze AA into leukotriene A4, which is then taken up and converted to LXA4 by 12-LO in platelets (22, 23); and [2] epithelial-monocyte 15-LO activity produces 15(S) hydroperoxyeicosatetraenoic acid from AA, which can then be converted by neutrophil 5-LO to generate LXA4 (24). To confirm the above data, we also detected the expressions of 5-LO, 12-LO, and 15-LO in peripheral blood. As shown by RT-PCR and real-time RT-PCR in Supplemental Figure 2A and B, the mRNA expressions of 5-LO, 12-LO, and 15-LO were strikingly down-regulated in PE women in all samples, compared with in women of normal pregnancy (the control group).

Down-regulation of Placenta LXA4-synthesizing Enzymes in PE
We detected the expressions of 5-LO, 12-LO, and 15-LO in placenta. As shown by RT-PCR and real-time RT-PCR in Figure 1A and B, the mRNA expressions of 5-LO, 12-LO, and 15-LO were also strikingly down-regulated in PE women. Similar changes of placental 5-LO, 12-LO, and 15-LO at the protein levels were also confirmed by immunohistochemical staining (Fig. 1C). The 5-LO, 12-LO, and 15-LO were distributed mainly in syncytiotrophoblast cells. Therefore, the down-regulation of LXA4-synthesizing enzymes may be the reason women with PE have much lower levels of LXA4.

FIGURE 1

Placental LXA4-synthesizing enzymes are down-regulated in PE. (A) Placenta samples were collected from women with normal pregnancy at about 36 gestational weeks and from women with PE. The expression of 5-LO, 12-LO, and 15-LO was analyzed by RT-PCR. (B) Placenta samples were collected (n = 15, each group), and the expression of 5-LO, 12-LO, and 15-LO was analyzed by real-time RT-PCR (*P < .001). (C) The placenta immunohistochemical examination of LXA4-synthesizing enzymes (original magnification ×400, bar = 25 μm) and the density mean of 5-LO, 12-LO, and 15-LO (n = 10; *P < .05).

Blocking LXA4 Signaling Induces PE in Pregnant Rats

But was LXA4 really significant in PE? A comparable approach was used to further elucidate the role of LXA4 in PE. The peptide N-Boc-Phe-Leu-Phe-Leu-Phe (Boc, Gen-script), an effective antagonist of FPR2/ALX (11), was used to block LXA4 signaling pathways in pregnant rats, beginning from day 14 of pregnancy at the rate of once per day for 7 days. It was found that the birth fetal weight was significantly lower but that SBP and the urine protein level of the placebo and LXA4 administration groups (Supplemental Fig. 3A–3C), suggesting that LXA4 signaling is critical for PE and that its blocking may result in symptoms of PE and fetal growth restriction.

LXA4 Treatment Improves Symptoms of Experimental PE Rats

To validate the above clinical data in animal models of PE, an LPS-induced rat PE model was constructed. Meanwhile, the rat PE model was treated with LXA4. As expected, the LPS injections resulted in a decrease of LXA4 levels (Fig. 2A). The fetal birth weight of the LPS group was significantly lower than that of the placebo and LXA4 administration groups (Fig. 2B). As shown in Figure 2C, SBP was significantly elevated after the infusion of LPS, compared with the control group. Three days after the LXA4 treatment, blood pressure conditions were obviously improved relative to the LPS group, but they could not be dropped to the control group level. The variation of urine protein is similar to SBP; the urine protein level was significantly higher in the LPS group on day 12 of pregnancy, compared with the control group. But after LXA4 treatment, the expressions of IL-6, TNF-α, and IFN-γ were enhanced, whereas those of IL-10 mRNA declined compared with the control group. Although LXA4 treatment significantly reduced the excretion of urine protein (Fig. 2D). These data suggest that LXA4 is effective in relieving symptoms in experimental PE rats.

LXA4 Ameliorates the Inflammatory Microenvironment in Experimental PE Rats

To address the question of how LXA4 prevented LPS-induced PE, the focus was turned to a set of inflammation-associated factors arising from the proinflammatory properties of LPS. What is interesting is that these inflammation-associated factors (IL-10, IL-6, TNF-α, IFN-γ) were involved in the pathogenesis of PE (25–27). On day 20 of pregnancy, inflammation-associated factors in the blood were detected by RT-PCR and real-time RT-PCR. As shown in Supplemental Figure 4A and B, in LPS–induced experimental PE rats, the expressions of IL-6, TNF-α, and IFN-γ were enhanced whereas those of IL-10 mRNA declined compared with the control group. But after LXA4 treatment, the expressions of IL-6, TNF-α, and IFN-γ were down-regulated, whereas IL-10 mRNA was up-regulated. ELISA results were also consistent (Supplemental Fig. 4C).

Down-regulation of FPR2/ALX in Women with PE and Experimental PE Rats

To address the question of how LXA4 plays a role in women with PE and experimental PE rats, focus was turned to FPR2/ALX. The mRNA expressions of FPR2/ALX in peripheral blood and placenta were detected by RT-PCR and real-time RT-PCR as shown in Figure 3A and B; the mRNA expressions of FPR2/ALX were strikingly down-regulated in women with PE. Similar changes of placental FPR2/ALX at the protein levels were also confirmed by immunohistochemical staining (Fig. 3C). The same happens in experimental PE rats (Fig. 3D–3F). Therefore, the down-regulation of FPR2/ALX inhibits the function of LXA4.
LXA4 is Involved in the Up-regulation of HSD2

Analyzing blood samples of clinical patients with PE showed none of the otherwise expected corresponding increases in the levels of cortisol (Fig. 4A). Given that 11β-hydroxylation is a prerequisite for glucocorticoid functions and is regulated by 11β-HSD1 and 11β-HSD2 (28), there is no doubt that when compared with normal pregnancies, the expression of 11β-HSD1 was not changed, whereas 11β-HSD2 was strikingly decreased in the placenta of PE at both the mRNA and protein levels, as evaluated by conventional RT-PCR, real-time RT-PCR, and immunohistochemical staining, respectively (Fig. 4B and C). Furthermore, 11β-HSD1 and 11β-HSD2 were distributed mainly in syncytiotrophoblast cells. Therefore, in women with PE, reductions in 11β-HSD2 might result in increases in the placenta local activity but not blood cortisol levels. The above data prompted further verification of whether there is some relationship between LXA4 and HSD2. Surprisingly, as shown in Figure 4D and E, in human TEV-1 cells, LXA4 up-regulates HSD2 mRNA expression and protein level, and the effect of up-regulation was related to the role time of LXA4: 4 hours after LXA4 treatment, it reaches the peak and then declines. The same thing happens in experimental PE rats (Fig. 4F and G).

DISCUSSION

The inflammatory response and the endothelial dysfunction are hallmarks of PE; however, the causes of such dysfunction are not fully understood. The present study provides evidence that PE is associated with a deficiency of LXA4, an endogenous anti-inflammatory mediator.

Inflammation has a key role in maintaining tissue homeostasis, while an exaggerated inflammatory reaction or
the lack of a response can lead to disease [29]. The imbalance between an inflammatory and anti-inflammatory pattern of monocytes may play a part in PE pathophysiology [30]. The successful resolution of inflammation and return to tissue homeostasis is a major therapeutic goal in disease management. In fact, the body has to employ an elaborate mechanism to optimally and advantageously regulate inflammation in pregnancy. LXA4, a local endogenous eicosanoid, might play such a role. In counter-regulating the evolvement of inflammation, LXA4 blocks dendritic cell IL-12 production by increasing suppressor of cytokine signaling-2 expression (31) and stimulates phagocytosis of apoptotic cells by reprograming macrophages from M1 to M2 types (32). Furthermore, LXA4 inhibits neutrophil and eosinophil recruitment and down-regulates NF-κB activation [33]. LXA4, inhibiting or quenching inflammation defection and promoting inflammatory resolution at different levels, may be the actual pathogenesis of PE. In this study, the measurements of LXA4 are based on ELISA, although with significant cross-reactivity to other compounds; thus, the identity of the compounds measured will need to be confirmed by another method. The serum and placenta LXA4 levels of women with PE are

**FIGURE 4**

LXA4 up-regulates HSD2. Human: (A) Levels of cortisol were not increased in patients with PE. Serum levels of cortisol were measured in patients with normal pregnancy and in patients with PE (n = 15; P > 0.05). (B) The immunohistochemical examinations of 11β-HSD1 and 11β-HSD2 in human placenta (original magnification ×400, bar = 25 μm). The density mean was calculated, as described in Materials and Methods (P < 0.001). (C) The mRNA expression of 11β-HSD2 was down-regulated in the placenta of PE patients. The human placenta tissue was used to isolate the total RNA, and the expression of 11β-HSD1 and 11β-HSD2 was analyzed by RT-PCR (top) and real-time RT-PCR (bottom, n = 15; P < 0.001). Human TEV-1 cells: Cells were divided into four groups according to different treatment times (0, 2, 4, and 8 hours) of LXA4 (200 nM). (D) The expression of 11β-HSD2 was analyzed by RT-PCR (top) and real-time RT-PCR (bottom, P < 0.001). (E) The levels of 11β-HSD2 were analyzed by ELISA (P < 0.05; P < 0.001). Rat: (F) The expression of 11β-HSD1 and 11β-HSD2 was analyzed by RT-PCR (top) and real-time RT-PCR (bottom, n = 10; P < 0.001). (G) The placenta immunohistochemical examination of 11β-HSD1 and 11β-HSD2 (original magnification ×400, bar = 25 μm) and the density mean of 11β-HSD1 and 11β-HSD2 (n = 10; P < 0.001).

significantly lower than those in women with normal pregnancies. LXA4 is biosynthesized by 5-LO/12-LO or 15-LO/5-LO. The mRNA and protein expressions of 5-LO, 12-LO, and 15-LO are strikingly down-regulated in PE women. What is more, blocking LXA4 signaling induces PE but the symptoms in the experimental PE rats treated by LXA4 improved. That is, in the LPS-induced rat PE model, LXA4 treatment improved symptoms similar to BML-111 treatment. It is suggested that a deficiency of LXA4 may be responsible for PE.

LXA4 may promote inflammatory resolution at different levels. However, whether LXA4 regulates PE inflammation remains unclear. In previous studies, BML-111 inhibited TNF-α and IL-8 generation in experimental PE rats; however, in this study, we further tested whether LXA4 has effects on inflammatory factors (IL-10, IL-6, and IFN-γ). Elevated levels of TNF-α and IL-6 were found in women with PE in previous studies [34, 35]. IL-10 is a pregnancy-compatible cytokine that plays a vital role in maintaining the balance of the anti-inflammatory and pro-inflammatory milieu of the maternal-fetal interface. Recent evidence indicates that IL-10 plays a protective role in PE [5]. IL-10 reduces inflammation and endothelial dysfunction, in addition to blunting hypertension [4], and its deficiency induces PE-like symptoms [27]. This study has demonstrated that LXA4 effectively inhibits LPS-induced proinflammatory factors, including IL-6, TNF-α, and IFN-γ, while increasing IL-10 expression. We propose in this work that endogenous LXA4 is a critical factor in keeping normal pregnancies and avoiding PE by regulating inflammation-associated factors.

Systemic cortisols are not changed, whereas their use in placenta is significantly enhanced in PE. Two distinct isozymes of 11β-HSD catalyze the interconversion of hormonally active cortisol and inactive cortisone: 11β-HSD1 uses NADPH to generate active glucocorticoid; in contrast, 11β-HSD2 uses NAD+ to produce a dehydroxy form of glucocorticoid. The two 11β-HSD enzymes form a metabolic placental barrier by regulating transplacental passage of glucocorticoids and protecting the fetus against excessive exposure [36, 37]. The reduced amount and/or activity of 11β-HSD2 in placenta, and the accompanying increase in exposure of the placenta and embryo/fetus to active germinal centers, could be the result of impaired placental perfusion and hypoxia [38, 39]. In this study, the placenta is characterized by 11β-HSD2 decreases with unchanged 11β-HSD1. However, the principal finding of this study lies in the discovery that LXA4 up-regulates HSD2. Therefore, we hypothesize that LXA4 increases the utility of glucocorticoid, protects placental functions, and avoids the side effect of a high concentration of glucocorticoid.

An increase in reactive oxygen species (ROS) early in pregnancy results in placental abnormalities leading to the maternal symptoms of PE [40]. Aspirin-triggered LXA4 has been found to attenuate LPS-induced intracellular ROS by inhibiting the function of NAPDH oxidase [41]. Combined with our research, we proposed that in normal pregnancy, such an interaction among AA-derived ROS, consequent activation of NF-kB, promotion in the transcription of IL-6 and TNF-α, stimulation of phospholipase A2 (PLA2), release of AA, and its conversion to LXA4 is a well-integrated feedback regulatory system to suppress excess production of ROS and inappropriate inflammation. This built-in feedback system fails when the tissue LXA4 is low, a situation that appears to exist in subjects with PE.

On the basis of the above findings, we conclude that a deficiency of LXA4 may result in PE, which might be ascribed to a reduction in the inflammatory response, oxidative stress, and regulation of 11β-HSD2, providing new insights into PE. This study also suggests that the administration of LXA4 might be a potential strategy against PE.

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Low levels of LXA4 in women with PE. (A) Comparison of serum LXA4 levels between PE and normal pregnancy (201.73 ± 71.91 vs. 552.82 ± 151.35 pg/mL; n = 15; *P<.001). (B) Comparison of placental tissue LXA4 levels between PE and normal pregnancy (140.68 ± 44.74 vs. 246.65 ± 57.91 pg/mL; n = 15; *P<.001).

Serum LXA4-synthesizing enzymes are down-regulated in PE.  
(A) Blood samples were collected from women with normal pregnancy at about 36 gestational weeks or from women with PE. The expression of 5-LO, 12-LO, and 15-LO was analyzed by RT-PCR.  
(B) Blood samples were collected (n = 15, each group), and the expression of 5-LO, 12-LO, and 15-LO was analyzed by real-time RT-PCR (*P < .001).

SUPPLEMENTAL FIGURE 3

Blocking LXA4 signaling induces PE. Rats on day 14 of pregnancy were treated with BOC (20 mg/kg) once per day for 7 days. (A) The fetal weight of the BOC group was significantly lower (3.52 ± 0.64 g), compared with that of the sterile saline administration group (4.12 ± 0.54 g; n = 10; P < .05). (B) The SBPs of the BOC group at days 18 and 20 of pregnancy were 112 ± 5 and 114 ± 6 mmHg, respectively (n = 10; *P < .01, vs. the control group, 105 ± 5 and 102 ± 4 mmHg at the corresponding time point). (C) Twenty-four-hour urinary protein excretions on days 12 and 18 of pregnancy were presented. There were no differences between the two groups on day 12 of pregnancy (P > .05), but on day 18 of pregnancy, the urine protein of the BOC group (0.61 ± 0.08 mg/24 hours) was significantly higher than that of the control group (0.42 ± 0.07 mg/24 hours; n = 10; *P < .001).

LXA4 antagonized the proinflammatory effects of LPS. Twenty-four hours after LXA4 treatment, (A) the inflammation-associated factors in the blood were analyzed by RT-PCR. (B) The analysis of inflammation-associated factors by real-time RT-PCR (n = 10; *P < .001). (C) The protein levels of IL-10, IL-6, TNF-α, and IFN-γ in the serum were analyzed by ELISA (n = 10; *P < .001).