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Correlation of long-chain fatty acid oxidation with oxidative stress and inflammation in pre-eclampsia-like mouse models

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ABSTRACT

Introduction: Pre-eclampsia has been further recognized as a syndrome during pregnancy. Recent studies have found that long-chain fatty acid oxidation (FAO) disorders may be associated with some of pre-eclampsia. However, the mechanism remains unclear. In this study, we investigated the role of FAO and its relationship with oxidative stress and inflammatory signaling pathways in the pathogenesis of pre-eclampsia.

Methods: PE-like groups included ApoC3 transgenic mice with abnormal fatty acid metabolism, classical PE-like models with injection of Nω-nitro-L-arginine-methyl ester (L-NA) or lipopolysaccharide (LPS), and antiphospholipid syndrome (APS) mouse model with β2GPI injection. The control group included wild-type mice with normal saline injection. Serum FFA was compared and placental and hepatic LCHAD, p47phox and NF-κB mRNA and protein were detected using real-time quantitative PCR and western blot.

Results: FFA levels were significantly increased and were positively correlated with P47phox and NF-κB mRNA and protein expression in liver of all groups ($p < 0.05$), except LPS group ($p < 0.05$) as compared to control. LCHAD mRNA and protein expression in the liver and placenta was significantly increased in ApoC3+NS, ApoC3+L-NA, and β2GPI group, whereas decreased in L-NA group ($p < 0.05$) as compared to the control group. P47phox mRNA, NF-κB mRNA, and protein expression in the liver of all groups, except in LPS and in the placenta of β2GPI and L-NA groups, significantly increased ($p < 0.05$). Discussion: FAO disorders were involved in the pathogenesis of pre-eclampsia through oxidative stress and inflammatory endothelial cell injury.

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1. Introduction

Pre-eclampsia, a serious obstetric complication is one of the major causes of maternal and perinatal mortality. In recent years, it has been further recognized that pre-eclampsia is a common syndrome during pregnancy; however, its pathogenesis remains unclear. Some studies, at home and abroad, including ours have demonstrated that long-chain fatty acid oxidation (FAO) is associated with pathogenesis of some pre-eclampsia.

Results from various *in vitro* experiments have shown reduced FAO capability in the placenta of pre-eclampsia patients [1]. Robinson et al. have demonstrated that the plasma of pre-eclampsia patients could cause lipid droplets aggregation and mitochondrial dehydrogenase activity decrease in *in vitro* cultured human umbilical vein endothelial cells [2]. In our previous study, it was reported that the expression of LCHAD was decreased in the placental tissue of patients with early onset severe pre-eclampsia, while there was no significant difference between late onset pre-eclampsia and the control group [3]. In animal models, we found obvious pathological changes and decreased LCHAD expression in the placenta of early- and mid-onset pre-eclampsia-like (PE-L) mouse models [4]. These studies showed that FAO disorders due to decreased LCHAD played an important role in the occurrence and development of pre-eclampsia.

We opined that the abnormal FAO may be one of the initiating factors in the occurrence and development of pre-eclampsia. Free fatty acids (FFA) can result in oxidative stress. Nicotinamide adenine dinucleotide phosphate (NADPH) oxidase is the main source of reactive oxygen species (ROS). NADPH is composed by 6 subunits, including gp91phox, p22phox, p47phox, p67phox, p40phox, and Rac [5]. In our previous study, it was reported that

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p47phox mRNA and protein expression was increased in placenta of early- and mid-onset PE-L mouse model, which proved that NADPH was associated with the pathogenesis of pre-eclampsia through p47phox [6]. Raijmakers et al. [7] found that NADPH activity and superoxide generation existed in human placenta. In addition, early-onset pre-eclampsia placenta exhibited higher total superoxide levels as compared to the late-onset pre-eclampsia. Peroxidation reaction, between the excessive free radicals and lipid, results in malondialdehyde (MDA) production. Many studies have reported higher MDA levels in serum and placenta of patients with pre-eclampsia as compared to normal pregnant women [8,9]. Free radicals generated during oxidative stress lead to mitochondrial damage, which affects the mitochondrial FAO. Above findings reported a correlation between oxidative stress and FAO [10].

ROS, as the second messenger in cellular signaling cascade, activates many redox-sensitive signaling pathways, including the nuclear factor-κB (NF-κB) [11]. Shah et al. [12] reported the expression of NF-κB and infiltration of neutrophil in blood vessels of subcutaneous adipose tissue of patients with pre-eclampsia by immunohistochemical staining. Adipocytes can activate NF-κB pathway in vascular endothelial cells by secreting inflammatory cytokines, which results in endothelial cell injury. The activated NF-κB regulates transcription of tumor necrosis factor alpha (TNF-α), intercellular adhesion molecule-1 (ICAM-1) and other inflammatory factors. There may be a positive-feedback relationship between FFA and NF-κB signaling pathway. Takacs et al. [13] reported serum MDA concentration increased in patients with pre-eclampsia and NF-κB activity and ICAM-1 expression increased in human umbilical vein endothelial cells cultured with plasma of pre-eclampsia patients. This study demonstrated that oxidative stress, lipid metabolism, and inflammatory endothelial injury were associated with the pathogenesis of pre-eclampsia.

In summary, the long-chain FAO disorders play an important role in oxidative stress and endothelial cell activation and injury. Our previous studies have shown the presence of different FAO disorders in different PE-L mouse models [14]. However, the relationship between FAO disorders and oxidative stress and endothelial injury in different factor-induced and different onset time pre-eclampsia is unclear, which is the focus of our present study.

2. Materials and methods

2.1. Establishment and identification of animal models

The animal experiments were approved by the Animal Care Committee and Medical Ethics Committee of Peking University, China. C57BL/6J mice were purchased from the Laboratory Animal Science Department of Peking University Health Science. ApoC3 transgenic mice were provided by the Institute of Cardiovascular Sciences, Peking University Health Science Center. Eight- to 10-week-old females and 10- to 14-week-old males were housed at Specific-pathogen-free (SPF) environment with the temperature (23 ± 2 °C), relative humidity (55 ± 10%), illumination time for 12L: 12D and with available chow and water *ad libitum*. The mice were mated at a ratio of 2:1 females to males. Females were inspected daily for vaginal plugs and were designated as day 1 of pregnancy.

Pregnant mice were randomized into ApoC3, L-NA, LPS, and β2GPI PE-L model groups. Furthermore, except β2GPI group, other PE-L groups were further divided into early pregnancy (Early) and mid pregnancy (Mid) sub-groups according to the time of injection. We chose 10 mice for each subgroup. The wild-type and transgenic mice were subcutaneously injected with nitric oxide synthase inhibitor L-NAME (Sigma, USA) (50 mg/kg/d) daily [15,16] from day 7 or 11 of pregnancy to establish L-NA and ApoC3+L-NA groups, respectively. Wild-type mice were injected with a single injection of an ultra-low dose of lipopolysaccharide (LPS) (1 μg/kg body weight, Sigma) [17,18] to establish the LPS group. The β2GPI group was established as our previous work [19]. Wild-type mice in the control group and transgenic mice in the ApoC3+NS group were injected daily with physiological saline on day 7 or 11 of pregnancy.

From day 2 of gestation, a CODA non-invasive tail-cuff acquisition system (Kent Scientific Corp., USA) was used to measure blood pressure every 2 days. The mice were placed in standard metabolism cages before the day of sample collection and 24-h urine was collected. The urinary protein was detected using a protein assay kit (Bio-Rad, USA).

2.2. Sample collection

All the mice were anesthetized with 10% chloral hydrate (3 ml/kg) on day 14 and 18 of pregnancy. Blood samples, taken immediately from the retro-orbital plexus, were centrifuged and serum was collected. Cesarean section was performed, and the placenta and liver tissues were collected and frozen at −80 °C for mRNA and protein detection. Finally, the mice were sacrificed by cervical dislocation.

2.3. Serum FFA assay

Serum FFA levels were assayed using a chemical analysis kit (Wako Chemicals, Japan) according to the instructions.

2.4. Quantitative real-time PCR

TRIzol reagent (Sigma, USA) was used to extract total RNA from liver and placenta. Total RNA, 1 μg, was reverse-transcribed to complementary deoxyribonucleic acid (cDNA) using Revert Aid First Strand cDNA Synthesis Kit (Thermo, USA). The real-time quantitative polymerase chain reaction (PCR) reaction system involved SYBR Select Master Mix reagent (Invitrogen Life Technologies, USA) and PCR amplification involved a 7500 Real-Time PCR System (Life Technology, USA). Primer sequences used for the synthesis of primer (Sangon Biotech, Shanghai) were as follows: LCHAD, forward: 5′-TGCATTTGCCGCAGCTTTAC-3′; reverse: 5′-GTTGGCCCAGATTTCGTTCA-3′; P47phox, forward: 5′-ACACCTT-CATTCGCCATATTGC-3′; reverse: 5′-CCTGCCACTTAACCAGGAACA-3′; NF-κB, forward: 5′-ATGGCAGACGATGATCCCTAC-3′; reverse: 5′-CGGAATCGAAATCCCCTCTGTT-3′; GAPDH (as an internal control) forward: 5′-TGATGACATCAAGAAGGTGGTGAAG-3′; reverse: 5′-TCCTTGGAGGCCATGTAGGCCAT-3′. PCR conditions were 94 °C for 2 min; 55–60 °C for 30 s, and 72 °C for 1 min, 40 cycles.

2.5. Western blot analysis

Protein was extracted from liver and placenta tissues by RIPA lysis buffer (cwbiotech, China) with addition of protease inhibitors (Pierce, USA). An equal amount of protein sample was used for electrophoresis in 8% or 10% polyacrylamide gel and was transferred to a 0.45-μm polyvinylidene difluoride (PVDF) membrane (Millipore, USA), which was blocked with 5% milk (BD, USA) at room temperature for 1 h. The membrane was incubated with primary antibodies rabbit anti-mouse LCHAD (Abcam, UK; 1:500), rabbit anti-mouse P47phox (Santa Cruz, USA; 1:500), NF-κB (Cell Signaling, USA; 1:1000), and rabbit anti-mouse β-actin (Cell Signaling, USA; 1:1000) at 4 °C overnight. The next day membranes were washed 5 times at room temperature for 5 min. This was followed by the addition of horseradish peroxidase-conjugated secondary antibody (1:10,000, Thermo, USA) for incubation at room temperature for 1 h. The membranes were again washed 5 times for 5 min. The KODAK gel logic 4000 MM PRO imaging system (Kodak, USA) was used for

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