Placenta 58 (2017) 9–16

Contents lists available at ScienceDirect

Placenta

journal homepage: www.elsevier.com/locate/placenta

Involvement of dysregulated IK_{Ca} and SK_{Ca} channels in preeclampsia

Fan-fan Li, Meng-zhou He, Yin Xie, Yuan-yuan Wu, Mei-tao Yang, Yao Fan, Fu-yuan Qiao, Dong-rui Deng*

Department of Gynecology and Obstetrics, Tongji Hospital, Tongji Medical College, Huazhong University of Science and Technology, Wuhan 430030, China

ARTICLE INFO

Article history:
Received 23 May 2017
Received in revised form
25 July 2017
Accepted 31 July 2017

Keywords:
Preeclampsia
CPAs
IK_{Ca} and SK_{Ca} channels
NO
Vasorelaxation

ABSTRACT

Introduction: Excessive constriction of placental chorionic plate arteries (CPAs) may be associated with preeclampsia (PE). Nitric oxide (NO) as well as intermediate and small Ca^{2+}-activated K^+ channels (IK_{Ca} and SK_{Ca}) plays vital roles in vasodilation of CPAs. We hypothesized that dysregulated IK_{Ca} and SK_{Ca} channels may be involved in the pathogenesis of PE mediated by the impaired NO system on CPAs.

Methods: The location of IK_{Ca} and SK_{Ca} channels, activities of NO synthases (NOS), and expression levels of these molecules were studied on CPAs from 30 normal pregnancies and 30 PE. The vasodilating function of CPAs was measured under openers or blockers of IK_{Ca}/SK_{Ca} channels in the presence or absence of NO donor or inhibitor.

Results: IK_{Ca} and SK_{Ca} channels were located both on endothelium and on smooth muscles of CPAs and the expressions of them were downregulated in PE women comparing to those in normal pregnant women. The protein expressions of endothelial NOS (eNOS) and inducible NOS (iNOS) were down-regulated on CPAs in PE accompanied by decreased activity of eNOS. Notably, the vasodilatory functions mediated by IK_{Ca}/SK_{Ca} channels and by NO were aberrant on preeclamptic CPAs. In addition, IK_{Ca} and SK_{Ca} channels were responsible for nitric oxide (NO)-attributable vasorelaxation and activity modulation of NO synthases.

Conclusions: This study provides evidence that dysregulated IK_{Ca} and SK_{Ca} channels might contribute to fetal pathogenesis of PE through direct promotion of vascular constriction of CPAs and through affecting functions of NO and activities of NOS.

1. Introduction

Preeclampsia (PE), as one of the most common complications in obstetrics, is the leading cause of maternal and perinatal morbidity and mortality [1,2]. Termination of pregnancy is still considered as the best treatment option in severe cases, regardless of fetal immaturity, which, in turn, gives rise to an elevated number of premature infants and conveys further developmental complications. Unfortunately, the incidence of PE has been increasing in the past decade [3]. Uncovering the pathogenesis and mechanisms of PE has persistently been a great challenge for obstetricians over the years.

Successful pregnancy requires complex vascular adaptions which decrease resistance and facilitate vasodilation throughout the systemic circulation [4] and the feto-placental circulation. The adaptations on the feto-placental vasculature promote the ultimate establishment of a low-resistance placental circulation, which is critical to enable the substantive increase in feto-placental blood flow [5]. It is necessary for sustaining the developing fetus with an effective supply of oxygen and nutrients, and adequate removal of metabolic waste products. Any impairment in the feto-placental circulation could increase the possibilities of growth retardation of the fetus in utero, fetal distress, and intrauterine fetal death [6]. Previous studies have shown that the control of vascular tone in the feto-placental circulation may be impaired in PE [7-9]. However, the regulatory mechanisms are incompletely understood. Remarkably, since the feto-placental blood vessels lack autonomic innervation and respond poorly to potent vasoactive agents of the systemic circulation [7,10], the regulation of vasorelaxation depends mainly on local vasodilating substances such as nitric oxide (NO), prostacyclin and endothelium-derived hyperpolarizing factors (EDHF). NO produced by endothelial cells causes vasodilated state apart from preventing platelet aggregation and adhesion of platelets to endothelial cells [11]. However, the relative contribution of NO to vasodilation is variable depending on the vascular bed

* Corresponding author.
E-mail address: tjdengdongrui@126.com (D.-r. Deng).

http://dx.doi.org/10.1016/j.placenta.2017.07.361
0143-4004/

and vessel size [12]. Accumulating evidence showed that EDHF were more potent than NO in terms of blood pressure regulation of small vessels and seemed predominant in endothelium-dependent vasorelaxation in the absence of NO pathway [13,14]. The endothelial cell intermediate- and small-conductance K_{Ca} channels (IK_{Ca} and SK_{Ca}, respectively) are major components of EDHF [13,15]. The notion that IK_{Ca} and SK_{Ca} channels contribute greatly to the regulation of blood pressure has been confirmed by a series of novel genetic approaches. For instance, IK_{Ca}-deficient mice exhibited a significant increase in arterial blood pressure and reduced endothelial and smooth muscle hyperpolarization in response to acetylcholine [16]. Mark S. Taylor et al. discovered that SK3, a subtype of SK_{Ca}, exerted a profound, tonic and hyperpolarizing influence on resistance arteries of $SK3^{T/T}$ mice and played a fundamental role in determining vascular tone and blood pressure [17]. More importantly, recent studies demonstrated that IK_{Ca}/SK_{Ca} channels might be associated with oxidative stress, which is considered involved in PE. It has been proved that hypoxia suppressed pregnancy-induced upregulation of SK_{Ca} channel on the uterine arteries of sheep [18], and in porcine coronary arteries, hypoxia markedly reduced endothelial K^+ currents related to IK_{Ca} and SK_{Ca} with downregulation of protein expression [19]. Although the roles of NO and IK_{Ca}/SK_{Ca} in maintaining vascular tone have been well recognized, the functional link between them in mediating vasorelaxation of placental chorionic plate resistance arteries (CPAs) and the relationship of them with PE are far from clear. In the present study, the expressions of IK_{Ca}/SK_{Ca} channels and NO syntheses (NOS) on CPAs, and the vasodilatory functions mediated by IK_{Ca}/SK_{Ca} and NO were compared between normal pregnant and PE women. Furthermore, the roles of IK_{Ca}/SK_{Ca} channels in NO-dependent vasodilation and in activity modulation of NOS were explored to figure out the mechanism by which the impaired NO pathway underlied PE [20].

2. Materials and methods

2.1. Patients

Placentas were collected from women who delivered at Tongji Hospital, Wuhan and they were divided into two groups: normal pregnancy and PE ($n = 30$ per group). The PE patients were classified according to the American College of Obstetricians and Gynecologist criteria [21]: (1) Blood pressure was greater than or equal to 140 mmHg systolic or greater than or equal to 90 mmHg diastolic on two occasions at least 4 h apart after 20 weeks of gestation in a woman with a previously normal blood pressure. (2) Proteinuria was greater than or equal to 300 mg per 24-h urine collection or protein/creatinine ration was greater than or equal to 0.3 plus dipstick reading of 1+. Or in the absence of proteinuria, diagnosis of hypertension with the onset of any of the following: thrombocytopenia, renal insufficiency, impaired liver function, pulmonary edema and cerebral or visual symptoms. All women were matched for age and gestational age. The study was approved by the local ethics committee. Patient consent was obtained prior to delivery.

2.2. Procurement of vascular tissue

CPAs (diameter$\leq$500 μm) were dissected as soon as the placenta was taken out from uterus after parturition. Vessels were carefully freed of connective tissues, washed in sterile PSS, and divided into three parts. A 3 mm vessel ring was cut off for the measurement of myogenic tone. A very small section of vessels was placed into paraformaldehyde for immunohistochemical analysis and the rest were kept at −80 °C awaiting further protein or mRNA analysis.

2.3. Immunohistochemistry

CPAs were fixed in 4% paraformaldehyde, embedded in paraffin, and sectioned. Immunohistochemistry was performed using the method of SP (steptavdin-perioxidase-biotin) staining. Sections were blocked with 2% BSA for 2 h and incubated overnight with a rabbit anti-IK1 and-SK3 poly antibody (Alomone labs, Israel). They were subsequently incubated for 30 min with appropriate secondary antibodies. The resulting products were visualized by peroxides-conjugated streptavidin system with 3,3-diaminobenzibine as substrate (Zhongshan Goldenbridge Biotechnology, China). Cells were considered positive if their cytoplasm or nucleus had brownish staining. Each slice randomly selected non-overlapping, equal area of five horizons, using Image J software to automate the K_{Ca} (IK_{Ca} or SK_{Ca}) identification. K_{Ca}-marked area (area +) and integrated optical density (IOD; antibody color intensity measure, which reflects the amount of K_{Ca} present in the tissue) were measured. The average optical density (AOD, equal to IOD/area +) was used to represent the expression intensities of IK_{Ca} and SK_{Ca}.

2.4. Western blot analysis

CPAs were homogenized in cold RIPA buffer [50 mmol/l Tris·HCl (pH 7.4), 0.15 mmol/l NaCl, 0.25% deoxycholic acid, 1% Nonidet P-40, and 1 mmol/l EDTA] enriched with 1 mmol/l cocktail. The homogenate was separated by centrifugation at 15,000 g for 20 min at 4 °C. The supernatant was kept on ice. Total protein concentration was measured with a protein assay kit (Bio-Rad, USA). Equal level of protein samples was loaded and separated by 10% SDS-PAGE and subsequently transferred to polyvinylidenedifluoride membrane (Millipore Biosciences, Singapore). Membranes were blocked by treatment with 5% BSA in Tris-buffered saline containing 0.05% Tween 20 (TBST) for 1 h at room temperature, probed with anti-IK1 (1:400), anti- SK3 (1:400), anti-eNOS (1:500; Abcam, UK) and anti-iNOS (1:500; Abcam, UK) and kept overnight at 4 °C. After washing (3 times for 10 min each) in TBST, blots were incubated with anti-mouse or anti-rabbit secondary antibodies (1:2000; Santa Cruz Biotechnology, USA) at room temperature for 1 h and then washed (3 times for 20 min each) in TBST. Immunoblotting signals were revealed with enhanced chemiluminescence (Amersham Biosciences, USA) and exposed to an X-film (Kodak, USA). Densitometry analysis was performed using ImageLab.

2.5. Real time RT-PCR amplification of K_{Ca} channel genes

CPAs were homogenized and total RNAs were isolated with TRIzol reagent (Invitrogen Life Technologies Incorporation, USA) according to the manufacturer's instructions. RNA concentration was measured by spectrophotometry, and equal amounts of RNAs were reversely transcribed. 10 μL of cDNAs were amplified with reverse kit (Toyobo Life Science Incorporation, Japan). Real-time quantitative PCR was performed in an Mx 3000pTM real-time PCR System (Applied Biosystems Incorporation, USA). Primer sequences for the IK1 gene were as follows: forward primer, 5′-GCTGCTGCGTCTCTACCTG-3′; reverse primer, 5′-AAGCGGACTTGATTGAGAGCG-3′. Primer sequences for the SK3 gene were as follows: forward primer, 5′-GGCGGATAGCCATGACCTAC-3′; reverse primer, 5′-CGTGCCGTCCAGAAGAACTT-3′. Primer sequences for GAPDH were as follows: forward primer, 5′-CGGAGTCAACGGATTTGGTCGTAT-3′; reverse primer, 5′-AGCCTTCTCCATGGTGGTGAAGAC-3′. 1 μL of cDNAs were amplified with SYBR Green Universal PCR Master mix (Toyobo Life Science, Japan) according to the following protocol: 95 °C for 5 min, 39 cycles at 60 °C for 30 s and at 72 °C for 30 s, following 95 °C for 15 s. The samples,