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Alterations in antioxidant system, mitochondrial biogenesis and autophagy in preeclamptic myometrium

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ABSTRACT

Preeclampsia is a pregnancy complication which causes significant maternal and fetal morbidity and mortality worldwide. Although intensive research has been performed in the last 40 years, the pathology of preeclampsia is still poorly understood. The present work is a comparative study of the myometrium of women with normal pregnancy, and those with late- and early-onset preeclampsia (n = 10 for each group). We observed significant changes in the levels of antioxidant enzymes, markers of mitochondrial biogenesis and autophagy proteins in preeclamptic myometrium. Levels of superoxide dismutase 1 and catalase were lower in both preeclamptic groups than the control group. In late-onset preeclampsia, expression levels of essential mitochondria-related proteins VDAC1, TFAM, hexokinase 1, PGC-1α and PGC-1β, and autophagy marker LC3A, were significantly elevated. In the myometrium of the early-onset preeclampsia group OPA1 and Bcl-2 were up-regulated compared to those of the control ($p < 0.05$). These findings suggest that crucial molecular changes in the maternal myometrium occur with the development of preeclampsia.

1. Introduction

Preeclampsia (PE) is a multisystem disorder which affects approximately 6% of pregnant women worldwide, and still remains a leading cause of maternal and perinatal morbidity and mortality [1,2]. Despite the large body of data on the molecular changes associated with PE, its etiology is still poorly understood. There are two clinically-distinct PE phenotypes that vary in the time of onset: early-onset PE (eoPE), which occurs before 34 weeks, and late-onset PE (loPE), which takes place after 34 weeks of gestation [3]. PE is thought to be associated with impaired trophoblast invasion into the myometrial segment of the spiral artery [4–6]. Subsequent disturbance of placental oxygenation results in permanent ischemia/reperfusion and induction of oxidative stress in the placenta and maternal blood [7–10]. The present study is an investigation into myometrial tissue from patients with normal pregnancy and those with PE. Myometrium is an uterine muscle, composed of three poorly defined layers (inner, outer and middle), and is rich in blood vessels [11]. A number of studies have demonstrated alterations in the expression of vascular tension modulators in myometrial tissue in PE [12–14]. However, very few studies have focused on the antioxidant system, mitochondrial apparatus and autophagy in myometrium, despite them being highly interconnected [15]. Free radicals, derived from preeclamptic placenta, induce the circulation of reactive oxygen species (ROS) and oxidation products of biomolecules in the blood. This may affect the functionality of myometrium and endothelial cells by influencing a wide range of cellular processes [10]. Since mitochondria and NADPH oxidase are considered major sources of ROS in PE placentas [16,17], it is important to evaluate the expression of antioxidant enzymes, state of ROS-sensitive mitochondrial network [18], and level of autophagy in the neighbouring myometrium.

2. Material and methods

2.1. Ethics statement

All procedures and experimental protocols involving myometrial tissue were conducted in accordance with the Declaration of Helsinki, Guidelines for Good Clinical Practice and Committee on Biomedical Research Ethics of Research Center for Obstetrics, Gynecology and Perinatology, Ministry of Healthcare of the Russian Federation. All the patients signed informed consent in accordance with the Ethics Committee requirements and Helsinki Declaration of the World Medical Association.

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2.2. Sample collection

Myometrial samples were collected immediately after delivery by elective caesarean section, proposed on clinical grounds for women with normal pregnancies, eoPE or loPE, in the Research Center for Obstetrics, Gynecology and Perinatology, Moscow. PE was diagnosed according to common medical criteria [3]. Myometrial biopsy (0.5 × 0.5 × 0.5 cm) was obtained from the upper edge of lower segment uterine incision, snap frozen in liquid nitrogen and stored at − 80 °C until used.

2.3. RNA extraction and reverse transcription

Total RNA was isolated using Extract RNA Reagent (Evrogen, Russia) after homogenisation of myometrial tissue in liquid nitrogen. RNA concentration and 260/280 ratio was measured using a spectrophotometer DS-11 (DeNovix, USA). The integrity of RNA was confirmed by 1.5% agarose gel electrophoresis. For the reverse transcription reaction, 0.5 μg of total RNA was reverse transcribed using the MMLV-RT kit (Evrogen, Russia) with random hexamer primers.

2.4. Quantitative real-time PCR

Quantification of mRNA was conducted using DT-96 Real-Time Detection Thermocycler (DNA-Technology LLC, Russia). The reactions were carried out in duplicate in volumes of 10 μl, containing 50 ng of cDNA, 300 nM of each primer, and 2 μl of 5xSybrGreen-mix (Evrogen, Russia). All primers (Supplementary Table S1) were generated by Primer-BLAST [19]. Specificity of the amplified fragments was confirmed by melting curve analysis and electrophoresis of the PCR products on 1.5% agarose gel. The PCR program consisted of an initial step at 95 °C for 5 min, followed by 45 cycles of denaturation at 95 °C for 10 s, annealing at 60 °C for 20 s, and elongation at 67 °C for 20 s, followed by melting at a gradient from 65 °C to 95 °C. Relative gene expression was determined as the ratio of the target gene to the internal reference gene expression (β-actin) based on Ct values, using QGENE software [20].

2.5. mtDNA copy number

DNA extraction from myometrial homogenate was conducted using DNA Extran-2 kit (Syntol, Russia). The mtDNA content was measured by Real-Time PCR, normalising the quantity of a non-polymorphic region of D-loop with a single copy nuclear gene (β-2-microglobulin). Primer sequences are shown in Supplementary Table S1. 100 ng of total DNA was analysed in duplicate under the following conditions: 50 °C for 2 min, 95 °C for 20 s, followed by 45 cycles of denaturation at 95 °C for 15 s, annealing at 63 °C for 20 s, and elongation at 67 °C for 20 s, followed by melting at a gradient from 65 °C to 95 °C. Relative quantification values were calculated by the $2^{-\Delta Ct}$ method [21].

2.6. Activity of citrate synthase

Citrate synthase activity was determined in myometrial tissue homogenate at a wavelength of 412 nm as described by Eigentler et al. [22].

2.7. Western blot analysis

Sample preparation and immunoblotting were performed as previously described [23]. Membranes were incubated with primary antibodies (anti-SOD1–ab13498; anti-SOD2–ab16956; anti-catalase-ab76024; anti-GPx1–ab108427; anti-VDAC1–ab154856; anti-TFAM–ab155240; anti-PGC-1α–ab77210; anti-PGC-1β–ab176328; anti-OPA1–ab119685; anti-MFN1–ab57602; anti-MFN2–ab56889; anti-DRP1–ab56788; anti-LC3A–ab52628; anti-HK1–ab55144, all–Abcam, USA; anti-beta-actin–MA5-15739, anti-Bcl-2-13-8800, Invitrogen, USA) overnight at 4 °C with gentle shaking. After washing, the membranes were incubated with peroxidase-conjugated secondary antibodies for 1 h at room temperature. Target proteins were detected using Novex ECL Kit (Invitrogen, USA) in ChemiDoc station (Biorad, USA). Optical densities of the protein bands were measured using ImageLab Software. Protein content was normalised on β-actin.

2.8. Statistical analysis

Data is presented as mean ± standard error mean (SEM). The Shapiro-Wilk normality test was used to estimate distribution [24]. One-way analysis of variance (ANOVA) followed by the Tukey's post-hoc test was used to identify differences among multiple groups with normal distribution. One-way Kruskal-Wallis non-parametric ANOVA followed by the post-hoc Dunn test was used to calculate statistical differences for non-normal distributions. All calculations were performed by Prism 7.0 software (GraphPad, USA) and Website VassarStats for Statistical Computation (www.vassarstats.net). p-Value < 0.05 was considered significant and was indicative of the differences in comparison to control.

3. Results

3.1. Clinical data

Clinical and demographic data of the study patients are summarized in Table 1. Women with early-onset and late-onset PE showed significantly increased systolic and diastolic blood pressure, and proteinuria, in comparison with normal pregnancies. Lower baby weight and intrauterine growth restriction (IUGR) were observed in both PE types.

3.2. Decline of antioxidant system in preeclamptic myometrium

To evaluate the antioxidant system in preeclamptic and control

Table 1
Clinical and demographic characteristics of patients. Data is listed as mean ± SEM.

Characteristics	Control	loPE	eoPE
Number	10	10	10
Maternal age, years	32.7 ± 1.4	30.2 ± 1.1	33.7 ± 1.2
Gestational age at delivery, weeks	39.8 ± 0.1	38.0 ± 0.3	31.8 ± 0.4*
Body mass index before delivery, kg/m^2	26.6 ± 1.0	28.7 ± 1.0	28.9 ± 1.5
Systolic blood pressure, mm Hg	112.3 ± 1.2	152.2 ± 3.1*	163.7 ± 4.5*
Diastolic blood pressure, mm Hg	73.2 ± 1.2	95.7 ± 2.4*	99.9 ± 2.1*
Proteinuria, mg/dL	ND	100.9 ± 50.0*	168.2 ± 40.2*
Sex of the baby (male/female), %	70/30	60/40	50/50
Intrauterine growth restriction, %	ND	30*	60*
Baby mass, g	3395.5 ± 128.1	2799.3 ± 192.8*	1523.1 ± 146.9*

ND–not detected.
* $p < 0.05$ versus control.