General and Comparative Endocrinology 225 (2016) 61–70

Contents lists available at ScienceDirect

General and Comparative Endocrinology

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

Glucocorticoid exposure in early placentation induces preeclampsia in rats via interfering trophoblast development

Dongxin Zhang [a,b,1], Haojing Liu [c,1], Ji Zeng [a], Xili Miao [a], Wei Huang [b], Hongxiang Chen [d], Yinping Huang [e], Yongsheng Li [f], Duyun Ye [b,*]

[a] Department of Clinical Laboratory, Puai Hospital, Tongji Medical College, Huazhong University of Science and Technology, Wuhan 430033, People's Republic of China
[b] Department of Pathophysiology, Tongji Medical College, Huazhong University of Science and Technology, Wuhan 430030, People's Republic of China
[c] Department of Internal Medicine, Puai Hospital, Tongji Medical College, Huazhong University of Science and Technology, Wuhan 430033, People's Republic of China
[d] Department of Dermatology, Union Hospital, Tongji Medical College, Huazhong University of Science and Technology, Wuhan 430022, People's Republic of China
[e] Department of Gynecology and Obstetrics, First Affiliated Hospital, Wenzhou Medical University, Wenzhou 325003, People's Republic of China
[f] Center for Experimental Therapeutics and Reperfusion Injury, Department of Anesthesiology, Perioperative and Pain Medicine, Brigham and Women's Hospital and Harvard Medical School, Boston, MA 02115, USA

ARTICLE INFO

Article history:
Received 3 July 2015
Revised 18 August 2015
Accepted 17 September 2015
Available online 25 September 2015

Keywords:
Glucocorticoid
Preeclampsia
Placentation
Trophoblast development
Epithelial–mesenchymal transition

ABSTRACT

In pregnancy, placenta can be exposed to glucocorticoids (GCs) *via* several ways, which may disturb placentation and adversely affect pregnancy. Preeclampsia (PE) is thought to be attributed, in part, to impaired trophoblast development. The purpose of the present study was to confirm that GC exposure in early placentation could lead to PE in rats, with the mechanisms involving dysregulated trophoblast development. In the study, pregnant rats were administered with 2.5 mg/kg Dex subcutaneously once per day from gestational day 7 to 13. Maternal systolic blood pressure and urinary albumin were increased, while both fetus and placenta were restricted after GC exposure relative to the control group. GC exposure also contributed to placental abnormalities and renal impairment. Moreover, placental oxidative damage was increased along with placental hypoxia-inducible factor 1-alpha (HIF1A) overexpression after GC treatment. Mechanically, GC induced PE in rat partially through inhibiting trophoblast proliferation, migration, invasion and epithelial–mesenchymal transition (EMT), which involved phospho-extracellular signal regulated kinase (p-ERK) downregulation. Furthermore, GC receptor was required for the inhibition of GC on trophoblast proliferation, migration, invasion and EMT *in vitro*. These findings suggest that GC exposure in early placentation could contribute to PE in pregnant rats, with the mechanisms involving inhibition of trophoblast proliferation, migration, invasion and EMT by GC.

1. Introduction

Preeclampsia (PE), characterized by maternal hypertension, proteinuria and other systemic disorders occurring after 20 weeks of gestation, is a leading cause of maternal and fetal morbidity and mortality (Noris et al., 2005; Redman and Sargent, 2005; Young et al., 2010). PE, originating in placenta abnormalities, is induced by the causative mediators derived from damaged placenta (Redman and Sargent, 2005; Warrington et al., 2013). During early placentation, invasive extravillous trophoblasts replace the endothelial layer of uterine spiral arteries, and transform them from high-resistance, small-diameter spiral arteries into high-capacitance, low-resistance vessels (Redman and Sargent, 2005; Warrington et al., 2013; Young et al., 2010). Although the exact pathogenesis of PE remains unclear, failure of trophoblasts to adequately invade and remodel spiral arteries is widely recognized as a major predisposing factor for PE (Redman and Sargent, 2005; Warrington et al., 2013).

During pregnancy, placenta can be exposed to glucocorticoids (GCs) *via* several ways, including maternal stress, synthetic GCs administration and cortisol metabolism damage (Michael and Papageorghiou, 2008; Xu et al., 2013). Maternal stress or GCs abnormality may result in adverse pregnancy outcomes (Klebanoff et al., 1990; László et al., 2013; Michael and Papageorghiou, 2008; Woods et al., 2010). We demonstrated recently that stress-mediated increase in GC levels is directly

* Corresponding author at: Department of Pathophysiology, Tongji Medical College, Huazhong University of Science and Technology, No. 13, Hangkong Road, Wuhan 430030, People's Republic of China.
E-mail address: yedy@mails.tjmu.edu.cn (D. Ye).
[1] These authors contributed equally to this work.

http://dx.doi.org/10.1016/j.ygcen.2015.09.019
0016-6480/

involved in spontaneous miscarriages (Xu et al., 2013), and GC exposure in mid-late gestation could lead to intrauterine growth restriction in pregnant rats (Lin et al., 2013). Interestingly, a previous work reported that stress in early pregnancy could induce the rat PE (Takiuti et al., 2002). Furthermore, László et al. showed that stress exposure in the first trimester of pregnancy may lead to an increased risk of early-onset PE *via* influencing early placentation (László et al., 2013), while Hogg et al. demonstrated that early onset PE is associated with altered GC-signaling (Hogg et al., 2013).

Epithelial–mesenchymal transition (EMT) is required for placentation (Kalluri and Weinberg, 2009; Kokkinos et al., 2010), since trophoblasts acquire proliferative, migratory and invasive capabilities to allow for continual growing into the decidua through it (Kokkinos et al., 2010). Increasing evidence indicate that trophoblast EMT is dysregulated in PE (Fedorova et al., 2012; Sun et al., 2011; Zhou et al., 1997). GC has been demonstrated to inhibit EMT (Zhang et al., 2010). In addition, GCs could negatively affect trophoblast growth and invasion (Gennari-Moser et al., 2011; Michael and Papageorghiou, 2008). Thus, GC-mediated inhibition of trophoblast development may contribute to abnormal placentation, which may partially explain the cause of PE.

The purpose of the present study was to confirm that exposure to GC in early placentation could induce PE in the pregnant rats, and the mechanisms underlying this induction are associated with dysregulated trophoblast development by GC.

2. Materials and methods

2.1. Reagents and antibodies

Dex, Dex sodium phosphate, mifepristone (RU486), 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide (MTT) and DMSO were from Sigma Aldrich (Allentown, PA, USA). Anti hypoxia-inducible factor 1-alpha (HIF1A, sc-10790), nuclear factor erythroid 2-related factor 2 (NRF2, sc-722), Osteopontin (OPN, sc-73631), β-actin (sc-1616) and Lamin B (sc-374015) antibodies were purchased from Santa Cruz Biotechnology (Santa Cruz, CA, USA). Anti kelch-like ECH-associated protein 1 (KEAP1, MAB3024) antibody was from R&D Systems (Minneapolis, MN, USA). Anti E-cadherin (E-cad, 610181) antibody was from BD Biosciences (San Diego, CA, USA). Anti vimentin (VIM, 10366-1-AP) and fibronectin 1 (FN1, 15613-1-AP) antibodies were from Proteintech Group, Inc. (Chicago, IL, USA). U0126, Anti phospho-extracellular signal regulated kinase (p-ERK, 4377) and ERK (4695) antibodies were from Cell Signaling Technology (Danvers, MA, USA). RIPA Lysis Buffer and Nuclear and Cytoplasmic Protein Extraction Kit were from Beyotime Institute of Biotechnology (Shanghai, China). BCA protein assay kit was from Pierce (Rockford, IL, USA). Trizol was purchased from Life Technologies (Grand Island, NY, USA).

2.2. Animals and experimental protocol

Female Sprague–Dawley rats (10–12-weeks old, weighing 220–250 g) were purchased from the Experimental Animal Center of Tongji Medical College, Huazhong University of Science and Technology. Animals were housed individually in plastic cages with wood chips as bedding under pathogen-free conditions, in a controlled environment of temperature at 20–25 °C and 12 h cycles of light and dark. Rats were fed a standard laboratory diet and water *ad libitum*. Pregnancy was obtained by mating female rats with fertile male rats at a ratio of 2:1 overnight. Daily vaginal smears were observed, and appearance of spermatozoa in vaginal smear was defined as gestational day (GD) 1. All animal work was conducted according to the recommendations in the Guide for the Care and Use of Laboratory Animals of the National Institutes of Health. All studies involving rats were approved by Animal Care and Use Committee of Huazhong University of Science and Technology.

2.2.1. Experimental protocol 1: continuous Dex administration

Pregnant rats were randomly divided into control and treatment groups. Treatment rats were injected subcutaneously (*s.c.*) with Dex sodium phosphate (2.5 mg/kg per day) from GD7 to 13, while controls were injected *s.c.* with equal saline. Here, 2.5 mg/kg Dex *s.c.* is selected because we referenced a previous report that this dose and route could result in circulating GC levels in rats over time that reproduce the circulating levels of GC in rats observed during and after exposure to acute stress (Frank et al., 2010). On GD21 (pregnancy time = 22 days), rats were fully anesthetized with chloral hydrate and the uterus was removed and placed in a chilled dish. Placenta and pup were rapidly extracted. All of the pups and placentas were weighed, and litter size noted. Kidney was also removed. All samples were analyzed individually.

2.2.2. Experimental protocol 2: single Dex administration in different doses

Rats were injected *s.c.* with different doses of Dex sodium phosphate (1, 2.5, 5 and 10 mg/kg) on GD13, while controls were injected *s.c.* with equal saline. At 24 h post-Dex injection, placentas were collected for different analyses.

2.3. Measurement of systolic blood pressure (SBP)

At indicated time (initial non-pregnant status, GD3-5, GD14 and GD20), the SBP was determined in conscious, restrained pregnant rats. An automated system with a photoelectric sensor linked to a dual channel recorder (BP-98A, Softron, Japan), tail cuff and sphygmomanometer was used to obtain indirect blood pressure measurements, which have been previously demonstrated to be closely correlated with direct arterial measurements (Mulvany and Halpern, 1977). The measurements were repeated three times for each rat, with the mean value recorded.

2.4. Determination of urinary albumin excretion

For 24-h urine collection, on GD5 and GD20, the pregnant rats were placed in metabolic cages. To avoid contaminating the collected urine, rats were restricted from food; however, they were allowed free access to water. To avoid the adverse effects of fasting, rats were fed in other cages for 30 min every 6 h. Urine samples were centrifuged at 3000 rpm for 20 min at room temperature, and the supernatant was collected for urinary albumin analysis. Urine protein concentrations were determined with a BCA protein assay kit using bovine serum albumin as standard.

2.5. H&E staining

For histological evaluation, placenta and kidney were fixed in neutral-buffered formalin. Hematoxylin and eosin staining was performed on 4-μm paraffin sections of placenta and kidney specimens for conventional morphological evaluation under light microscope (Olympus BX60, Japan).

2.6. Measurement of thiobarbituric acid reactive substances (TBARS)

Placental TBARS was measured by using a commercially available kit (QuantiChrom™ TBARS Assay Kit, DTBA-100) according to manufacturer's instruction (BioAssay Systems, USA). Briefly, placentas (~20 mg) were placed into 200 μL ice-cold phosphate buffered saline with protease inhibitors. The tissues were first homogenized thoroughly and then sonicated for 20 s on ice.