Placenta 49 (2017) 23–32

Contents lists available at ScienceDirect

Placenta

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

Activation of the cholinergic anti-inflammatory pathway by nicotine ameliorates lipopolysaccharide-induced preeclampsia-like symptoms in pregnant rats

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ARTICLE INFO

Article history:
Received 5 May 2016
Received in revised form
18 October 2016
Accepted 6 November 2016

Keywords:
Preeclampsia
Inflammation
Nicotine
α7nAChR
NF-κB

ABSTRACT

Introduction: Preeclampsia (PE) exerts a more intense systemic inflammatory response than normal pregnancy. Recently, the role of the cholinergic anti-inflammatory pathway (CAP) in regulating inflammation has been extensively studied. The aim of this study was to investigate the effect of nicotine, a selective cholinergic agonist, on lipopolysaccharide (LPS)-induced preeclampsia-like symptoms in pregnant rats and to determine the molecular mechanism underlying it.

Methods: Rats were administered LPS (1.0 μg/kg) via tail vein injection on gestational day 14 to induce preeclampsia-like symptoms. Nicotine (1.0 mg/kg/d) and α-bungarotoxin (1.0 μg/kg/d) were injected subcutaneously into the rats from gestational day 14–19. Clinical symptoms were recorded. Serum and placentas were collected to determine cytokine levels using Luminex. The mRNA and protein expression levels of α7 nicotinic acetylcholine receptor (α7nAChR) were determined using Real time-PCR and Western blot analysis. Immunohistochemistry was performed to determine the level of activation of nuclear factor-κB (NF-κB) in placentas.

Results: Nicotine significantly ameliorated LPS-induced preeclampsia-like symptoms in pregnant rats ($P < 0.05$). Nicotine treatment decreased the levels of LPS-induced pro-inflammatory cytokines in the serum ($P < 0.05$) and placenta ($P < 0.05$). Nicotine significantly increased the expression of α7nAChR ($P < 0.01$) and attenuated the activation of NF-κB p65 in the placenta in LPS-induced preeclampsia ($P < 0.01$). Meanwhile, these protective effects of nicotine were abolished by the administration of the cholinergic antagonist α-bungarotoxin in preeclampsia rats.

Discussion: Our findings suggest that the activation of α7nAChR by nicotine attenuates preeclampsia-like symptoms, and this protective effect is likely the result of the inhibition of inflammation via the NF-κB p65 pathway.

1. Introduction

Preeclampsia (PE) is a multisystemic disorder that can occur during pregnancy and is characterized by hypertension, proteinuria, and sometimes edema. It is a severe obstetric problem that affects about 2%–8% of pregnancies and is the primary global cause of prematurity, neonatal and maternal morbidity and mortality. However, treatment for PE remains a challenge, and there is no effective treatment for this pregnancy-related disease except for the early delivery of the foetus [1–3].

Normal pregnancy elicits a mild systemic inflammatory response, while PE is associated with an exaggerated inflammatory response [4]. Consistent with the excessive inflammation response in PE, clinical studies indicate that preeclamptic women exhibit higher serum and placental levels of pro-inflammatory T helper type 1 (Th1) cytokines than women with normotensive pregnancies [5]. Furthermore, studies have demonstrated that maternal immune cell activation via NF-κB was a critical regulator of inflammation [6,7].

An accumulating amount of evidence shows that inflammatory responses can be efficiently regulated by the vagus nerve, a concept

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http://dx.doi.org/10.1016/j.placenta.2016.11.003
0143-4004/

that is referred to as the cholinergic or nicotinic antiinflammatory pathway [8–10]. α7nAChR plays a key role in this regulatory pathway [11]. Nicotine, an α7nAChR agonist, has been shown to attenuate inflammatory responses during experimentally induced endotoxemia in rodents by reducing the release of pro-inflammatory cytokines [12]. In our previous study, we found that nicotine treatment significantly reduced the LPS-induced concentrations of TNF-α and IL-6 in the serum but did not change IL-10 levels, and it also protected foetuses in pregnant rats [13]. The study showed that chronically treating spontaneously hypertensive rats (SHRs) with the α7 nAChR agonist PNU-282987 inhibited tissue levels of pro-inflammatory cytokines in a two-kidney, one-clip hypertension model [14]. Studies about the relationship between smoking and preeclampsia have conflicted results, numerous studies have demonstrated that maternal smoking protects against pregnancy-induced PE and that it has been shown to be associated with a 30% reduction in the risk of PE [15–17], while some studies found that smoking during pregnancy increased preeclampsia incidence [18]. However, the precise mechanism underlying the nicotine on preeclampsia remains unknown.

Data from animal and human studies revealed an important role of inflammatory mediators in the pathogenesis of PE [19–21]. Inflammation related models of PE included ultra low dose endotoxin and TNF-α infusion in pregnant rat [22], low dose LPS to induce preeclampsia in pregnant rats which we used in our previous study [23]. Besides, vascular growth factor inhibitor model, uteroplacental ischemia models, nitric oxide synthase (NOS) inhibition models, combined reduced uteroplacental perfusion pressure (RUPP) with a high cholesterol diet (HC) models also have been proposed to mimic the key features of disease such as systemic inflammatory changes, hypertension and proteinuria [24–26].

We sought to determine whether the activation of α7nAChR by nicotine improves preeclampsia-like symptoms in pregnant rats. In the present study, we use the α7nAChR agonist nicotine and antagonist α-bungarotoxin to interfere with LPS-induced preeclampsia in a rat model, and we then analysed changes in preeclampsia-like symptoms and investigated their underlying molecular mechanisms.

2. Materials and methods

2.1. Animals

The experimental procedures used in this study were performed in strict accordance with the guidelines of the Care and Use of Laboratory Animals (Guangdong Province, China). The Guangzhou Medical University Animal Ethics Committee approved all of the experimental protocols (Permit No. 2015-025).

Female Sprague-Dawley rats (8–10 weeks old and weighing 200–240 g) that were purchased from the Medical Experimental Animal Centre of Guangdong, China. The animals were housed under controlled laboratory conditions (temperature 23-26 °C, relative humidity 50–60%, light between 6:00 a.m. and 6:00 p.m.) with free access to standard rat chow and water. The female rats were mated overnight with fertile male rats. A positive vaginal smear for sperm defined day 0 of pregnancy (GD 0).

2.2. Reagents

LPS and nicotine hydrogen tartrate were obtained from Sigma-Aldrich (St Louis, MO, USA). α-bungarotoxin was provided by Shanghai Kemin Biological Technology Company (China). Anti-α7nAChR was purchased from Abcam (ab24644, Grand Island, NY, USA). Luminex kits specific for tumor necrosis factor-α (TNF-α), interferon-γ (IFN-γ), interleukin (IL)-1β, IL-2, IL-6, IL-10, IL-17A were obtained from Bio-Rad (CA, USA).

2.3. Study design

A total of 57 Sprague-Dawley rats were randomly divided into the following seven groups: non-pregnant (NP group, n = 7), pregnant (P group, n = 8), pregnant + nicotine (P + Nic group, n = 8), pregnant + α-bungarotoxin (P + α-BGT group, n = 7), preeclampsia (PE group, n = 10), preeclampsia + nicotine (PE + Nic group, n = 10) and preeclampsia + α-bungarotoxin + nicotine (PE + α-BGT + Nic group, n = 7). Based on the report of Faas et al. [22], on GD 14, the experimental PE rats were induced by infusing LPS (1 μg/kg body weight) in 2 ml of sterile saline through an infusion pump into the tail vein (infusion rate, 2 ml/h). The rats were subcutaneously treated with nicotine twice per day (1 mg/kg/d at 9:00 a.m. and 3:00 p.m.) and α-BGT twice per day (1.0 μg/kg/d one half hour prior to the nicotine injections) from day 14 until 19 of gestation. The control rats received an equivalent volume of physiological saline solution (0.9% NaCl) at comparable times.

2.4. Measurement of SBP

SBP was measured by volume-pressure recording (VPR) noninvasive blood pressure monitoring system (CODA, Kent Scientific, Torrington, CT) (tail cuff and sphygmomanometer) on GDs 6, 11, 14 (just before LPS infusion), 16, and 18 (between 9 a.m. and 12 a.m.). The rats were exposed to the measurement processes to adapt them to the conditions before the actual measurements were taken, and they were also allowed to relax for 30 min before the study BP measurements commenced. For each rat, BP measurements were performed three times, and the mean value was recorded.

2.5. Measurement of biochemical parameters

On GDs 7, 12, 17 and 19, the rats were placed in individual metabolic cages for 24 h to obtain urine samples. Urine samples were centrifuged at 2000 rpm for 15 min at room temperature, and the top layer was stored at −20 °C for subsequent analysis of protein levels. The urine protein concentration was measured using an automatic analyser (HIACHI 7600-020, Japan).

On GD 20, after the rats were anesthetized, blood samples were obtained from the inferior cava vena. The foetal pups and placentas were removed and weighed. All samples were stored at −80 °C for further assessment. Pup viability, resorption and gross morphology were also recorded.

2.6. Luminex assays

Maternal serum and placentas were collected as described above. Total lysates were prepared from the placentas by homogenizing 60 mg of placental tissue in 600 μl of RIPA lysis buffer. The protein samples were then assessed to determine cytokine levels using a Bio-Plex Pro Rat Cytokine 8-Plex Panel (Bio-Rad, USA). In accordance with the manufacturer's instructions, multiplex assays were performed using a Luminex 200 system and Bioplex HTF (Bio-Rad, CA, USA). The standards and samples were analysed in duplicate. The data analysis was performed using Bio-Plex Manager, version 5.0 (Bio-Rad), and is presented as concentrations (pg/ml or pg/ml/mg).

2.7. Real-time PCR

The placenta was disrupted using a motorized pestle and mortar, and total RNA was extracted using an RNeasy mini kit (Qiagen, cat. number. 74104, Germany). RNA concentration was