Oxidative Stress in the Rostral Ventrolateral Medulla Contributes To Cardiovascular Regulation in Preeclampsia

Jiu-Qiong Yan 1†, Fang Huang 2†, Fan Hao 1, Xiao-Ling Su 1, Qi Meng 1 and Ming-Juan Xu 1*

1 Department of Obstetrics and Gynecology, Changhai Hospital, Second Military Medical University, Shanghai, China, 2 Department of Geriatrics, Jinling Hospital, Nanjing, China

Background: It has been demonstrated that preeclampsia, a pregnancy-specific hypertension disorder, is characterized by high blood pressure (BP) and sympathetic overactivity. Increased reactive oxygen species (ROS) in the rostral ventrolateral medulla (RVLM), a key region for controlling sympathetic tone, has been reported to contribute to high level of BP and sympathetic outflow. The aim of the present study was to determine the role of the RVLM ROS in mediating the preeclampsia-associated cardiovascular dysfunction.

Methods: The animal model of preeclampsia was produced by administration of desoxycorticosterone acetate (DOCA) to pregnant rats.

Results: Compared with normal pregnant rats without DOCA treatment (NP), the protein concentration and norepinephrine excretion in 24-h urine, as well as BP in pregnant rats with DOCA treatment (PDS) were significantly increased. The levels of superoxide anion and the protein expression of NADPH oxidase subtype (NOX4) in the RVLM were significantly increased in PDS than in NP groups. Furthermore, microinjection of the superoxide dismutase (SOD) mimic Tempol (5 nmol) into the RVLM significantly decreased BP, heart rate, and renal sympathetic nerve activity in PDS but not in NP group.

Conclusion: The present data suggest that high BP and sympathetic overactivity in preeclampsia rats is associated with increased oxidative stress in the RVLM via upregulation of NOX4 expression.

Keywords: preeclampsia, rostral ventrolateral medulla, reactive oxygen species, sympathetic overactivity, blood pressure

INTRODUCTION

Preeclampsia is a pregnancy-specific disorder with the de novo onset of hypertension and proteinuria after 20 weeks of gestation (Pridjian and Puschett, 2002a,b). This disease has become to be a major cause resulting in morbidity and mortality in pregnant women and neonates (Lenfant, 2001; Roberts et al., 2016). In addition to high blood pressure (BP), preeclampsia has been reported to exhibit an obvious sympathetic overactivity (Fischer et al., 2004; Jarvis et al., 2012; Logue et al., 2016). Furthermore, a prospective...
study in pregnant women has revealed that pregnancy-associated with sympathetic overactivity precedes preeclampsia (Fischer et al., 2004). However, the underlying mechanism of increased sympathetic outflow involved in cardiovascular dysfunction in preeclampsia remains unclear.

Sympathetic nerve overactivity is recognized as an important mechanism responsible for pathological process of cardiovascular diseases such as hypertension and chronic heart failure (Greenwood et al., 2003; Dell’Oro et al., 2017). It is well-known that the rostral ventrolateral medulla (RVLM) plays a key role in mediating tonic and reflex control of the cardiovascular activity (Dampey, 1994; Guyenet, 2006). RVLM has been widely accepted to be involved in maintaining resting BP and sympathetic tone (Guyenet, 2006). Moreover, accumulating studies indicate that oxidative stress, which is produced by the imbalance between mechanisms of reactive oxygen species (ROS) production (e.g., NADPH oxidase) and mechanisms of ROS clearance (e.g., superoxide dismutase, SOD), contributes to the enhanced central sympathetic outflow in the RVLM in experimental animal models of hypertension and chronic heart failure (Peterson et al., 2006; Hirooka et al., 2011). Interestingly, oxidative stress in peripheral system has been reported to be associated with the pathophysiology of preeclampsia (Siddiqui et al., 2010; Guerby et al., 2015). For example, the oxidative stress level in serum of patients was increased during preeclampsia (Medrano Rodriguez et al., 2008; Genc et al., 2011), while an reduction in the oxidative stress in placentas significantly improves N-omega-nitro-L-arginine methyl ester (L-NAME)-induced preeclamptic symptoms in the preeclamptic Sprague-Dawley (SD) rat model (Xuan et al., 2016). However, there is no direct evidence showing the relationship between oxidative stress in the RVLM and cardiovascular dysfunction including high BP and sympathetic overactivity in preeclampsia.

Therefore, the main goal of this study was to determine the role of the oxidative stress-mediated sympathetic overactivity in cardiovascular dysfunction in preeclampsia. First, we determined the level of oxidative stress in the RVLM in rat model with preeclampsia. Second, we detected the possible sources of oxidative stress in the RVLM in preeclampsia. Finally, we observed the effects of microinjection of Tempol (superoxide dismutase mimic) into the RVLM on cardiovascular activity in preeclampsia.

**METHODS**

**Experimental Protocols**

A total of 90 rats were used in this study. Female SD rats (200–250 g) and male SD rats (275–300 g) were supplied by Sino-British SIPPRI/BK Laboratory Animal Ltd (Shanghai, China). All experimental procedures were approved by the Institutional Care and Use Committee of the Second Military Medical University.

**Animal Model with Preeclampsia**

Desoxycorticosterone acetate plus increased NaCl intake produced hypertension, proteinuria, rapid weight gain, convulsions, decreased litter size, decreased offspring weight, increased fetal and maternal mortality, and renal lesions similar to those seen in human preeclampsia (Douglas, 1976). Therefore, the animal model of preeclampsia was produced by administration of DOCA to pregnant rats. The female animals were mated with male SD rats. Pregnancy of female rats was confirmed by vaginal smearing. The animals were divided into four groups: Group 1 was non-pregnant animals (control); Group 2 was non-pregnant animals treated with DOCA and saline (NPS). On based on previous study (Ianosi-Irimie et al., 2005; Uddin et al., 2010; Moraloglu et al., 2012), NPS group was injected initially with 12.5 mg of DOCA intraperitoneally in a depot form, followed by 6.5 mg on a weekly basis. Their drinking water was replaced with 0.9% saline. Group 3 was normal pregnant without DOCA treatment (NP). Pregnant animals were given by tap water and libitum. Group 4 was normal pregnant treated with DOCA and saline (PDS). All 4 groups were maintained on normal rat chow. At 18–19 days of pregnancy, 24 h-urine was collected in the absence of food. Each animal was housed separately in a metabolic cage. Before the animals euthanized by pentobarbital sodium (200 mg/kg, i.p.), BP and heart rate (HR) were measured. At the end of experiment, the brain was quickly removed for further analysis.

**General Surgery, Recording of RSNA, and RVLM Microinjection**

The protocol for RVLM microinjection was based on previous study (Peng et al., 2011). Briefly, rats were anesthetized by intraperitoneal injection of urethane (800 mg/kg) and a-chloralose (40 mg/kg). The trachea of rats was cannulated for mechanical ventilation. The right femoral artery was catheterized for measuring BP and HR via a Powerlab system. The procedure for recording of RSNA was described previously (Peng et al., 2009). The left renal sympathetic nerves were exposed, identified and dissected free of the surrounding connective tissue, and placed on a pair of recording electrodes. Both the nerve and the electrodes were covered with a fast-setting silicone (Wacker Sil-Gel). The signal was amplified, monitored, and analyzed by Powerlab system. Noise levels were subtracted from the nerve recording data before percent changes from baseline were calculated. Integrated RSNA was normalized as 100% baseline in the control period.

The anesthetized rats were placed in a stereotaxic frame, and the dorsal surface of the medulla was surgically exposed. Microinjections were made from a three-barrel micropipette (20–50 µm diameter). According to rat atlas (Paxinos and Watson, 1998), the coordinates for RVLM were 2.0–2.5 mm rostral to obex, 1.8–2.1 mm lateral to midline, and 2.8–3.2 mm ventral to the dorsal surface of the medulla. The injection was made over a period of 5–10 s and the injection volume (100 nl) was carefully measured by observing the movement of the fluid meniscus along a reticule in a microscope. The RVLM was chemically identified by a pressor response (>20 mmHg) to L-glutamate (1 nmol) microinjection. The changes in BP, HR, and renal sympathetic nerve activity (RSNA) were recorded 10 min after injection of Tempol (5 nmol), which was based on the previous study (Wang et al., 2016; Zahid et al., 2016). At the end
of each experiment, 100 nl of 2% pontamine sky blue solution was injected into the RVLM to mark the site. The injection sites in the study were confirmed to be located within the RVLM.

**Western Blot Analysis**

Rats were euthanized and the brains were removed for western blot analysis. The protocol for Western blot analysis was described in our previous study (Hao et al., 2016). In brief, the rat brains were removed and immediately frozen, blocked in the coronal plane, and sectioned at 100 µm thickness in a cryostat from 2.0 to 3.0 mm rostral to the Obex. The total span of punches was 1 mm, from 1.6 to 2.6 mm lateral to the midline. The RVLM was then punched using a punch-needle (0.6 mm inside diameter) from 10 sections. The punched tissues from the 10 sections were pooled for protein extraction. The protein concentration was measured with BCA kit. The protein samples were loaded onto a 10% SDS–PAGE gel and then transferred to PVDF membrane. One lane on the right contained a molecular weight marker, which provides a visual check of transfer efficiency and marks band position for target protein. The membrane was incubated with primary antibody (SOD1 rabbit anti-mouse, Santa Cruz, sc-650, 1:2,000; NOX4 rabbit anti-mouse, Santa Cruz, sc-650, 1:1,000) and secondary antibodies (1:10,000). The protein bands were visually detected and analyzed. The levels of target proteins were normalized to tubulin, which served as a loading control.

**Measurement of Urinary Norepinephrine Excretion by High-Performance Liquid Chromatography (HPLC)**

Urinary norepinephrine content was measured using HPLC at the 18–19 days of pregnancy. HPLC (model 582 pump, ESA) with electrochemical detection (model 5300, ESA) was used to detect content of norepinephrine (NE) in 24-h urinary excretion, as described previously (Peng et al., 2011). Briefly, 24-h urinary samples were collected after rats were placed in metabolism cages for 24 h and acidified with glacial acetic acid in 15-ml centrifuge tubes, which were embedded in crushed ice. Dihydroxybenzylamine (Sigma) was used as the internal standard. NE was absorbed onto acid-washed alumina with 3 mol/L Tris (hydroxymethyl) aminomethane buffer at pH 8.6 in 2% EDTA. The alumina was then washed three times with 3 ml of distilled water. NE was extracted with 400 µl of 0.2 M glacial acetic acid with 5 min of shaking and a final 30-min settlement. The supernatant (50 µl) was injected into the HPLC column [reverse phase, ESA, 150 × 3.2 mm, 3 um C18 (P/N70–0636)], and NE was eluted with the mobile phase (80 mM citric acid monohydrate, 73.4 mM citric acid trisodium salt, 0.12 mM 1-octanesulfonic acid sodium salt, and 0.1 mM EDTA adjust to pH 4.3 with phosphoric acid). The flow rate was set at 0.5 ml/min.

**Measurement of ROS Generation in the RVLM**

We performed fluorescence microtopography to detect ROS content in the RVLM as previously described (Peng et al., 2009). The rats were euthanized and perfused through the aorta with 0.9% NaCl solution. The brain stem were removed and rapidly frozen. According to the rat atlas (Paxinos and Watson, 1998), the target area RVLM sections of 10 µm thickness were cut in a cryostat, and placed on glass slides and incubated at room temperature in the dark for 30 min with Dihydroethidium (DHE, 5 µmol/L). DHE, an oxidant-sensitive probe, is widely used for detection of ROS. Two products of DHE oxidation, ethidium, and 2-hydroxyethidium, can bind to the nuclear DNA, thereby forming a strong red fluorescent complex. The reaction usually takes place about 30 min (He et al., 2011; Zhou et al., 2013). The microslide with brain slice were washed three times 5 min in PBS (0.01 mol/L, pH 7.4). Fluorescence was determined using a microscope (Nikon, Japan) at excitation and emission wavelengths of 535 and 590 nm, respectively. Fluorescence intensity was semi-quantitatively analyzed by Leica LAS-AF lite 2.1.1. A total of five rats were used to measure ROS in the RVLM by DHE each group. Fluorescence value of each animal was detected by a brain slice at the same Bragma parameter (~12.24 mm) for statistical analysis.

Furthermore, brain tissues the RVLM was punched on coronal sections and was further performed to detect the ROS level in the RVLM by the GENMED lucigenin chemiluminescence quantitative determination kit (GEMED, GSM101113.5, USA). These procedures were performed according to the manufacturer’s instruction. In brief, after RVLM tissue was punched and weighed from the rat which was euthanized (pentobarbital sodium, 300 mg/kg, i.p.), 80 µL Protein Lysis

| Table 1 | Baseline values of BP and HR before microinjection of Tempol into the RVLM in four group rats. |
|---------|---------------------------------------------------------------------------------------------|
| Group   | BP (mmHg) | HR (bpm) |
| Con     | 116 ± 5  | 375 ± 13 |
| NPS     | 118 ± 4  | 372 ± 12 |
| NP      | 124 ± 6  | 368 ± 17 |
| PDS     | 143 ± 3† | 376 ± 14 |

Data are presented as mean ± SEM, bpm, beats per minute. n = 5/group, *p < 0.05 vs. Con.
Buffer (Cell Signaling Technology, USA) was added into the test tube, tissue was polished by electric homogenizer and then centrifuging for 20 min. Supernatant was collected for analysis by lucigenin chemiluminescence quantitative assay. Chemiluminescence was expressed as arbitrary units.

Measurement of Urinary Protein Concentration in 24 h Urine
Urine was collected for 24 h urinary protein quantization. The 24-h protein excretion was measured using the enzyme-linked immunosorbent assay kit (purchased from Beyotime Biotechnology, China). Measurement of urinary protein concentration was completed according to the manufacturer's instructions. In brief, 24 h urine was collected for each rat, Protein concentration was determined using Bradford method with Bradford Protein Assay. Absorbance was measured at 595 nm with Microplate Reader and BSA was used as standard.

Statistical Analysis
All values in this study were expressed as mean ± SEM. Statistical comparisons between different groups were made by the one-way ANOVA, and followed by Bonferroni’s post-hoc analysis. All analyses were performed by software (SPSS18.0). A value of $P < 0.05$ was considered statistically significant.

RESULTS
Cardiovascular Dysfunction in Preeclampsia Rats
Rats with pregnancy were confirmed by vaginal smearing at first day after mating (Figure 1). At 18 days of pregnancy, PDS group showed a significant increase in concentration of urinary protein ($114 \pm 19.6$ vs. $48.5 \pm 3.76 \mu$mol/L, $p < 0.05$), mean arterial pressure (MAP, $148 \pm 3.99$ vs. $117 \pm 3.03$ mmHg, $p < 0.05$), HR ($475 \pm 9.01$ vs. $371 \pm 19.3$ bpm, $p < 0.05$), and the content of norepinephrine in the 24 h urine ($1.94 \pm 0.477$ vs. $0.269 \pm 0.117 \mu$g, $p < 0.05$; Figure 2) compared with the rats in Con group. However, these parameters were no significant differences between NPS group and NP group.

The Level of Oxidative Stress in the RVLM in Preeclampsia Rats
As indicated in Figure 3, it was shown that the ROS content in the RVLM using DHE probe in PDS group was increased remarkably compared with Con groups ($4.27 \pm 0.248$ vs. $1.00 \pm 0.134$, $p < 0.05$). Furthermore, we used the tissue super oxide anion lucigenin chemiluminescence quantitative detection kit to detect the ROS level in the RVLM, it was observed that ROS levels in RVLM was significantly ($p < 0.05$) higher ($\approx$7-fold) in PDS group compared with Con groups. As shown in Figure 4, the protein expression of NOX4 in the RVLM was upregulated in PDS group compared with Con group. The protein expression of SOD1 had no significant changes in the RVLM of PDS group compared with Con group. In additional, the protein expression of NOX4 and SOD1 between NPS group and NP group also had no significant differences.

The Effects of Microinjection of Tempol into the RVLM on Cardiovascular Activity in Preeclampsia
The baseline BP and HR in four groups for microinjection of Tempol into the RVLM was shown in Table 1. As shown in
Figures 3, 4, microinjection of Tempol (5 nmol) into the RVLM significantly decreased MAP (−31.8 ± 5.09 vs. −1.06 ± 2.02 mmHg, p < 0.05), HR (−31.3 ± 2.00 vs. −2.72 ± 4.29 bpm, p < 0.05), and RSNA (−12.2 ± 0.609 vs. 4.38 ± 2.17 %, p < 0.05) in PDS group compared with Con group. The peak changes in MAP, HR, and RSNA induced by microinjection of Tempol into
FIGURE 5 | The effects of RVLM microinjection of Tempol on cardiovascular activity in preeclampsia. (A) Representative traces showing the responses of MAP, HR, and RSNA to microinjection of Tempol (5 nmol) into the RVLM in four groups. (B) The original picture showing the RVLM microinjection site stained by pontamine sky, and the standard atlas of RVLM region in rat brainstem.

In this study, the rat model of preeclampsia was successfully established by administrating DOCA and saline water to pregnant rats, as described in previous study (Ianosi-Irimie et al., 2005). Although, many animal models share features with the hypertensive disorders of pregnancy, most of them do not include the complete spectrum of symptoms present in the human disease. It has been suggested that the increase of extracellular fluid can lead to hypertension, which is due to the activation of the renin-angiotensin system (RAS; Kobori et al., 2007). All of the RAS components are present and intra-renal angiotensin II (Ang II) is formed by independent multiple mechanism. Ang II exerts a cardinal role in the pathogenesis of hypertension (Kobori et al., 2007). Many studies have reported associations between RAS and preeclampsia (Li et al., 2016; Aung et al., 2017), indicating that RAS may also play a role in the pathogenesis of preeclampsia. Although, the peripheral RAS (plasma levels of active renin, renin and Ang II) is downregulated in the DOCA preeclampsia model (Uddin et al., 2010); the association of gene polymorphisms of RAS and preeclampsia was observed in human, moreover, T allele of angiotensinogen may involve in the pathogenesis of PE (Aung et al., 2017). During pregnancy, an increase in extracellular fluid volume occurs, which reaches a 40–50% increase by the end of gestation (Scott, 1972; Gallery and Brown, 1987). Since pregnancy represents a condition in which spontaneous volume expansion of the extracellular fluid occurs (Scott, 1972; Gallery and Brown, 1987). It has also been reported that the burden of volume expansion represented by pregnancy would be sufficient to cause the development of hypertension (Ianosi-Irimie et al., 2005). Therefore, the rat model of preeclampsia by administrating DOCA and saline was performed in this study. Furthermore, the present study has confirmed that the changes in MAP, HR and urinary protein concentration of this model were consistent with preeclampsia patients. In this study, the gestation period of rat is about 20–22 days. At 18 days after pregnant
FIGURE 6 | The peak changes in MAP (A), HR (B), and RSNA (C) induced by microinjection of Tempol (5 nmol) into the RVLM in four groups. n = 5/group. *P < 0.05 vs. Con.

abdominal cavity space occupied by enlarged uterus of rats. To confirm the rat model of preeclampsia sympathetic nerve activity increased, we measured the content of NE in the 24 h urine. The content of NE was significantly higher in preeclampsia model group compared with Con groups, suggesting that sympathetic tone was increased in the rat with preeclampsia.

The balance between ROS generation and anti-oxidant mechanism determines the degree of oxidative stress and ROS (Espinosa-Diez et al., 2015; Matsubara et al., 2015; Venza et al., 2015). It has been reported that decrease in placental blood perfusion induces oxidation stress, vascular active factor imbalances, and endothelial cell injury, which plays an important role in the development and maintenance of preeclampsia (Jauniaux et al., 2006; Sedeek et al., 2008). Chappell et al. have also reported that supplementation with vitamins C and E, anti-oxidative vitamins, can be beneficial to preeclampsia in women at increased risk of the disease (Chappell et al., 1999). Indeed, it is well-known that ROS plays a key role in the mechanism of preeclampsia (D’Souza et al., 2016; Elliot, 2016). However, there is little study reported that whether the central oxidative stress has involved in the pathogenesis of preeclampsia, especially in oxidative stress in the RVLM. The enhanced oxidation stress contributes to the increase in sympathetic nerve activity and hypertension (Zimmerman et al., 2004; Oliveira-Sales et al., 2009). In the present study, we had observed the increased level of oxidative stress in the RVLM in preeclampsia rats. These results indicated that the increased generation of ROS in the RVLM might be involved in the pathogenesis of preeclampsia. It is suggested that the oxidative stress could increase the RVLM sympathetic outflow in the preeclampsia, in which there is an exaggerated super oxide anion generation. Moreover, it has demonstrated that microinjection of an antioxidant, vitamin C, into the RVLM resulted in a decrease in arterial pressure and sympathetic activity in hypertensive rats (Oliveira-Sales et al., 2008). In this work, microinjection of Tempol, a superoxide dismutase mimic, into the RVLM caused a significant decrease in BP, HR, and RSNA in preeclampsia rats but not in the control rats. In fact, this study was to determine whether oxidative stress was involved in cardiovascular abnormalities in preeclampsia, so acute microinjection of Tempol was to determine whether increased oxidative stress is involved in the maintenance of resting BP and sympathetic tone in preeclampsia state. We have realized that chronic infusion of Tempol is also very useful to detect the importance of oxidative stress in preeclampsia-associated high BP. This experiment would clarify if chronic decrease in oxidative stress improves the cardiovascular abnormalities induced by preeclampsia. Taken altogether, the results suggest that increased oxidative stress is involved in maintaining the cardiovascular activity in the preeclampsia.

There are many possible mechanisms involved in DOCA-induced increase in ROS in the RVLM. For example, DOCA can increase blood volume, which induces an increase in cardiac sympathetic afferent impulses, then causing changes in release of neurotransmitter in the central nervous system. It is reported that the central Ang II-associated oxidative stress was induced by activation of cardiac sympathetic afferent (Campese et al., 2005). To further explore the mechanism of ROS production in the RVLM in preeclampsia rats. It has been demonstrated that increased NADPH oxidase activity enhances ROS production, leading to hypertension (Oliveira-Sales et al., 2009). In this study, we had observed a significant increase in the NADPH oxidase subunits-NOX4 protein expression in the RVLM in preeclampsia rats. However, the superoxide dismutase subtype SOD1 expression in the RVLM in preeclampsia rats was unchanged compared to the Control groups. It is suggested that enhanced ROS production in the RVLM in preeclampsia rats is highly associated with the NADPH oxidase, especially NOX4 subunit. On the other hand, NADPH oxidase includes seven subtypes, NOX1, NOX2, NOX3, NOX4, NOX5, DUOX1, and DUOX2 (Bedard and Krause, 2007). Superoxide dismutase has main two subtypes, including SOD1 and SOD2 (Fukai and Ushio-Fukai, 2011). Our
previous study has demonstrated that the ROS overproduction caused by upregulating protein expression of NOX4 and downregulating protein expression of SOD1 in the RVLM, which increased sympathetic tone in ovariectomized rats (Hao et al., 2016). In this work, there is a limitation that only the protein expressions of NOX4 subtype and SOD1 were detected. Further experiments are needed to determine whether other subtypes of NADPH oxidase and superoxide dismutase involved in the ROS production in the RVLM in preeclampsia.

In conclusion, our results have revealed that the level of ROS in the RVLM was enhanced in preeclampsia rats, and reduction in oxidative stress in the RVLM via microinjection of Tempol could ameliorate cardiovascular dysfunction in preeclampsia rats. Taken together, it is suggested that oxidative stress in the RVLM is involved in sympathetic overactivity and high BP, which contributes to the pathogenesis of preeclampsia.

**REFERENCES**

Aung, M., Konoshita, T., Moodley, J., and Gathiram, P. (2017). Association of gene polymorphisms of four components of renin-angiotensin-aldosterone system and preeclampsia in South African black women. Eur. J. Obstet. Gynecol. Reprod. Biol. 215, 180–187. doi: 10.1016/j.ejogrb.2017.05.011

Bedard, K., and Krause, K. H. (2007). The NOX family of ROS-generating NADPH oxidases: physiology and pathophysiology. Physiol. Rev. 87, 245–313. doi: 10.1152/physrev.00044.2005

Campese, V. M., Shaohua, Y., and Huiquin, Z. (2005). Oxidative stress mediates angiotensin II-dependent stimulation of sympathetic nerve activity. Hypertension 46, 533–539. doi: 10.1161/01.HYP.0000179088.57586.26

Chappell, L. C. S., Briley, P. T., Kelly, A. L., Lee, F. J., Hunt, R., Parmar, B. J., et al. (1999). Effect of antioxidants on the occurrence of pre-eclampsia in women at increased risk: a randomised trial. Lancet 354, 9810–9186. doi: 10.1016/S0140-6736(99)80010-5

Dampey, R. A. (1994). Functional organization of central pathways regulating the cardiovascular system. Physiol. Rev. 74, 323–364.

Dell’Oro, R., Gronda, E., Seravalle, G., Costantino, G., Alberti, L., Baronio, B., et al. (2014). Functional organization of central pathways regulating the cardiovascular system. Physiol. Rev. 94, 235–300. doi: 10.1152/physrev.00044.2005

Elliot, M. G. (2016). Oxidative stress and the evolutionary origins of preeclampsia. J. Reprod. Immunol. 114, 75–80. doi: 10.1016/j.jri.2016.02.003

Espinosa-Diez, C., Miguel, V., Mennerich, D., Kietzmann, T., Sanchez-Perez, P., Cadenas, S., et al. (2015). Antioxidant responses and cellular adjustments to oxidative stress. Redox Biol. 6, 183–197. doi: 10.1016/j.redox.2015.07.008

Fischer, T., Schobel, H. P., Frank, H., Andreae, M., Schneider, K. T., and Heusser, K. (2004). Pregnancy-induced sympathetic overactivity: a precursor of preeclampsia. Eur. J. Clin. Invest. 34, 443–448. doi: 10.1111/j.1365-2362.2004.01350.x

Fukai, T., and Ushio-Fukai, M. (2011). Superoxide dismutases: role in redox signaling, vascular function, and diseases. Antioxid. Redox. Sign. 15, 1583–1606. doi: 10.1089/ars.2011.3999

Gallery, E. D., and Brown, M. A. (1987). Volume homeostasis in normal and hypertensive human pregnancy. Baillieres Clin. Obstet. Gynaecol. 1, 835–851. doi: 10.1016/S0950-3552(87)80037-8

Genc, H., Uzun, H., Benian, A., Simsek, G., Gelisgen, R., Madazli, R., et al. (2011). Evaluation of oxidative stress markers in first trimester for assessment of preeclampsia risk. Arch. Gynecol. Obstet. 284, 1367–1373. doi: 10.1007/s00404-011-1865-2

Greenwood, J. P., Scott, E. M., Walker, J. J., Stoker, J. B., and Mary, D. A. (2003). The magnitude of sympathetic hyperactivity in pregnancy-induced hypertension and preeclampsia. Am. J. Hypertens. 16, 194–199. doi: 10.1016/S0895-7061(02)03256-9

Guery, P., Vital, F., Garoby-Salom, S., Vaysiere, C., Salvayre, R., Parant, O., et al. (2015). Oxidative stress and preeclampsia: a review. Gynecol. Obstet. Fertil. 43, 751–756. doi: 10.1016/j.ygobf.2015.09.011

Guyenet, P. G. (2006). The sympathetic control of blood pressure. Nat. Rev. Neurosci. 7, 335–346. doi: 10.1038/nrn1902

Hao, F., Gu, Y., Tan, X., Deng, Y., Wu, Z. T., Xu, M. J., et al. (2016). Estrogen replacement reduces oxidative stress in the rostral ventrolateral medulla of ovariectomized rats. Oxid. Med. Cell. Longev. 2016:2158971. doi: 10.1155/2016/2158971

He, X., Zhang, H. L., Zhao, M., Yang, J. L., Cheng, G., Sun, L., et al. (2011). Amlodipine ameliorates endothelial dysfunction in mesenteric arteries from spontaneously hypertensive rats. Clin. Exp. Pharmacol. Physiol. 38, 255–261. doi: 10.1111/j.1440-1681.2011.05495.x

Hirokou, Y. K., Sakai, T., Takeshita, K., and Sunagawa, A. K. (2011). Imbalance of central nitric oxide and reactive oxygen species in the regulation of sympathoexcitation and neural mechanisms of hypertension. Am. J. Physiol. Regul. Integr. Comp. Physiol. 300, 818–826. doi: 10.1152/ajpregu.00426.2010

Ianos-irinie, M., Vu, H. V., Whitbred, J. M., Pridjian, C. A., Nadig, J. D., Williams, M. Y., et al. (2005). A rat model of preeclampsia. Clin. Exp. Hypertens. 27, 605–617. doi: 10.1080/10641960500298608

Jarvis, S. S., Shibata, S., Bivens, T. B., Okada, Y., Casey, B. M., Levine, B. D., et al. (2012). Sympathetic activation during early pregnancy in humans. J. Physiol. 590, 3353–3354. doi: 10.1113/jphysiol.2012.228262

Jauniaux, E., Poston, L., and Burton, G. J. (2006). Placental-re related diseases (from the Department of Statistical Analysis, SMMU).

**AUTHOR CONTRIBUTIONS**

Study design: JY, MX. Performing experiments: JY, FangH, FanH, and XS. Data collection and analysis: JY, QM, and MX. Drafting manuscript: JY. Revising manuscript content: FangH and MX. Approving final version of manuscript: JY, FangH, FanH, XS, and MX.

**FUNDING**

This work was supported by the National Natural Science Foundation of China (No. 81770421, No. 81600329).

**ACKNOWLEDGMENTS**

We gratefully acknowledge the statistical assistance of Dr. Jian Lu (from the Department of Statistical Analysis, SMMU).

This work has been supported by the National Natural Science Foundation of China (No. 81770421, No. 81600329).
Logue, O. C., George, E. M., and Bidwell, G. L. (2016). Preeclampsia and the brain: neural control of cardiovascular changes during pregnancy and neurologological outcomes of preeclampsia. *Clin. Sci. 130, 1417–1434. doi: 10.1042/CS2016108*

Matsubara, K., Higaki, T., Matsubara, Y., and Nawa, A. (2015). Nitric oxide and reactive oxygen species in the pathogenesis of preeclampsia. *Int. J. Mol. Sci. 16, 4600–4614. doi:10.3390/ijms16036400*

Medrano Rodriguez, J. C., Yahuaca Mendoza, P., Presno Bernal, M., and Alvarado Acosta, J. L. (2008). Oxidative stress level and placental histological changes during preeclampsia. *Gynecol. Obstet. Mex. 76, 319–326. Available online at: https://ginecologiyabyobstetricia.org.mx/secciones/articulos-originales-numero83/grado-de-estres-oxidativo-y-cambios-histologicos-placentarios-durante-la-preeclampsia/

Moraloglu, O., Engin-Ustun, Y., Tonguç, E., Var, T., Tapısız, O. L., Ergün, H., et al. (2012). The effect of resveratrol on blood pressure in a rat model of preeclampsia. *J. Matern. Fetal Neonatal Med. 25, 845–848. doi: 10.3109/14767058.2011.599081*

Oliveira-Sales, E. B., Dugaich, A. P., Carillo, B. A., Abreu, N. P., Boim, M. A., Martins, P. J., et al. (2008). Oxidative stress contributes to renovascular hypertension. *Am. J. Hypertens. 21, 98–104. doi: 10.1038/ajh.2007.12*

Oliveira-Sales, E. B., Nishi, E. E., Carillo, B. A., Boim, M. A., Dolnikoff, M. S., Bergamaschi, C. T., et al. (2009). Oxidative stress in the sympathetic premotor neurons contributes to sympathetic activation in renovascular hypertension. *Am. J. Hypertens. 22, 484–492. doi: 10.1038/ajh.2009.17*

Patino, G., and Watson, C. (1998). The Rat Brain in Stereotaxic Coordinates, 3rd Edn. New York, NY: Academic Press.

Peng, J. F., Wu, Z. T., Wang, Y. K., Yuan, W. J., Sun, T., Ni, X., et al. (2011). GABAergic mechanism in the rostral ventrolateral medulla contributes to the hypotension of moxonidine. *Cardiovasc. Res. 89, 473–481. doi: 10.1093/cvr/cvq289*

Peng, J., Wang, Y. K., Wang, L. G., Yuan, W. J., Su, D. F., Ni, X., et al. (2009). Sympathoinhibitory mechanism of moxonidine: role of the inducible nitric oxide synthase in the rostral ventrolateral medulla. *Cardiovasc. Res. 84, 283–291. doi: 10.1093/cvr/cvp202*

Peterson, J. R., Sharma, R. V., and Davison R. L. (2006). Reactive oxygen species in the neuropathogenesis of hypertension. *Curr. Hypertens. Rep. 8, 232–241. doi: 10.1007/s11906-006-0056-1*

Pridjian, G., and Puschett, J. B. (2002a). Preeclampsia. Part 1: clinical and pathophysiological considerations. *Obstet. Gynecol. Surv. 57, 598–618. doi:10.1097/00006254-200209000-00023*

Pridjian, G., and Puschett, J. B. (2002b). Preeclampsia. Part 2: experimental and genetic considerations. *Obstet. Gynecol. Surv. 57, 619–640. doi:10.1097/00006254-200209000-00024*

Roberts, J. M., Mascalzoni, D., Ness, R. B., Poston, L., and Global Pregnancy Collaboration (2016). Collaboration to understand complex diseases: preeclampsia and adverse pregnancy outcomes. *Hypertension 67, 681–687. doi: 10.1161/HYPERTENSIONAHA.115.06133*

Scott, D. E. (1972). Anemia in pregnancy. *Obstet. Gynecol. Annu. 1, 219–244.*

Sedeek, M., Gilbert, J. S., LaMarca, B. B., Sholook, M., Chandler, D. L., Wang, Y., et al. (2008). Role of reactive oxygen species in hypertension produced by reduced uterine perfusion in pregnant rats. *Am. J. Hypertens. 21, 1152–1156. doi: 10.1038/ajh.2008.239*

Siddiqui, I. A., Jaleel, A., Tamimi, W., and Al Kadri, H. M. (2010). Role of oxidative stress in the pathogenesis of preeclampsia. *Arch. Gynecol. Obstet. 282, 469–474. doi: 10.1007/s00404-010-1358-6*

Uddin, M. N., Agunanenne, E., Horvat, D., and Puschett, J. B. (2010). Alterations in the renin-angiotensin system in a rat model of human preeclampsia. *Am. J. Nephrol. 31, 171–177. doi: 10.1159/000267099*

Venza, M., Visalli, M., Beninati, C., De Gaetano, G. V., Teti, D., and Venza, I. (2015). Cellular mechanisms of oxidative stress and action in melanoma. *Oxid. Med. Cell. Longev. 2015:481782. doi: 10.1155/2015/481782*

Wang, Y. K., Yu, Q., Tan, X., Wu, Z. T., Zhang, R. W., Yang, Y. H., et al. (2016). Centrally acting drug moxonidine decreases reactive oxygen species via inactivation of the phosphoinositide-3 kinase signaling in the rostral ventrolateral medulla in hypertensive rats. *J. Hypertens. 34, 993–1004. doi: 10.1097/HJH.0000000000000887*

Xuan, R. R., Niu, T. T., and Chen, H. M. (2016). Astaxanthin blocks preeclampsia progression by suppressing oxidative stress and inflammation. *Mol. Med. Rep. 14, 2697–2704. doi: 10.3892/mmr.2016.3569*

Zahid, H. M., Ferdaus, M. Z., Ohara, H., Isomura, M., and Nabika, T. (2016). Effect of p22phox depletion on sympathetic regulation of blood pressure in SHRSP: evaluation in a new congenic strain. *Sci. Rep. 6:36739. doi: 10.1038/srep36739*

Zhou, L., Dong, H., Huang, Y., Xu, L., Zou, X., Wang, K., et al. (2013). Attenuates diabetic nephropathy in Type 2 diabetic rats through PKC-α/NADPH oxidase signaling pathway. *Evid. Based Complement. Alternat. Med. 2013:504642.* doi: 10.1155/2013/504642*

Zimmerman, M. C., Lazartigues, E., Sharma, R. V., and Davisson, R. L. (2004). Hypertension caused by angiotensin II infusion increases superoxide production in the central nervous system. *Circ. Res. 95, 210–216. doi: 10.1161/01.RES.0000135483.12297.e4*

Conflict of Interest Statement: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

The reviewer HW and handling Editor declared their shared affiliation.