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Effects of lipopolysaccharide stimulation during pregnancy on kidneys and blood vessels in offspring rats

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Abstract: *Objective: To investigate the effect of lipopolysaccharide (LPS) inflammatory stimulation during pregnancy on offspring rats. Methods 20 SD pregnant rats were randomly divided into control and LPS groups, with 10 rats in each group. Rats in LPS group received intraperitoneal injection of LPS 0.79 mg/kg on the Days 8th, 10th, and 12th of gestation, and rats in control group received intraperitoneal injection of the same volume of sterile saline. The blood pressure of offspring rats was measured once every two weeks from 8 weeks of age to 16 weeks of age. Protein expression of NF-κB and NOX2 was detected by immunohistochemistry. ROS and NO levels in vascular tissues were observed by staining with DHE and DAF-2DA cular tissues were observed by staining with DHE and DAF-2DA fluorescent probes, respectively. Results At 8, 10, 12, 14 and 16 weeks of age,the systolic blood pressure of offspring in the LPS group was higher than that of the control group at the same age (P< 0.01).The urine volume and urinary sodium excretion rate of the LPS group were significantly lower than those of the control group (P < 0.05), while the urine creatinine and urine protein were not statistically significant as compared with those of the control group. Compared with the control group, the expression of NF-κB, NOX2, Ang II in thoracic aorta, and ROS in mesenteric artery were higher in LPS group. However, mesenteric artery NO expression was lower (P < 0.01) Conclusion LPS inflammatory stimulation during pregnancy causes kidneys inflammation and increased oxidative stress in blood vessels in offspring rats.*

Keywords: *Lipopolysaccharide; Oxidative stress; Offspring rat hypertension; Inflammatory stimulation; Programmed blood pressure*

1. Introduction

Hypertension is a prominent risk factor for mortality and morbidity worldwide and is widely associated with cardiovascular disease (CVD) such as atherosclerosis, acute myocardial infarction, and cardiomyopathy[1]. Maternal exposure to adverse environments such as drugs (e.g., cocaine, caffeine, dexamethasone), environmental toxicants (e.g., environmental organic volatiles), poor diet (e.g., alcohol, coffee, etc.), and adverse emotions have the greatest impact on fetal development. An adverse maternal environment during pregnancy impairs fetal development and may increase the risk of cardiovascular and renal disease in the offspring in adulthood[2,3]. Clinical epidemiological studies have also confirmed that inflammatory stimulation during pregnancy are closely related to the development of hypertension in the offspring[4].Our previous studies have found that LPS exposure during pregnancy caused increased blood pressure in offspring rats[5]. However, the specific mechanism is still unclear. In this study, we intended to investigate the effect of oxidative stress in arteries in the programming of hypertension induced by prenatal LPS exposure in offspring rats.

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2. Materials and methods

2.1 Animals

SPF grade SD rats were purchased from Sikebas Biotechnology Co., Ltd., license number: SCXK (Lu) 20190003. Ten male and twenty female rats were mated at the rate of 1:2 at room temperature (24±1℃) and under a 12 h light-dark cycle. The vaginas of the females were examined at 7:00 and 19:00 on the following day. A positive vaginal smear for sperm or having a vaginal plug was defined as day 0 of pregnancy. All pregnant rats were randomly divided into 2 groups with 5 rats in each group. Rats in LPS group was injected intraperitoneally with LPS 0.79 mg/kg on days 8th, 10th and 12th of pregnancy, and the control group was injected intraperitoneally with the same volume of sterile saline. After birth, the rats were breastfed for 1 month and then fed with the standard diet of the experimental rats.

The present study was conducted in accordance with the principles outlined in the National Institutes of Health (NIH) Guide for the Care and Use of Laboratory Animals and was approved by the local animal ethics committee at Henan University of Science and Technology, China. Every effort was made to minimize the number of animals used and their suffering.

2.2 Systolic Blood pressure(SBP) measurement

SBP was measured at 15:00 to 18:00 o'clock once every two weeks from 8 weeks of age until 16 weeks of age in offspring rats, using the standard tail-cuff method. Before measurement of SBP, rats were placed inside a warming chamber (~34℃) for 15 min and were then placed in plastic restraints. A cuff with a pneumatic pulse sensor was attached to the tail. In each rat, mean SBP was calculated from five consecutive SBP recordings. The rats had been trained at least three times before formal measurement.

2.3 Metabolic cage urinalysis

Monitoring of urinary sodium excretion in offspring rats started at week 16. Rats were acclimated to metabolic cages for 1 day before formal urine collection began. The feed was ground into powder powder with a flour machine and mixed with water to form a paste. During urine collection, rats in each group were fed pasty food and water ad libitum and at an ambient temperature of approximately 24 ℃. One rat was placed in a metabolism cage for 24 h for urine collection. Urine was then centrifuged at 2500 × g for 10 min at 4 ℃ and the supernatant was taken. Renal function parameters were measured by an automatic biochemical analyzer in the Department of Clinical Laboratory of the First Affiliated Hospital of Henan University of Science and Technology.

2.4 Immunohistochemistry and Immunofluorescence

The tissues were incubated in 4%paraformaldehyde for 24 h and embedded in paraffin wax. The sections (4μm) were rinsed and rehydrated in phosphate-buffered saline (PBS) for 5 min. An indirect immunoperoxidase staining technique was performed using the two-step IHC Detection reagent. Briefly, endogenous peroxidase was inhibited by treatment with 3% H_2O_2 in PBS for 15 min. Then, blocking solution with 5% bovine serum albumin was applied to the sections for 15 min at room temperature to avoid nonspecific binding of the biotinylated antibody. The tissues were incubated with rabbit anti-COX2 antibody, rabbit anti-NF-κB antibody, rabbit anti-Ang II antibody and rabbit anti-NOX2 antibody at 4 °C overnight. A second goat anti-rabbit IgG antibody and goat anti-mouse Alexa-488 were then added and incubated for 1 hour. Peroxidase activity was visualized using a DAB kit. Finally, the slides were dehydrated, mounted in an aqueous-based mounting medium and examined by light microscopy. Twenty high magnification fields were randomly selected from each section and positive signal areas and mean grayscale values were measured to calculate optical density (OD) values using Image-Pro Plus software ((Media Cybernetics, Silver Spring, MD, USA). The expression of Ang II in the thoracic aorta was observed under a fluorescence microscope (Zeiss Axio-vert100M, Carl Zeiss).

2.5 Measurement of ROS

ROS was measured with DHE probe. Offspring rats were anesthesized with 1% sodium pentobarbital (45 mg/kg). The thoracic aorta was rapidly isolated, incubated in 2 μmol/L DHE (Beyotime Biotech, China) for 30 min at 37°C in a light-proof container. DHE has cellular permeability and reacts

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specifically with intracellular superoxide to create ethidium which is embedded in DNA and thus exhibits red fluorescence. Images were captured with an inverted fluorescence microscope (TE-2000U; Nikon, Tokyo, Japan) and analyzed using Image-PRO Plus 5.0 (Media Cybernetics, Silver Spring, MD, USA). The integral optical density (OD) was calculated.

2.6 Measurement of NO

NO was measured with DAF-2 DA probe. After anesthesia, the thoracic aorta was rapidly isolated, placed on slides. The sections were incubated in 2 μmol/L DAF-2 DA (Beyotime Biotech, China) for 30 min at 37°C in a light-proof container. DAF-2 DA enters the cells and is catalyzed by intracellular esterases to form DAF-2 which cannot pass through the cell membrane. DAF-2 has very weak fluorescence. When oxygen is present, DAF-2 reacts with NO to form the strongly fluorescent triazole fluorescein (DAF-2T). Images were captured with an inverted fluorescence microscope (TE-2000U; Nikon, Tokyo, Japan) and analyzed using Image-PRO Plus 5.0 (Media Cybernetics, Silver Spring, MD, USA). The integral optical density (OD) was calculated.

2.7 Statistical analysis

The data are expressed as mean ± S.D. One-way analysis of variance and Tukey's post hoc test were used for all analyses. $P<0.05$ was considered significant.

3. Results

3.1 Changes of systolic blood pressure(SBP) in offspring rats

Systolic blood pressure in offspring of LPS group was higher than that in control group at the same age. They are (125.3 ± 1.7) vs. (109.8 ± 4.9), (137.9 ± 4.1) vs. (114.8 ± 4.3), (134.3 ± 3.7) vs. (11.8 ± 3.02), (135.4 ± 5.8) vs. (113.8 ± 3.6), (137.9 ± 4.1) vs. (114.8 ± 4.3) mmHg at 8, 10, 12, 14 and 16 weeks of age, respectively (Fig 1).

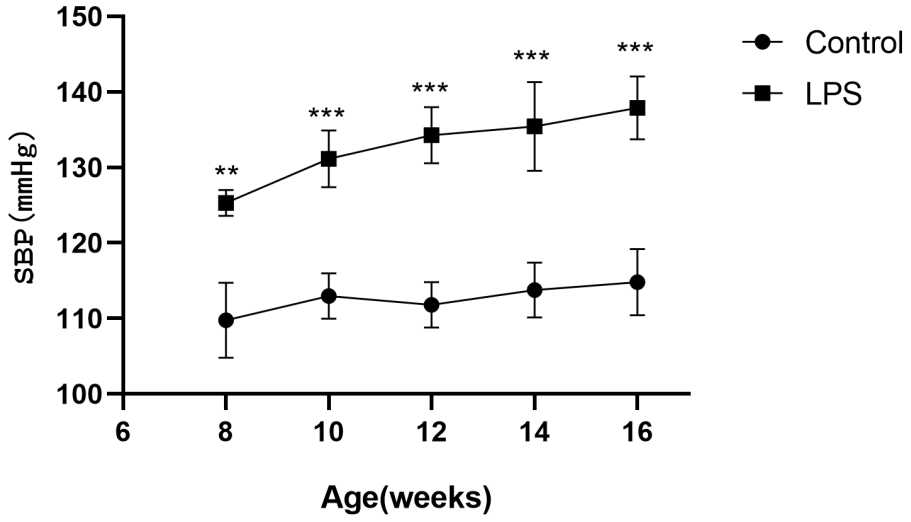

*Figure 1: SBP was measured in offspring rats of prenatal LPS-exposed and control groups once every two weeks from 8 weeks of age to 16 weeks of age using the tail-cuff method (** P<0.01, *** P<0.001 vs control, n=10/group)*

3.2 Metabolic cage urinalysis

Urine was collected and analyzed by 24 h metabolic cages, and it was found that the urine volume and urinary sodium excretion rate in the LPS group were significantly lower than those in the control group ($P < 0.05$), while urinary creatinine and urinary protein were not statistically significant ($P > 0.05$, Table 1)

ISSN 2616-5791 Vol.4, Issue 5: 53-59, DOI: 10.25236/AJMHS.2023.040509

Table 1: Comparison of kidney function parameters between control group and LPS group at 20 weeks of age

Group	Cr (mg/dL)	Urine protein (mg/24h)	Urine volume (mL/kg)	Natriuresis (μmol/kg)
Control	0.58±0.1	15.38±3.3	25.3±0.8	658±23
LPS	0.7±0.13	17.74±3.0	18.2±1.5[a]	495±38[a]

a: $P<0.05$,Compared with control group

3.3 The protein expression of COX2 in the thoracic aorta and NF-κB and NOX2 in the kidney

The protein expression of COX2 in LPS was significantly higher than that in Control group [(45.9 ± 6.5) vs (348 ± 10.7), ($P < 0.001$)]. (Fig 2).

NF-κB expression of kidney in LPS group was significantly higher than in Control group [(0.20 ± 0.03) vs (1.53 ± 0.07), ($P < 0.001$)]. (Fig 3).

The levels of NOX2 expression and LPS in kidney of offspring rats were significantly higher than those in Control group [(347.5 ± 13.5) vs (35.3 ± 3.1), ($P < 0.001$)], (Fig 4).

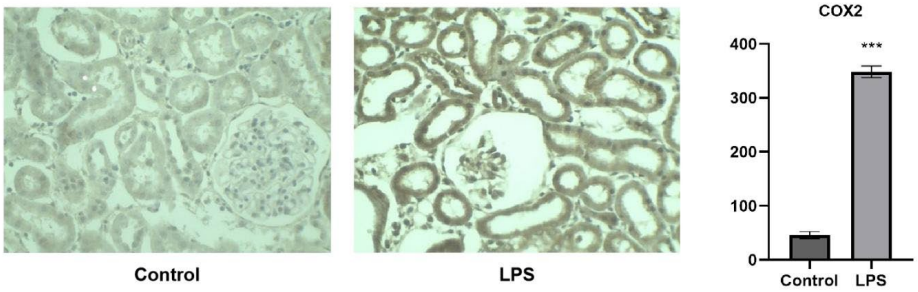

Figure 2: Effect of LPS stimulation during pregnancy on COX2 protein expression in the thoracic aorta of offspring rats.

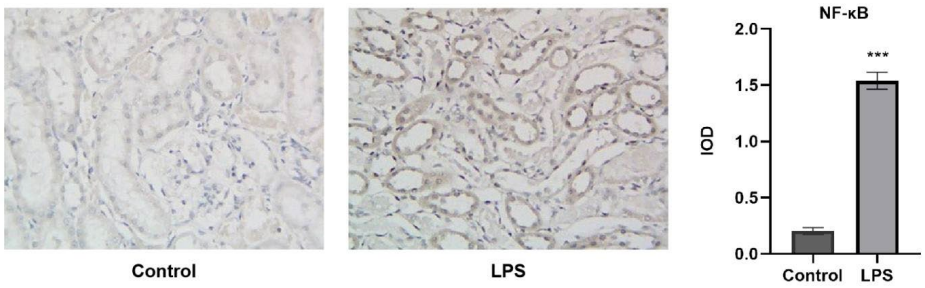

Figure 3: Effect of LPS stimulation during pregnancy on NF-κB protein expression in kidney of offspring rats. (n = 6 , Mean ± SD).

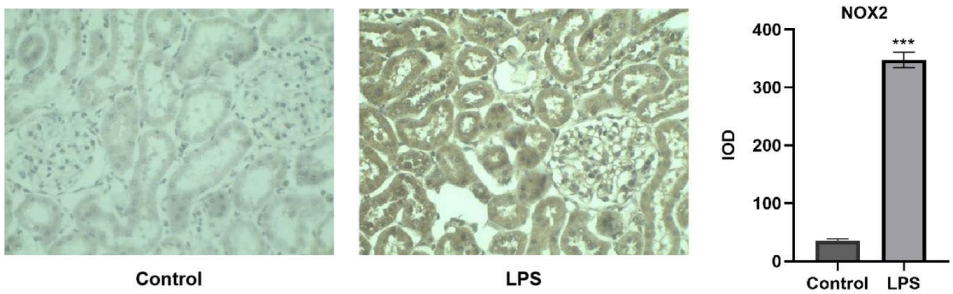

Figure 4: Effect of LPS in pregnancy stimulation on NOX-2 protein expression in kidney of offspring rats. (n = 6 , Mean ± SD).

ISSN 2616-5791 Vol.4, Issue 5: 53-59, DOI: 10.25236/AJMHS.2023.040509

3.4 Expression of AngII , ROS and NO

AngII protein expression of thoracic aorta in LPS group was significantly higher than that in Control group [(535 ± 13.42) vs (11.08 ± 1.4), ($P < 0.001$)]. (Fig 5).

Compared with control group, the expression of ROS in mesenteric arteries was significantly increased in the LPS group [(297.3 ± 6.2) vs. (0.5 ± 0.08), $P < 0.001$], (Fig 6).

The expression of NO in mesenteric arteries was significantly decreased in the LPS group [(40.4 ± 7.4) vs. (796.5 ± 24.1),($P < 0.001$)], (Fig 7)

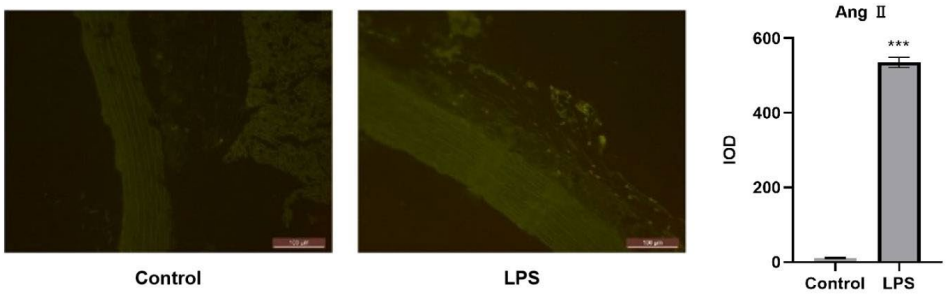

Figure 5: Influence of LPS stimulation during pregnancy on Ang II protein expression in thoracic aorta of offspring rats. (n = 6 , Mean ± SD).

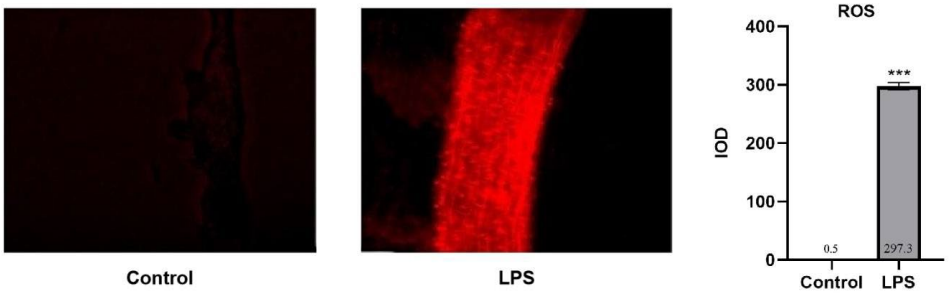

Figure 6: Impact of LPS stimulation during maternal period on ROS level in mesenteric arteries of offspring rats.(n = 6 , Mean ± SD)

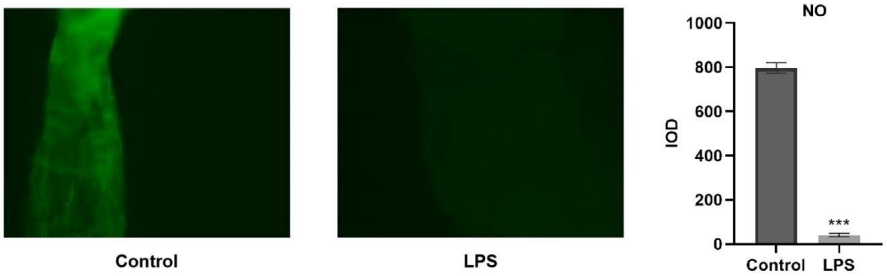

Figure 7: The influence of LPS stimulation during pregnancy on NO level in mesenteric arteries of offspring rats (n = 6, Mean ± SD).

4. Discussion

Previous explorations of the etiology of hypertension have focused on adult individuals, ignoring the influence of fetal environmental factors early in life on the susceptibility to adult hypertension[6]. Barker studied the relationship between birth weight and systolic blood pressure in adulthood and found that low weight infants are susceptible to hypertension in adulthood. This study of the inverse relationship between blood pressure and weight proposed the concept of programmed hypertension, i.e., the embryonic origin theory of hypertension. Many experimental studies on maternal malnutrition during pregnancy were investigate to illustrate the etiology of hypertension, such as inadequate maternal protein

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intake or high-fat and high-cholesterol diet [7,8] and maternal administration of glucocorticoids in gestation [5]. In our previous study, maternal intraperitoneal injection of LPS caused elevated blood pressure in adult offspring[9], suggesting that LPS, like other adverse stimuli during pregnancy, can cause elevated blood pressure in adult offspring, but the mechanism remains to be elucidated. In this experimental study, we found that the blood pressure of the offspring rats was significantly higher than that of the control group. Meanwhile, we found that the activity of NF-κB and COX2 in the kidney of the LPS-treated rats was significantly higher than that of the control group. NF-κB is a major regulator of inflammatory immunity and is involved in the early stages of the immune response and in all phases of the inflammatory response. Many molecules are regulated by NF-κB such as COX2. COX2 is induced by inflammatory stimuli and other factors such as angiotensin II, suggesting that NF-κB may be involved in hypertension by regulating the release of inflammatory mediators. We found that Ang II expression is high in the thoracic aorta of the offspring rats, which suggests that inflammation overstimulates the renin-angiotensin-aldosterone system (RAAS). Inflammatory markers such as C-reactive protein (CRP) [10], TNF-α and IL-1β[11] have been found to upregulate AT1R. Ang II regulates the immune system [12] and activates NADPH oxidase [13]. The main enzyme family of ROS is NADPH oxidase, in which NOX2 is the main functional subunit of NADPH oxidase in single nucleated cells. In the present study, we found that the level of oxidative stress of NOX2 was significantly higher in the LPS-treated group than in the control group. NADPH oxidase is a central mediator of ROS production in hypertension and renal disease [14] by acting in the blood vessels and kidneys [15]. NOX2-dependent ROS production takes part in this process, since this NADPH oxidase subunit is linked to impaired sodium handling, increased renal vascular resistance and renal fibrosis [16,17]. By assessing mesenteric artery and renal tissue ROS, we found significantiy higher levels of oxidative stress in LPS than in controls, and we can infer increased vascular reactivity in the efferent small arteries. Indeed, reduced blood flow to the kidney decrease renal function and can lead to chronic kidney disease [18], and oxidative stress plays a major role in its development [19]. We also speculated that the elevated blood pressure in the offspring rats may be associated with impaired vascular endothelial function, restricted nitric oxide synthesis, and elevated levels of oxidative stress and consequently impaired acetylcholine-mediated mesenteric arterial diastolic function. These processes are regulated by complex interacting systems such as the renin-angiotensin-aldosterone system, the sympathetic nervous system, oxidative stress, and immune activation.

5. Conclusion

Overall, the present results suggest a link between increased ROS and Ang II hyperactivity, inflammation. Inflammatory stimulation of LPS during pregnancy causes elevated oxidative stress in kidney and blood vessels in offspring rats, which acts as a key trigger for inflammation and hypertension.

Acknowledgements

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