Folic Acid Combined with L-Carnitine Alleviates Vascular Endothelial Injury in Preeclampsia Rats by Regulating Oxidative Stress Injury and Inflammatory Factor Expression

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To investigate the effect of folic acid combined with L-carnitine on vascular endothelial injury in preeclampsia rats by regulating oxidative stress injury and inflammatory factor expression. 42 female Sprague-Dawley rats were randomly divided into normal control group, preeclampsia group and folic acid+L-carnitine group with 14 rats in each group. The rats in folic acid+L-carnitine group were treated with folic acid 3 mg/kg and 400 mg/kg respectively the rats in normal control group and preeclampsia group were intragastrically given the same amount of normal saline as folic acid+L-carnitine group; from 9 d to 20 d, 75 mg/kg N-nitro-L-arginine methyl arginine was subcutaneously injected into the neck and back of rats in preeclampsia group and folic acid+L-carnitine group from 9 d to 20 d. The gestational d 0 and d 5 of each group were compared after 21 d, the percentage of malondialdehyde, 8-iso-prostaglandin F2 alpha, interleukin-6, monocyte chemotactic protein-1, interleukin-1 beta and vascular endothelial microparticles were measured. The changes of femoral artery fibromuscular dysplasia were observed at 0 s, 60 s, 120 s, 180 s, 240 s and 300 s after 21 d. The levels of malondialdehyde and 8-iso-prostaglandin F2 alpha in preeclampsia rats were increased than those in normal control group; and those in folic acid+L-carnitine group were reduced than those in preeclampsia group. The levels of interleukin-6 and monocyte chemotactic protein-1 in preeclampsia rats were raised than those in normal control group; while these in folic acid+L-carnitine group were reduced than those in preeclampsia group. Folic acid combined with L-carnitine can alleviate vascular endothelial injury in preeclampsia rats by regulating oxidative stress injury and expression of inflammatory factors.

Key words: Folic acid, L-carnitine, preeclampsia, vascular endothelial damage, hypertension

Preeclampsia is one of the complications of gestational hypertension, which can cause hypertension and proteinuria at the same time. However, the specific pathogenesis of preeclampsia is not clear, so no targeted measures have been found to effectively treat the disease. However, it is feasible to detect the related indicators of preeclampsia in the early stage and then give symptomatic treatment[1]. Therefore, the exploration of indicators that can reflect the incidence of early preeclampsia and the development of new preventive drugs for preeclampsia has become the focus of clinical scholars. Clinical studies have shown that vascular endothelial destruction plays a key role in the occurrence of preeclampsia, which can secrete vasoconstrictor and vasodilator, and then play a role in regulating smooth muscle tension; in addition, endothelium can also control anticoagulation and antiplatelet by secreting many types of soluble factors. According to the clinical literature, one of the most important causes of vascular endothelial damage is preeclampsia placental ischemia and hypoxia. In addition, hyperhomocysteinemia and inflammatory factors can induce endothelial destruction[2,3]. The abnormal content of plasma homocysteine is one of the most important biomarkers leading to adverse pregnancy outcome in pregnant women, which can lead to the destruction of vascular

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endothelial cells and the increase of Oxidative Stress (OS), which in turn leads to the increase of intraplacental vascular tension, and finally leads to placental ischemia and hypoxia. Folic Acid (FA) has a significant effect on reducing the level of plasma homocysteine[4,5]. L-Carnitine (LC) can be involved in lipid metabolism. At present, it is often used in the treatment of heart failure and asthenospermia[6]. It has also been reported that LC can alleviate OS and inflammatory injury[7]. However, the role of FA and LC in Vascular Endothelial Injury (VEI) in preeclampsia has not been clarified, so the purpose of this study is to explore the protective effect of FA combined with LC on VEI in preeclampsia rats and its mechanism. 42 female Sprague-Dawley (SD) rats were purchased from Reed liver Disease Research (Shanghai) Co., Ltd., with batch number SCXK (Shanghai) 2019-0025. All the selected rats were fed adaptively for 1 w and were fed in clean experimental animal rooms. The time point of light was 700 mm 21V, the noise was 60 dB or less, the room temperature was (23°±1°), the relative humidity was (50±10) %, and the relevant padding and drinking bottles were given, and the padding and drinking bottles were changed every 3 d. Bicinchoninic Acid (BCA) protein concentration detection kit was purchased from Beijing Kangjia Hongyuan Biotechnology Co., Ltd.; Enzyme-Linked Immunosorbent Assay (ELISA) kit was purchased from Jiangxi Jianglan Pure Biological Reagent Co., Ltd.; Bio LEGENDplex™ multi-factor flow detection kit was purchased from Beijing Baitai Paike Biotechnology Co., Ltd. Flow cytometry was purchased from Changzhou Bidake Biotechnology Co., Ltd.; cryogenic refrigerator was purchased from Hangzhou Nuoding Scientific equipment Co., Ltd.; non-invasive rat tail electronic sphygmomanometer was purchased from Shenzhen Zhongtu instrument Co., Ltd.; electron microscope was purchased from Quantum Quantum Science instrument Trading (Beijing) Co., Ltd. 42 female SD rats were randomly divided into normal control group (n=14), preeclampsia group (n=14) and FA+LC group (n=14). All the three groups were caged at 19:00 according to the ratio of female to male of 2:1. The vaginal secretions of female SD rats were examined by smear in the early morning of the 2nd d. If sperm were observed to indicate successful conception, the rats in FA+LC group were given intragastric administration of 3 mg/kg FA and 400 mg/kg once a day. The normal control group and preeclampsia rats were treated with the same amount of normal saline as FA+LC group. On the 20th d, preeclampsia rats and FA+LC group rats were subcutaneously injected with 75 mg/kg L-Nitro Arginine Methyl Ester (L-NAME) on the back of neck. The blood pressure was measured by noninvasive rat tail sphygmomanometer. The systolic blood pressure of some prognostic rats was higher than that before intervention and elevated to 115 mmHg was regarded as hypertension. The urine of three groups of rats were collected on the 5th d of gestation, the 15th d after the 10th d and the 24th d after the 20th d, and put into the centrifuge tube. The rats were centrifuged at 4° at the speed of 3000 r/min. The total urine volume of rats in each group was calculated by the pipette, and the 24 h urine protein was detected by BCA protein concentration detection kit. All the rats in the three groups were treated by cesarean section on the 21st d after pregnancy and their abdominal aortic blood were taken. The levels of Malondialdehyde (MDA) and 8-iso-prostaglandin were measured by ELISA kit. The levels of Interleukin (IL)-6, Monocyte Chemoattractant Protein-1 (MCP-1) and IL-1 Beta (β) in rats of each group were measured by Bio LEGENDplex™ multifactor flow detection kit, and the level of vascular endothelial microparticles was measured by flow cytometry. On the 20th d of pregnancy, the hollow annular head of the rat vascular occlude was used to oppress the distal end of the probe, which led to femoral artery ischemia after 5 min. At the same time, the Flow-Mediated Vasodilation Function (FMD) of the femoral artery was measured by small animal ultrasound. The relevant statistical data of this study were processed by Statistical Package for the Social Sciences (SPSS) 23.0, and the measurement data of OS related indexes of rats in each group were expressed as ($\bar{x}$±s). T-test was used for comparison between the two groups, and one-way Analysis of Variance (ANOVA) was used for comparison between multiple groups. Compared with the normal control group, *$p<0.05$ and compared with the preeclampsia group, $^{\#}p<0.05$. The 24 h urinary protein level of preeclampsia rats was raised than that of normal control group at 10 d, 15 d and 20 d, and that of FA+LC group was reduced than that of preeclampsia group as shown in Table 1. After

perfusion of 5 min, FMD increased gradually and then decreased gradually in normal control group and FA+LC group, and reached the peak at 120 s, while that in preeclampsia group reached the peak at 300 s. The peak level of FMD in FA+LC group was raised than that in preeclampsia group (Table 2). The percentage of endothelial microparticles in preeclampsia group was raised than that in normal control group, while this in FA+LC group was reduced than that in preeclampsia group as shown in Table 3. The levels of MDA and 8-iso-Prostaglandin F2 Alpha (8-iso-PGF2α) in preeclampsia rats were raised than those in normal control group, while these in FA+LC group were reduced than those in preeclampsia group (Table 4). The levels of IL-6 and MCP-1 in preeclampsia rats were raised than those in normal control group, while these in FA+LC group were reduced than those in preeclampsia group (Table 5). Preeclampsia can increase the risk of maternal death. However, there are few reports on the prevention and treatment of preeclampsia. Therefore, looking for a method that can effectively reflect maternal vascular injury in patients with preeclampsia has important clinical value and social benefits for the prevention and treatment of the disease. LC can be synthesized by itself, which can improve the cell activity by improving the function of mitochondria[8]. Clinical literature shows that FA is mainly used in clinical practice to prevent fetal neural tube malformations, and low-dose FA in early pregnancy can prevent fetal neural tube defects caused by FA deficiency to a certain extent[9]. Another report shows that FA and LC are natural nutrients, and the combination of FA and LC can effectively prevent the onset of preeclampsia and has certain safety[10]. Here, we monitored the 24 h urinary protein level of rats in each group. It was found that the 24 h urinary protein level in the FA+LC group was reduced than that in the preeclampsia group. It is suggested that FA combined with LC can improve the increase of urinary protein induced by L-NAME. Brachial artery FMD is a repeatable and low-cost method to detect vascular endothelial function, which is non-invasive. Many reports show that in normal pregnancy, FMD increases gradually, but FMD in patients with preeclampsia decreases. At present, this test is mainly used to evaluate preeclampsia. In this study, we did not find the synergistic effect of FA combined with LC, which may be due to the fact that the rats were only pregnant for about 21 d and the drug intervention time was short, which led to the unsatisfactory effect. However, from the data of this study, there is a downward trend. Clinical studies have shown that when endothelial cells are activated or apoptosis, bubbling is usually used to exfoliate micro particles from the capsule, i.e., endothelial particles, which are directly derived from activated or apoptotic endothelial cells, which is conducive to the direct detection of endothelial cell injury[11]. Clinical studies have shown that endothelial particles cannot only reflect endothelial cell dysfunction, but also mediate vascular endothelial cell injury. We monitored the level of vascular endothelial micro particles in preeclampsia rats. The results showed that the percentage of endothelial micro particles in FA+LC group was reduced than that in preeclampsia group. It is suggested that FA combined with LC can protect vascular endothelium of rats with preeclampsia and prevent the occurrence of preeclampsia. It has been confirmed that endothelial cell destruction in patients with preeclampsia occurs rapidly in the early stage of pregnancy, and OS injury and inflammatory response can lead to vascular endothelial cell activation and eventually endothelial cell destruction[12]. Clinical studies have shown that the levels of OS and inflammatory response related indexes in plasma of patients with preeclampsia are significantly increased, while the levels of endothelial protection related factors are significantly decreased, which leads to the destruction of vascular endothelial cells. This may be due to the ischemia and hypoxia of placental trophoblasts[13]. It has been reported that vascular endothelial cells are damaged under the stimulation of many factors, such as physical or chemical signals, and then release many types of cytokines to control vascular tension, thrombosis, cell proliferation and vascular wall inflammation[14]. The levels of MDA, 8-iso-PGF2α, IL-6, MCP-1 and IL-1 β in FA+LC group were reduced than those in preeclampsia group. It is suggested that FA combined with LC can significantly alleviate OS injury and inflammatory response in rats with preeclampsia. To sum up, FA combined with LC has the effect of VEI in rats with preeclampsia, which may be achieved by alleviating OS injury and reducing the expression levels of IL-6, MCP-1 and IL-1β.

TABLE 1: URINARY PROTEIN OF RATS IN EACH GROUP (x̄±s)

Group	n	0 d (mg/24 h)	5 d (mg/24 h)	10 d (mg/24 h)	15 d (mg/24 h)	20 d (mg/24 h)
Normal control	14	6.87±0.01	6.89±0.03	6.98±0.05	7.05±0.01	7.25±0.03
Preeclampsia	14	6.88±0.01	6.87±0.03	8.05±0.04*	11.56±0.15*	11.68±0.11*
FA+LC	14	6.89±0.01	6.66±0.05	7.02±0.03#	9.05±0.07#	9.16±0.06#

Note: Compared with the normal control group, *p<0.05 and compared with the preeclampsia group, #p<0.05

TABLE 2: FMD CHANGES IN FEMORAL ARTERY OF RATS (x̄±s)

Group	n	0 s (%)	60 s (%)	120 s (%)	180 s (%)	240 s (%)	300 s (%)
Normal control	14	3.01±0.05	7.25±0.11	12.03±0.23	6.98±0.05	4.95±0.09	2.51±0.05
Preeclampsia	14	2.53±0.06	6.15±0.08	6.99±0.11	7.89±0.11	9.05±0.15	12.43±0.16
FA+LC	14	5.01±0.06	9.99±0.15	16.12±0.25#	10.56±0.19	7.45±0.11	3.01±0.08

Note: Compared with the preeclampsia group, #p<0.05

TABLE 3: ENDOTHELIAL MICROPARTICLES IN DIFFERENT GROUPS OF RATS (x̄±s)

Group	n	Percentage of endothelial micro particles (%)
Normal control	14	4.99±0.10
Preeclampsia	14	25.12±3.58*
FA+LC	14	7.45±0.05#

Note: Compared with the preeclampsia group, *p<0.05 and compared with the FA+LC, #p<0.05

TABLE 4: LEVELS OF RELATED INDEXES OF OS IN RATS (x̄±s)

Group	n	MDA (nmol/ml)	8-iso-PGF2α (ng/l)
Normal control	14	4.63±0.27	94.84±4.19
Preeclampsia	14	12.79±0.66*	124.23±15.48*
FA+LC	14	6.74±0.68#	103.90±13.26#

Note: Compared with the preeclampsia group, *p<0.05 and compared with the FA+LC, #p<0.05

TABLE 5: INFLAMMATION RELATED FACTORS IN RATS (x̄±s)

Group	n	IL-6 (pg/ml)	MCP-1 (pg/ml)	IL-1β (pg/ml)
Normal control	14	101.07±1.84	87.19±16.60	33.12±1.89
Preeclampsia	14	134.52±6.76*	198.28±8.95*	27.67±2.54
FA+LC	14	124.34±4.57#	119.82±10.30#	27.55±2.12

Note: Compared with the preeclampsia group, *p<0.05 and compared with the FA+LC, #p<0.05

Conflict of interests:

The authors declared no conflict of interests.

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