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The Effects of Naoxintong Capsule on the Vascular Endothelial Function and Inflammatory Factors in Spontaneously Hypertensive Rats

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Abstract: Objective: To investigate the effects of Naoxintong (NXT) capsule on vascular endothelial function and inflammatory mechanism in spontaneously hypertensive rats. Methods: A total of 100 spontaneously hypertensive rats were divided into model group, positive control group, NXT low-dose group, NXT medium-dose group and NXT high-dose group. Rats in the model group were given an equal dose of normal saline once a day; the positive control group was given telmisartan 50 mg/(kg·d) once a day; the low, middle and high dose groups were given the NTX 0.5, 1.0, 2.0 mg/(kg·d) once a day, respectively. Rats in each group were continuously intragastrically administered for 12 weeks. The vascular endothelial function index, inflammation index and blood pressure of each group were observed at the end of 8 weeks. Results: Endothelin (ET), interleukin-6 (IL-6), tumor necrosis factor-α (TNF-α), C-reactive protein (CRP) and systolic blood pressure levels were lower in the positive control group and NXT group compared with model group. In the model group, the NO level was lower than the positive control group and NXT group; the levels of ET, IL-6, TNF-α, CRP and systolic blood pressure in the middle-dose group and the high-dose group were lower than those in the positive control group and NXT low-dose group. Furthermore, the level of NO was higher in positive control group and NXT middle and high dose group compared with model group. The levels of ET, IL-6, TNF-α, CRP and systolic pressure in NXT high dose group were lower than in NXT middle dose group, while the level of NO was higher in NXT high group than in NXT middle dose group and the difference was statistically significant ($p < 0.05$). Conclusions: NXT has obviously antihypertensive effect on spontaneously hypertensive rats, and its mechanism may be related to the improvement of vascular endothelial function and inflammatory factors.

Key words: Naoxintong capsule, spontaneous hypertension, vascular endothelial function, inflammatory factor.

1. Introduction

Hypertension is a common chronic disease which is mainly characterized as continuous increase of arterial blood pressure. Hypertension has become the most familiar “epidemic disease” in the world [1], which has caused great economic and social burden [2]. Hypertension often leads to dysfunction of kidney [3], brain [4], heart [5] and other important organs. Because of its harmful complications in the cardiovascular system, hypertension has been identified as the major risk factor for high cardiac mortality.

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Hypertension is closely related to the synthesis and release of endothelin and angiotensin II from vascular endothelial cells [6]. Previous study has shown that vascular endothelium is damaged during hypertension, which further leads to an imbalance between endothelin and nitric oxide (NO) secreted by the vascular endothelium [7]. During the hypertension, vascular endothelial dysfunction is mainly characterized by decreased endothelium-dependent diastolic function and/or enhanced endothelium-dependent systolic function due to the decreased bioavailability of endogenous NO [8]. NO is the most important vasoactive substance, which can result in increase blood pressure [9]. The production of NO is closely linked to L-arginine and nitric oxide

synthase (NOS), and the NOS which regulates vascular tone is mainly endothelial nitric oxide synthase (eNOS) [10]. The level of NO in the human body is decreased during hypertension, and the severity of its descent is positively correlated with the severity of blood pressure. Houston Mar et al. [11] reported that use of NO oral agents could effectively reduce systemic blood pressure.

Traditional Chinese herb has been used to treat cardiovascular and cerebrovascular diseases in China for more than 2,000 years. Advanced pharmaceutical technologies have developed many oral agents of traditional Chinese patent medicines based on the famous traditional formulae for the prevention and treatment of diseases, including hypertension. Naoxintong (NXT) capsule is a traditional Chinese herb compound according to the theory of traditional Chinese medicine. It could activate the blood circulation, clear collaterals, improve endothelial function and plays a pivotal role in anti-atherosclerosis [12, 13]. In recent years, more and more clinical studies indicated that NXT could reduce the plaque area and carotid intima-media thickness [14], and it could relieve the symptoms of hypertension as well. However, the specific mechanism by which NXT controls the blood pressure is completely unknown. The current study attempted to investigate the effects of NXT on vascular endothelial function and inflammatory mechanism in spontaneously hypertensive rats. Our results provided solider evidence for application of this traditional drug in clinical and basic research and treatment of hypertension.

2. Materials and Methods

2.1 Animal Study

The experimental protocol was in accordance with the guidelines of the Fudan University for Animal Experimentation and was approved by the Ethical Committee of the Fudan University Affiliated Zhongshan Hospital from January 2017 to January 2018. Totally, 100 spontaneously hypertensive rats were purchased from Shanghai Slack Laboratory Animals Co. Ltd. These rats were clean grade and half male and half female. Body mass were 200.48 ± 20.18 g and they were fed with food and water *ad libitum*, in room temperature of 23~25 °C, relative humidity (70%), adaptive feeding for 1 week. They were divided into model group, positive control group, NXT low-dose group, NXT medium-dose group and NXT high-dose group.

2.2 Reagents

Endothelin (ET) and NO assay kits were purchased from Shanghai Enzyme Biotechnology Co. Ltd. Interleukin-6 (IL-6), tumor necrosis factor-α (TNF-α), C-reactive protein (CRP) ELISA kit was purchased from Wuhan Boster Bioengineering Co. Ltd. NXT was purchased from Shaanxi Buchang Pharmaceutical Co. Ltd. (specification: 0.4 g per tablet). Telmisartan tablets were purchased from Yichang Changjiang Pharmaceutical Co. Ltd. (specification: 40 mg).

2.3 Animal Treatments

Rats in the model group were given an equal dose of normal saline once a day; the positive control group was given telmisartan with 50 mg/(kg·d) once a day; the lower, middle and high dose of NXT groups were give NXT with 0.5, 1.0, 2.0 mg/(kg·d), respectively, once a day. All rats were continuously administrated for 8 weeks. Serum samples were collected at the end of 8 weeks. Rats were anesthetized with ulatan, 6 mL of blood from abdominal aorta was taken into the EP tube, and the serum was centrifuged at 3,000 r/min for 10 min at 4 °C. The serum was separated and stored at -80 °C for further testing.

2.3.1 Blood Pressure Measurements in Rats

Indirect systolic blood pressure (SBP) was measured using tail-cuff plethysmography (Power Lab, AD Instruments, Pty Ltda, CO), as described by Rodrigues et al. [15]. Mean SBP was calculated from an average of four successive measurements in each rat.

2.3.2 Serum Nitrite and Nitrate (NO)

Serum NO levels were obtained by measuring the serum concentrations of its stable end-products nitrite (NO_2^-) and nitrate (NO_3^-), as described previously [16]. The NO/ozone chemiluminescence was performed using the NO Analyzer 280i (Sievers, Boulder, CO, USA).

2.3.3 Measurement of Inflammatory Cytokines

Quantification of inflammatory cytokines such as TNF-α, IL-6 and CRP in serum was performed using the indicated ELISA kit. IL-6 and TNF-α were evaluated using commercial OptEIA kits (BD Biosciences, Pharmingen, USA). Adiponectin and CRP were analyzed using Duo Set kits (R&D Systems, USA). All kits were used according to the manufacturers' instructions, and the results were expressed in pg/mL for all cytokines evaluated.

2.3.4 Statistical Analysis

All statistical analyses were performed using GraphPad Prism (https://www.graphpad.com/). The data were represented as means ± S.E.M. Differences between two groups were analyzed by unpaired two-tailed Student's *t* test. Difference with $p < 0.05$ was considered statistically significant. Chi-square test was used for the comparison of the count data. $p < 0.05$ was considered statistically significant.

3. Results

3.1 The Effects of NXT on Serum Inflammatory Cytokines in Spontaneously Hypertensive Rats

In order to evaluate the effects of NXT on the expression levels of inflammatory cytokines, the spontaneously hypertensive rats were treated with or without of NXT and telmisartan and then the serum of treated rats were collected. The indicated cytokines were detected by ELISA. We found that the expression levels of ET, IL-6, TNF-α and CRP in the positive control group and all NXT group were lower than those in the model group. The levels of serum ET, IL-6, TNF-α and CRP in the middle and high dose groups of NXT were lower than the positive control group and the low dose group of NXT was higher than that of the positive control group; The level of serum ET, IL-6, TNF-α and CRP in the high dose group was lower than the middle dose group, and the difference was statistically significant ($p < 0.05$). All the results were shown in Figs. 1A-1D.

3.2 NXT Ameliorates Systolic Blood Pressure in Spontaneously Hypertensive Rats

In order to evaluate the effects of NXT on systolic blood pressure, the spontaneously hypertensive rats were treated with or without of NXT and telmisartan and then the systolic blood pressure was measured. We found that systolic blood pressure was no significant difference between NXT low-dose group and the positive control group ($p > 0.05$). The systolic blood pressure in the positive control group and the all NXT groups was lower than that of the model group; the systolic blood pressure in the middle dose group and the high dose group was lower than that of the positive control group; the systolic blood pressure was lower in NXT high dose group than in NXT middle dose group. The difference was statistically significant ($p < 0.05$). All the results were shown in Fig. 2.

3.3 NXT Induces Serum NO Expression in Spontaneously Hypertensive Rats

Finally, in order to evaluate the effects of NXT on NO expression, the spontaneously hypertensive rats were treated with or without of NXT and telmisartan and then the NO expression level was measured. By quantification of serum NO expression, we observed that the serum level of NO in the positive control group and the all NXT groups was higher than that of the model group; the serum level of NO in the middle dose group and the high dose group was higher than that of the positive control group and NXT low dose group; the serum level of NO was higher in NXT high dose group than in NXT middle dose group. The difference was statistically significant ($p < 0.05$) and the results were shown in Fig. 3.

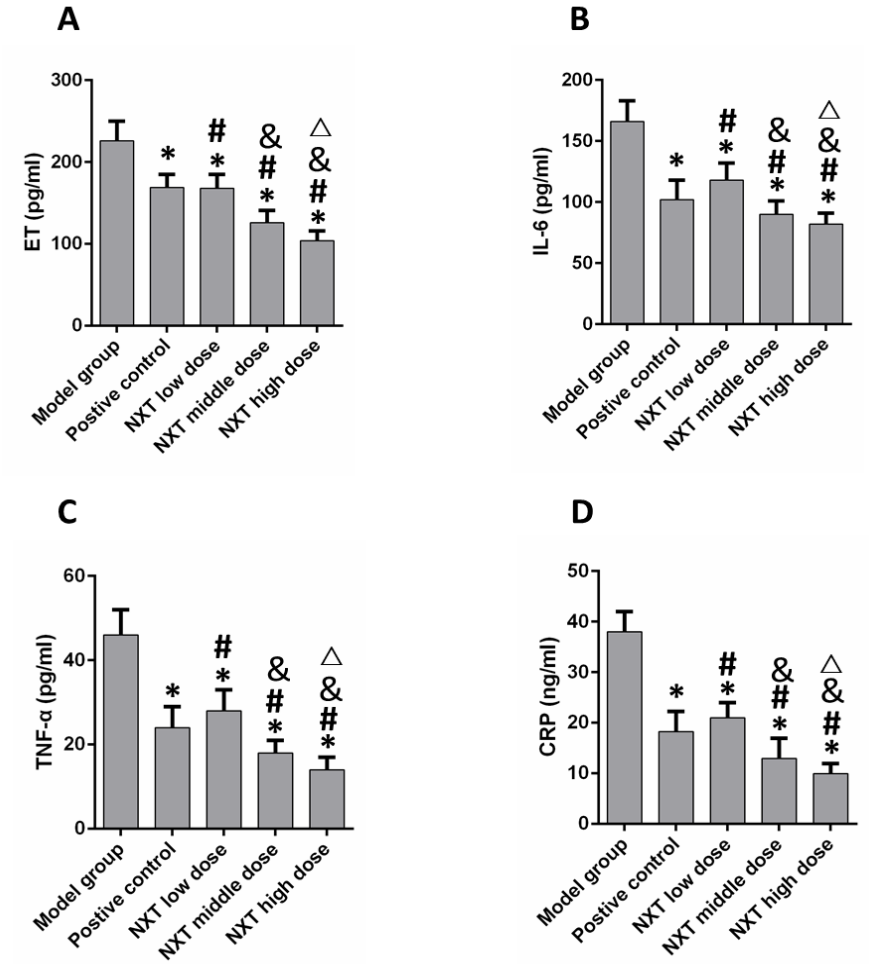

Fig. 1 Compared with model group.

$^{*}p < 0.05$; compared with positive control, $^{\#}p < 0.05$;compared with NXT low dose group, $^{\&}p < 0.05$; compared with NXT middle group, $^{\triangle}p < 0.05$.

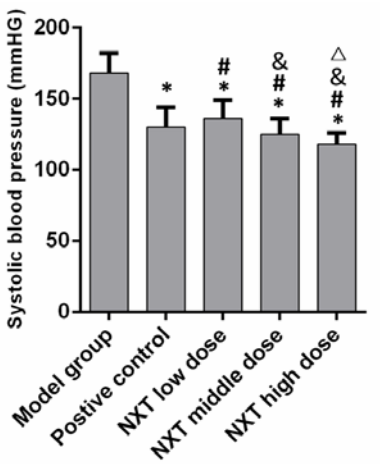

Fig. 2 Compared with model group.

$^{*}p < 0.05$; compared with positive control group, $^{\#}p < 0.05$; compared with NXT low dose group, $^{\&}p < 0.05$; compared with NXT middle group, $^{\triangle}p < 0.05$.

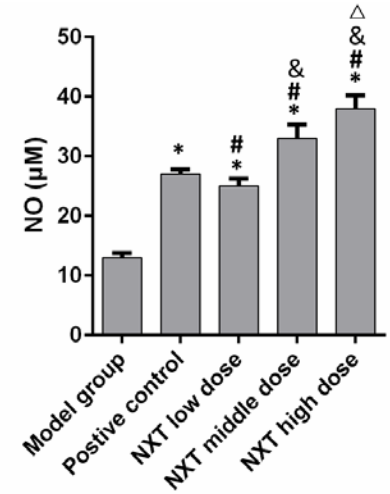

Fig. 3 Serum nitric oxide (NO) concentration in rats of the model control, positive control and NXT groups.

$^{*}p < 0.05$.

4. Discussions

Although many antihypertensive medications have been developed, ventricular hypertrophy and its subsequent congestive heart failure are still common occurrences in most hypertensive patients. NXT is a Chinese herb, and its main ingredients include 16 kinds of botanicals and insects, such as astragalus, salvia, angelica, chuanxiong, safflower, red peony, peach kernel, frankincense, myrrh, spatholobus, mulberry, leeches, earthworm, scorpion, cassia twig and achyranthes [17]. NXT has the functions of dilating blood vessels, anticoagulation, lipid lowering and maintaining vascular patency. NXT can ameliorate the blood pressure of spontaneously hypertensive rats and delay the onset of hypertension, and the potential mechanism might be as following [18]: vascular endothelial dysfunction is important in the pathogenesis of hypertension, and in spontaneously hypertensive rats. There are a variety of pathological factors which caused vascular endothelial cell damage, decreased NO secretion, increased ET secretion, promoted and aggravated the occurrence of hypertension. The latest pharmacological studies showed that NXT improved the endothelial function of blood vessels, improved hemorheology and relieved vasospasm; abnormalities of renin-angiotensin system were independent factors during the occurrence and development of hypertension [19]. Studies have shown that NXT regulated vasoactive substances in blood vessels. To date, there is no study on the treatment of spontaneously hypertensive rats with NXT.

The vascular endothelium is a barrier between the blood vessel wall and the blood, which can maintain vascular patency and smooth blood vessel walls, mediate immune responses and inflammation, and regulate vascular smooth muscle cells [20]. Endothelial dysfunction is considered to be the cause of hypertension, and its dysfunction is an important feature of early manifestations and initiation of hypertension [21]. A large number of clinical studies have shown that hypertensive patients were always associated with decreased endothelial function. The effects of vascular endothelium on hypertension could be achieved by secreting a variety of vasoactive substances, thereby regulating the relaxation and contraction of blood vessel walls and the proliferation of smooth muscle cells. These active substances mainly include ET, NO and Ang II. NO can maintain the stability of blood pressure and maintain the constancy of vascular tone [22]; ET is a vasoconstrictor secreted by the vascular endothelium when the vascular endothelium is damaged. This, the level of vascular endothelial ET was increased [23]; Ang II is secreted by vascular endothelial cells, which can cause stimuli contraction of vascular smooth muscle and lead to increase vascular arterial pressure and peripheral resistance [24]. The results of current study indicated that NXT improved endothelial function by reducing the level of serum ET and elevated the level of NO.

Interleukins play important roles in the activation and regulation of immune cells during the development of inflammation, and they also mediate the activation, differentiation and proliferation of T and B lymph cells. In recent years, the relationship between inflammatory cytokines and hypertension has been widely recognized [25]. Clinical studies have shown that hypertension was associated with inflammatory factors, such as IL-6, TNF-α and CRP [26]. These factors are involved in promoting vascular endothelial cell proliferation, thickening, and releasing of vasoactive substances, and promoting the occurrence of hypertension. Shafi Dar M et al. [27] found that people with high level of hs-CRP were more likely to suffer from hypertension, and hs-CRP was positively correlated with the development of hypertension. Studies have shown that IL-6 was significantly higher in patients with hypertension than normal people, and it was positively correlated with blood pressure.

IL-6 can stimulate and activate inflammatory cytokines and release inflammatory mediators. It is a

multi-effect cytokine that can induce production and secretion of CRP, platelet growth factor, endothelial cell adhesion factor, resulting in the destruction of vascular endothelial cell structure, reduction of vascular endothelial prostacyclin, elevation of thromboxane, change of blood rheology, peripheral blood vessels contraction and increasing blood pressure [28]. TNF-α is an inflammatory cytokine with endotoxin-like anti-tumor effect, which can exert various biological effects on T cells, B cells and NK cells. Previous studies have shown that TNF-α was increased with rising of blood pressure, and stimulated the hypothalamic thermoregulatory center and macrophages to release inflammatory cytokines such as IL-6 and IL-8, and led to the production of inflammatory response and induced matrix metalloproteinase [26]. At the same time, TNF-α destroyed the integrity of vascular endothelial cells, which resulted in thickening of the vessel wall, decreased elasticity, increased peripheral resistance, and elevated blood pressure. High serum levels of IL-6, TNF-α, and CRP may be the pathophysiological basis of hypertension [29]. The results of the current study indicated that NXT reduced serum IL-6, TNF-α, CRP levels and decreased inflammation.

In summary, there is a close relationship between vascular endothelial dysfunction and hypertension. Maintaining the normal function of vascular endothelial cells can help to control hypertension more effectively. NXT has obviously antihypertensive effect on spontaneously hypertensive rats, and its molecular mechanism may be related to the improvement of vascular endothelial function and change of the expression level of inflammatory factors.

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