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Original Research Article

Effect of enalapril maleate-folic acid tablets on inflammatory response and myocardial endoplasmic reticulum stress-related factors in hypertensive rats

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Abstract

Purpose: *To determine the effect of enalapril maleate folate on inflammatory reaction and myocardial endoplasmic reticulum stress-related factors in hypertensive rats.*
Methods: *Eighty (80) hypertensive rats with SBP > 140 mmHg were equally assigned to control and study groups. Rats in control group were given normal saline by gavage, while the observation group was given enalapril maleate powder (10 mg/kg/day). After 4 weeks of treatment, 2 mL of inferior vena cava blood was collected from each of the two rat groups. The level of homocysteine (Hcy) was determined with Hcy assay kit, while C-reactive protein (CRP) levels in patients were determined by latex immunoturbidimetry. Serum FBG was assessed using automatic biochemical analyzer. The expression levels of GRP78, CRP94, chop and caspase-12 in aortic smooth muscle cells were assayed immunohistochemically.*
Results: *Central arterial pressure (MAP), aortic media thickness, Ivwi, HWI FBG and CRP were significantly higher in the study rats, while Hcy level was lower, than in controls ($p < 0.05$). There were significantly lower levels of glucose regulatory protein 78 (GRP78), CRP94, CHOP and caspase-12 in study rats than in control rats ($p < 0.05$).*
Conclusion: *Enalapril maleate-folate slows down inflammatory reaction by reducing the levels of Hcy, CRP and FBG. It inhibits the expressions of GRP78, CRP94, CHOP and caspase-12 in myocardium, reduces damage to myocardial cells, and alleviates or reverses left ventricular hypertrophy in rats with high blood pressure. This provides new insight into the research and development of other drugs.*

Keywords: *Enalapril maleate-folic acid, Hypertension, Inflammatory response, Endoplasmic reticulum stress factors*

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INTRODUCTION

Hypertension-linked myocardial remodeling is manifested mainly as left ventricular hypertrophy (LVH), and the hypertrophy and apoptosis of myocardial cells are its important cytological bases [1]. It has been found that endoplasmic reticulum stress (ERS) induces cardiomyocyte apoptosis, and the expression levels of ERS-related factors, GRP78, glucose regulatory protein 94 (GrP94), caspase-12, and CHOP are of great significance to cell survival [2,3]. Studies

have shown that homocysteine (Hcy), C-reactive protein (CRP), fasting blood glucose (FBG) and other inflammatory factors influence the proliferation and differentiation of smooth muscle cells and induce thickening of vascular intima and middle layer [4]. Enalapril maleate folate tablets are second-generation angiotensin converting enzyme inhibitors which inhibit vascular remodeling and reverse left ventricular hypertrophy. However, their effects on inflammatory response and factors related to myocardial endoplasmic reticulum stress are not completely clear [5-7]. This investigation was focused on determination of the effect of enalapril maleate + folate on inflammatory response and endoplasmic reticulum stress-related factors in 90 SD adult male rats.

EXPERIMENTAL

Animals

Eighty adult male Sprague Dawley rats were selected and used for this study. The average body weight of the rats was 220 ± 20 g, and their mean age was 9 ± 1 weeks.

Reagents

Rabbit anti-rat GRP78 antibody, rabbit anti-rat CRP94 antibody and rabbit anti-rat CHOP antibody were provided by Beijing Yiqiao Shenzhou Technology Co. Ltd. Rabbit anti-rat caspase-12 antibody was provided by Shenzhen Haodi Huatuo Biotechnology Co. Ltd. Biotinylated sheep anti-rabbit IgG was purchased from Beijing Keriji Biotechnology Co., Ltd; SABC was obtained from Beijing Keriji Biotechnology Co. Ltd; Enalapril maleate folic acid tablet was supplied by Shenzhen Aokang Pharmaceutical Co. Ltd, while Hcy test kit was provided by Shanghai Yiyan Biotechnology Co. Ltd.

Equipment

Optical microscope was provided by Shenzhen Zike Biotechnology Co. Ltd. Image analysis software was provided by Guangzhou Kesite Scientific Instrument Co. Ltd, while intelligent physiological recording and analysis processing system was purchased from Nanjing Calvin Biotechnology Co. Ltd.

Establishment of rat model and animal grouping

Ninety Sprague Dawley rats were subjected to abdominal aortic coarctation in order to establish hypertensive rat model. The specific procedures were as follows: the rats were anesthetized with intraperitoneal injection of 1 % pentobarbital sodium, and fixed in supine position. The specific procedures were as follows: the rats were anesthetized with intraperitoneal injection of 1 % pentobarbital sodium, and fixed in supine position. The surgical area was disinfected, after which the abdominal area was cut along the midline into the abdominal cavity, layer by layer.

The surgical area was fully exposed, and the abdominal aorta of the surgical segment was stripped. The tip of the injection needle was folded into an L-shape, and the long handle was placed parallel to the abdominal aorta. Partial constriction of the abdominal aorta was done through ligation with surgical thread and needle. Thereafter, the rats were fed in a clean rat cage. Two weeks after surgery, the blood pressure of rats was determined using tail pressure measuring system. Eighty hypertensive rats with SBP > 140 mmHg were assigned to control and observation groups (n = 40 each). Control rats were given normal saline, while rats in the other group were given enalapril maleate tablet at a dose of 15 mg/kg/day via intragastric administration.

Evaluation of indicators

Determination of mean arterial pressure (MAP)

The blood pressure of each rat was determined using non-invasive tail artery blood pressure measurement and analysis system, 4 weeks after treatment. The rats were fixed with an appropriate barrel-shaped fixator and placed in a dark quiet environment. When the rats were calm for about 30 minutes, they were placed in a pre-warming tank at 37 ℃ for 30 min to effect blood vessel dilation in the tail of each rat. Then, blood pressure was measured after the rat pulse stabilized. The systolic blood pressure (SBP) and diastolic blood pressure (DBP) were recorded. Then, MAP was calculated using Eq 1.

MAP = (DBP + 1/3 (SBP-DBP)) …… (1)

Measurement of serum Hcy, FBG and CRP levels

Blood pressure of rats was measured 4 weeks after treatment. Then, 2 mL of inferior venous blood was collected from each rat in the two groups. The blood samples were allowed to stand at room temperature for 30 min, and centrifuged at 3000 rpm for 10 min. The supernatants (sera) were carefully separated and stored in a refrigerator at -80 ℃. Before centralized assay, the samples were thawed in

cold water, and the Hcy level was determined according to the procedure indicated in Hcy assay kit. The level of CRP was determined using latex immunoturbidimetry. Serum FBG was determined with automatic biochemical analyzer.

Determination of muscle hypertrophic index (HWI) and LVWI

Body mass (BW) was measured after 12 h of fasting and water restriction, and the heart of each anesthetized rat was excised after opening the chest. The heart was repeatedly rinsed in PBS at 4 ℃, after which whole heart quality (HW) was measured. Left ventricular mass (LVW) was measured by cutting the ventricular wall tissue of the right ventricle and preserving the ventricular septum and left ventricular wall tissue. The total cardiac hypertrophy index HWI (HW/BW) and left ventricular hypertrophy index LVWI (LVW/BW) were calculated.

Aortic middle thickness

The thoracic aorta of each anesthetized rat was quickly opened, and thoracic aorta length of about 1.0 cm was cut out, washed with PBS, fixed with paraformaldehyde, and embedded in paraffin. Five paraffin sections of each thoracic aorta and three fields were randomly selected for each section for measurement of the thickness of rat aortic middle layer using medical image analysis system. The mean thickness of thoracic aorta was obtained.

Assessment of expressions of GRP78, CRP94, CHOP and caspase-12 in aortic vascular smooth muscle cells

Specimens were collected for immunohistochemical staining. For GRP78 expression, paraffin sections were routinely dewaxed and treated with 3 % H_2O_2. Tissue antigens were repaired with citrate buffer in microwave oven, and the sections were washed with PBS after cooling. Then, the sections were incubated with 5 % BSA blocking solution at room temperature for 20 min, to block non-specific binding. Thereafter, the sections were incubated successively with 1:100 dilutions of anti-rat GRP78 antibody, biotinylated sheep anti-rabbit IgG and SABC. Thereafter, the sectioms were stained with DAB, followed by re-staining with hematoxylin, dehydration, clearing and sealing. Then, the sections were examined under a light microscope. The expression levels of CRP94, CHOP, and Caspase-12 in aortic vascular smooth muscle cells were determined in the same way.

Statistical analysis

The SPSS20.0 software package was used for statistical analysis of data. All measurement data are expressed as mean ± SD. Groups were compared with *t*-test. Statistical results are presented as percentage, and groups were compared with χ^2. Values of $p < 0.05$ were considered significant differences.

RESULTS

Myocardium-related indices

Table 1 shows that MAP, aortic middle thickness, LVWI and HWI in study rats were significantly higher than those in control rats ($p < 0.01$).

Inflammatory response levels

As presented in Table 2, the levels of FBG and CRP were markedly higher in study rats than in control rats, while the level of Hcy was significantly lower than that in control rats.

Table 1: Levels of myocardial related indices in both groups (mean ± SD)

Group	MAP (mmHg)	Aortic medium thickness (μm)	LVWI	HWI
Control	172.46±8.46	108.43±4.15	3.49±0.11	3.84±0.13
Study	131.46±5.28	96.48±5.42	2.41±0.08	2.79±0.12
t	26.002	11.071	50.219	37.535
P-value	<0.001	<0.001	<0.001	<0.001

Table 2: Inflammatory response levels in both groups (mean ± SD)

Group	Hcy (μmol/L)	FBG (mmol/L)	CRP (mmol/L)
Control	7.59±1.75	6.72±1.15	27.46±5.18
Study	16.83±5.42	5.03±0.52	18.76±3.46
t	10.260	8.468	8.833
P-value	<0.001	<0.001	<0.001

GRP78, CRP94, CHOP and caspase-12 levels in myocardium

The levels of GRP78, CRP94, CHOP and caspase-12 were markedly lower in study group than in control rats ($p < 0.01$). These results are shown in Figure 1, Figure 2, Figure 3 and Figure 4, and Table 3.

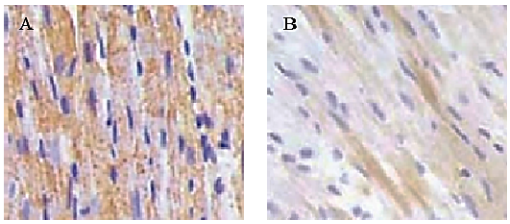

Figure 1: Immunohistochemical staining of GRP78 expression in myocardium of rats in the two groups. A: immunohistochemical staining image of GRP78 expression in myocardium of control rats; B: immunohistochemical staining image of myocardial GRP78 expression in observation group

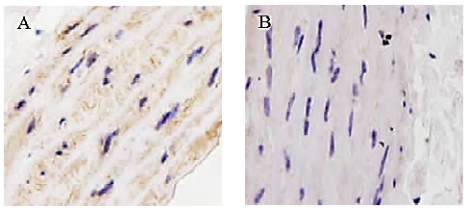

Figure 2: Immunohistochemical staining of CRP94 expression in myocardia of rats in the two groups. A: immunohistochemical staining image of GRP94 expression in myocardium of control rats; B: immunohistochemical staining image of GRP94 expression in myocardium of study group

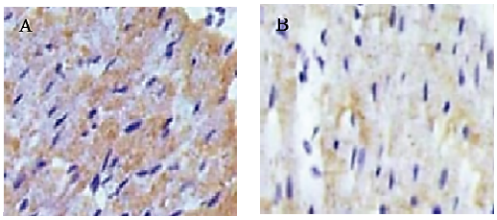

Figure 3: Immunohistochemical staining of myocardial CHOP expression in the two groups. A: Immunohistochemical staining image of CHOP expression in control group; B: immunohistochemical staining image of CHOP expression in the observation group

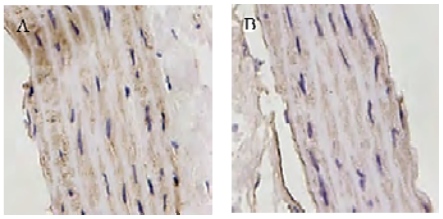

Figure 4: Immunohistochemical staining of myocardial caspase-12 expression in the two groups. A: Immunohistochemical staining image of caspase-12 expression in control rats; B: immunohistochemical staining image of caspase-12 expression in the study group

DISCUSSION

Hypertension, the most common chronic disease in man, is a clinical syndrome characterized by increased arterial blood pressure in the systemic circulation, and it leads to functional or organic damage to organs such as heart, brain and kidney [8]. Cardiovascular disease is a serious threat to human life and health, and it is one of the major causes of death in the world. Hypertension is a risk factor for cardiovascular disease which has aroused extensive attention of medical professionals in China and abroad [9].

Enalapril maleate folic acid tablet is a compound derived from enalapril and folic acid. Studies have shown that enalapril maleate folic acid tablet steadily reduces blood pressure in patients with hypertension [10]. The relationship between enalapril maleate and hypertension has become the focus of attention by medical scholars all over the world. Recent studies have found that enalapril maleate folic acid tablets also reduced arterial sclerosis in patients with hypertension [11]. In the present study, MAP, aortic middle thickness, LVWI and HWI of rats were markedly decreased by enalapril maleate folic acid. These data indicate that enalapril maleate folate tablets may inhibit ER stress response in rats, prevent ER-induced myocardial cell damage, inhibit vascular remodeling, and reverse left ventricular hypertrophy, which are in agreement with a previous report [12].

The endoplasmic reticulum stress pathway is a recently discovered apoptosis signaling pathway which is involved in a variety of pathological and

Table 3: Comparison of levels of endoplasmic reticulum stress-related factors between the two groups

Group	GRP78	GRP94	CHOP	Caspase-12
Control	0.89 ±0.01	0.49±0.01	0.56±0.01	0.52±0.02
Study	0.38±0.01	0.31±0.02	0.32±0.03	0.37±0.01
t	228.078	50.911	48.000	42.426
P-value	<0.001	<0.001	<0.001	<0.001

physiological events *in vivo*, and it is linked to the pathogenesis of cardiovascular diseases. Endoplasmic reticulum stress (ERS) induces up-regulations of GRP78 and GRP94 in vascular and arterial smooth muscle cells at the initial stage, and promotes degradation of unfolded or misfolded proteins, thereby relieving endoplasmic reticulum pressure; maintains cellular homeostasis, and protect the cells [13]. However, excessive or prolonged ERS may activate the expressions of pro-apoptotic factors such as caspase-12 and CHOP, thereby causing cell metabolic disorders. Homocysteine (Hcy) is a sulfur-containing amino acid and an intermediate metabolite of methionine in humans. Studies have found that hypertension and increases in serum Hcy level have a synergistic effect on cardiovascular events, which may increase the risk of death from cardiovascular diseases.

Studies have found that the incidence of cardiovascular and cerebrovascular events in patients with high Hcy-induced hypertension are about 5 times higher than that in patients with simple hypertension [14]. It is known that CRP is the most valuable reactive protein in the acute stage of inflammation. During acute infection or acute tissue damage, serum CRP increases sharply, thereby playing a protective role in natural immunity. Studies have found that CRP is not only a marker of inflammatory response, but it is also involved in the pathogenesis of atherosclerosis to varying degrees [15].

Moreover, FBG is associated with the occurrence and development of arterial paper-like sclerosis in hypertension, which may be related to the involvement of FBG in inflammatory response. In this study, enalapril maleate folic acid tablet markedly increased FBG and CRP in rats, while it decreased the levels of GRP78, CRP94, CHOP and caspase-12. These results indicate that enalapril maleate folate tablets inhibited vascular remodeling and reversed left ventricular hypertrophy, most probably due to slowing down of inflammatory response and inhibition of the expressions of factors related to cardiac endoplasmic reticulum stress.

CONCLUSION

Enalapril maleate folate tablets lower inflammatory response in hypertensive rats by reducing the levels of Hcy, CRP and FBG; inhibiting the expression of endoplasmic reticulum stress-related factors such as GRP78, CRP94, CHOP and caspase-12 in myocardium; reducing myocardial cell damage, and relieving or reversing left ventricular hypertrophy.

DECLARATIONS

Conflict of Interest

No conflict of interest associated with this work.

Contribution of Authors

We declare that this work was performed by the authors named in this article and all liabilities pertaining to claims relating to the content of this article will be borne by the authors. Cuina Feng designed the study, supervised the data collection, and analyzed the data. Yugang Zu interpreted the data and prepared the manuscript for publication. Yugang Zu and Xiaoqiong Zhang supervised the data collection, analyzed the data and reviewed the draft of the manuscript. Yugang Zu and Xiaoqiong Zhang contributed equally to this work and should be considered as co-first authors.

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