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Comparison of the antihypertensive effects of folic acid and resveratrol in spontaneously hypertensive rats combined with hyperhomocysteinemia

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Abstract

Objectives: Studies have found that both folic acid and resveratrol have potential benefits in reducing complications of hypertension. The aim of this study was to compare the effects of resveratrol and folic acid on blood pressure in spontaneously hypertensive rats combined with hyperhomocystinemia, and to explore their potential mechanisms.

Methods: Twenty-four male specific pathogen free (SPF) SPF grade spontaneously hypertensive rats were randomly divided into four groups: the SHR group, the hypertension combined with hyperhomocystinemia group (SHR + HHcy), the folic acid intervention group (SHR + HHcy + FA), and the resveratrol intervention group (SHR + HHcy + Res). The rat model of hypertension combined with hyperhomocystinemia was constructed, and then folic acid or resveratrol were given by gavage. Rat tail artery blood pressure, serum homocysteine concentration, superoxide dismutase activity, malondialdehyde levels, and mRNA transcription and protein expression of endothelial nitric oxide synthase and angiotensin II were detected.

Result: Compared with the SHR group, the SHR + HHcy group significantly increased hyperhomocystinemia and malondialdehyde levels, and inhibited superoxide dismutase activity and endothelial nitric oxide synthase expression. Compared with the SHR + HHcy group, the SHR + HHcy + FA group significantly reduced hyperhomocystinemia and malondialdehyde levels, and significantly increased superoxide dismutase activity and endothelial nitric oxide synthase expression; the SHR + HHcy + Res group also inhibited malondialdehyde levels and promoted endothelial nitric oxide synthase expression, but did not reduce hyperhomocystinemia. When comparing between the SHR + HHcy + FA group and the SHR + HHcy + Res group, folic acid significantly decreased hyperhomocystinemia and increased superoxide dismutase activity, while resveratrol significantly decreased blood pressure and angiotensin II expression.

Conclusions: Both resveratrol and folic acid reduced the levels of oxidative stress and promoted the expression of endothelial nitric oxide synthase in SHRs combined with hyperhomocystinemia. Moreover, resveratrol exhibited superior antihypertensive efficacy compared to folic acid, potentially attributed to its ability to inhibit angiotensin II expression.

Keywords

Resveratrol, folic acid, SHR, HHcy, eNOS, Ang II

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Introduction

Hypertension combined with hyperhomocystinemia (HHcy) accounts for about 75% of hypertensive patients in China,[1] and hypertension combined with HHcy significantly increased cardiovascular and cerebrovascular complications.[2] HHcy is an abnormal accumulation of homocysteine (Hcy), an intermediate product of methionine metabolism. According to the American Heart Association criteria, Hcy > 15 μmol/L is defined as HHcy.[3] Studies have found that HHcy aggravates the arterial damage of hypertension.[4]

It is well known that folic acid (FA) can alleviate HHcy.[5] Folic acid is one of the essential vitamins of the human body, and participates in the coenzyme of many human enzymes.[6] Folic acid is also an important antioxidant in the body.[7] In addition, resveratrol (Res), a food-derived polyphenol, is also known for its antioxidant properties.[8,9] Both folic acid and resveratrol have been found to have potential benefits in reducing complications of hypertension, particularly by reducing oxidative stress.[10–12] However, there are few studies on the effects of folic acid and resveratrol against blood pressure in patients with hypertension and HHcy, especially the comparison of their advantages in combating the risk of hypertension.

In this study, a rat model of hypertension combined with HHcy was constructed, and the effects of folic acid and resveratrol on blood pressure (BP), HHcy, malondialdehyde (MDA), and superoxide dismutase (SOD) of reflecting circulating oxidative stress, and endothelial nitric oxide synthase (eNOS) and angiotensin II (Ang II) of regulating vascular function were observed. The purpose of this study was to compare the advantages of folic acid and resveratrol in reducing the risk of BP, so as to guide the clinical adjunctive medication and daily nutrient intake in patients with hypertension and HHcy.

Animals and methods

Rat grouping and model construction

According to the resource equation determination method,[13] the total experimental sample size should be 16–24. Therefore, 24 spontaneously hypertensive rats (SHR) (BP 180 ± 3.5/120 ± 3.5 mmHg, male, 260 ± 10 g, aged 12 weeks) were provided by Beijing Weitong Lihua Experimental Animal Technology Co., Ltd. (NO. 11400700269870). All rats were free to obtain food and water in a 12/12 h light/dark cycle at room temperature of 22 ± 2°C, and were adaptively reared in a well-ventilated clean animal room for 1 week. Afterward, 24 SHRs were randomly divided into the SHR group, the SHR + HHcy group, the SHR + HHcy + FA group, and the SHR + HHcy + Res group, with six rats in each group. The experimental animal ethics review number was kyks-070101.

Four groups of rats were subjected to experimental intervention for 12 weeks, as follows: (1) The SHR group, as the control group, was intraperitoneally injected with normal saline (NS) (5 ml/kg d) for 12 weeks, and then was given NS (0.5 ml/d) by gavage in the last 8 weeks of the experiment. (2) The SHR + HHcy group was intraperitoneally injected with 2% DL-Hcy (5 ml/kg d) for 12 weeks to construct the model of hypertension combined with HHcy (BP ⩾ 180/120 mHg, Hcy ⩾ 15 μmol/L), referring to the experimental model of Zhang et al.[14] At the same time, NS (0.5 ml/d) was given by gavage in the last 8 weeks of the experiment. (3) The SHR + HHcy + FA group was given folic acid (20 mg/kg d, referring to the model dose of Wang et al.[15]) by gavage in the last 8 weeks of the experiment, on the basis of maintaining the model of hypertension combined with HHcy for 12 weeks. (4) The SHR + HHcy + Res group was given resveratrol (80 mg/kg d, referring to the model dose of Locatelli et al.[16]) by gavage in the last 8 weeks of the experiment, on the basis of maintaining the model of hypertension combined with HHcy for 12 weeks. (Experimental grouping is shown in Figure 1).

Determination of rat tail artery BP

The noninvasive BP measurement system (Anhui Yaokun Biotechnology Co., Ltd., ZL-620-F) was used to measure systolic blood pressure (SBP) and diastolic blood pressure (DBP) of rat tail artery. Each rat was acclimated at 37~39°C for 10 min before measurement, then SBP and DBP were measured at least three times, with an interval of 5 min. The average BP of the three times was taken as the final BP value.

Experimental sample collection

At the end of the 12th week of the experiment, all rats were fasted overnight, and were intraperitoneally injected with pentobarbital sodium (50 mg/kg) the next day. Until the pain response disappeared, the rat's chest and abdomen walls were dissected layer by layer along the anterior median line to fully expose its heart. Arterial blood was extracted from the rat's left ventricle, which was used for detecting serum Hcy concentration, SOD activity, and MDA levels. Afterward, the rat's abdominal vein was cutoff, and NS was injected into its left ventricle to flush the blood out of the arteries. Finally, the rat's thoracic aorta was carefully isolated and immediately submerged in liquid nitrogen before being transferred to a refrigerator at −80°C for subsequent eNOS and Ang II detection using quantitative real-time polymerase chain reaction (qRT-PCR) and Western blotting (WB) analysis (Sample collection is shown in Figure 1).

Determination of biochemical indexes

The rat's left ventricular arterial blood was placed in a non-anticoagulant tube for half an hour. After centrifugation, the supernatant was collected to obtain the serum. Serum Hcy concentration was determined using Cobas8000 automatic biochemical analyzer (Roche, Switzerland). Serum SOD activity and MDA levels were measured with commercial kits according to the instructions from the manufacturer

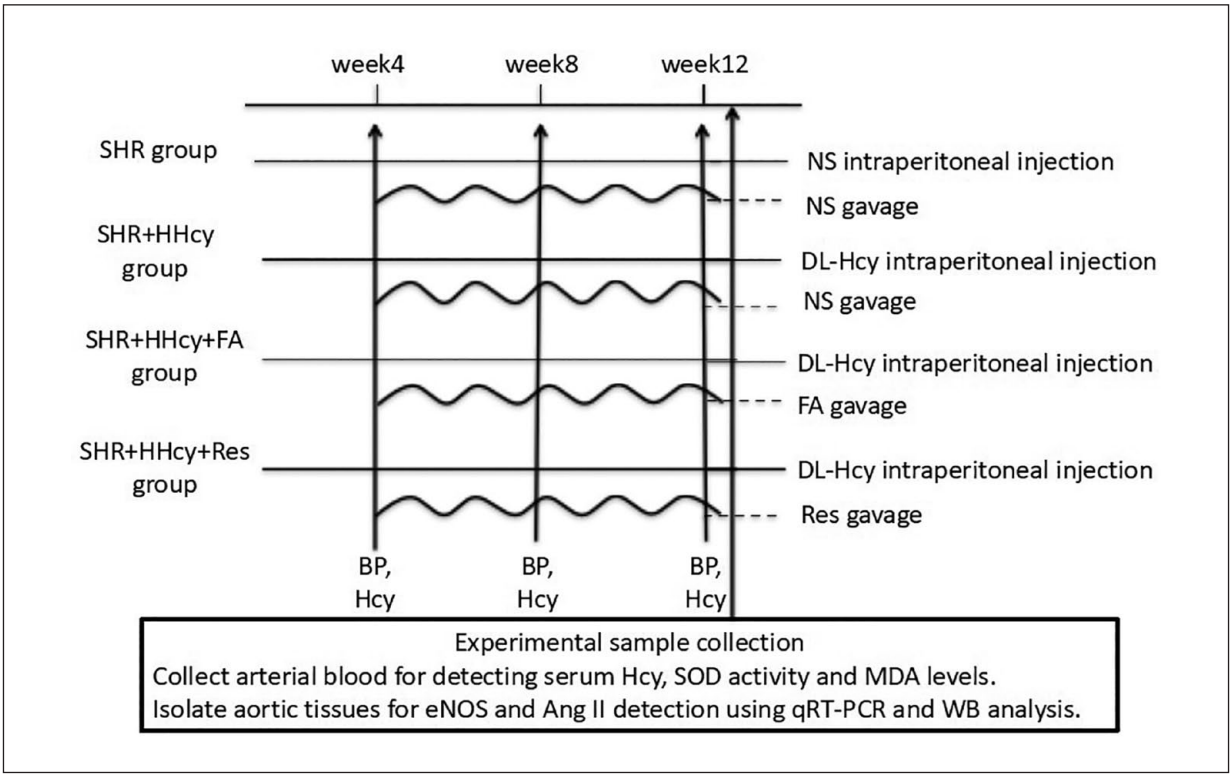

Figure 1. Flow diagram of experiment.
SHR: spontaneously hypertensive rat; HHcy: hyperhomocysteinemia; FA: folic acid; Res: resveratrol; NS: normal saline; Hcy: homocysteine; SOD: superoxide dismutase; MDA: malondialdehyde; eNOS: endothelial nitric oxide synthase; Ang II: angiotensin II.
The straight line represents the duration of intraperitoneal injection and the wavy line represents the duration of gavage. Arrows represent experimental intervention points.

Table 1. Primer sequences for qRT-PCR from Takara.

Name	Sequence-F	Sequence-R
eNOS	CAAGACCGATTACACGACATTGAGA	TGAGGACTTGTCCAAACACTCCAC
Ang II	GCTTGTCTGGGCTGGAGCTA	ACCCAGGTCAGGATGCAGAA
GAPDH	GATGCTGGTGCTGAGTATGRCG	GTGGTGCAGGATGCATTGCTCTGA

(Jiancheng Institute of Biological Technology, Nanjing, Jiangsu, China). Among them, the content of MDA was determined by thibabituric acid method, and the activity of SOD was determined by hydroxylamine method.

Quantitative real-time polymerase chain reaction

Total RNA was extracted from the rat's thoracic aorta with Trizol (Invitrogen, 15596026), and then the RNA was reversely transcribed into cDNA with a gDNA Eraser kit (Takara, RR047A). Subsequently, the cDNA, the primer sequences of target genes (Table 1) and the fluorescent contrast agent TB Green Premix Ex Taq II (Takara, RR820A), according to a certain ratio, were placed in a qRT-PCR instrument (ABI ViiA 7, Applied Biosystems, Foster City, CA) to determine the circulation threshold (CT value) of target genes. The relative expression levels of target genes were calculated by the cycle threshold ($2^{-\triangle\triangle Ct}$).

WB analysis

Total protein was extracted from the rat's thoracic aorta with a protein extraction kit (invent, SA-03-BV). Afterward, protein samples were separated by SDS-PAGE sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) polyvinylidene fluoride (PVDF) electrophoresis, and target protein bands in the gel were transferred to PVDF membrane. Subsequently, the PVDF membrane was blocked with 5% milk for 1 h, and was incubated with primary antibody (anti-eNOS, 1:1000, Abcam, ab199956, Cambridge, MA, USA; anti-AngII, 1:1000, Abcam, ab124734, Cambridge, MA, USA) overnight at 4°C. Finally, the PVDF membrane strip was incubated with horseradish peroxidase (HRP)-conjugated second antibody (Wuhan Sanying, SA00001-2) for 1 h, and was visualized by ChemiDoc™ Touch Gel imaging system (Bio-Rad, Hercules, CA, USA). The expression of target protein was measured using Image-Pro Plus 6.0 software,[17] and was compared with GAPDH.

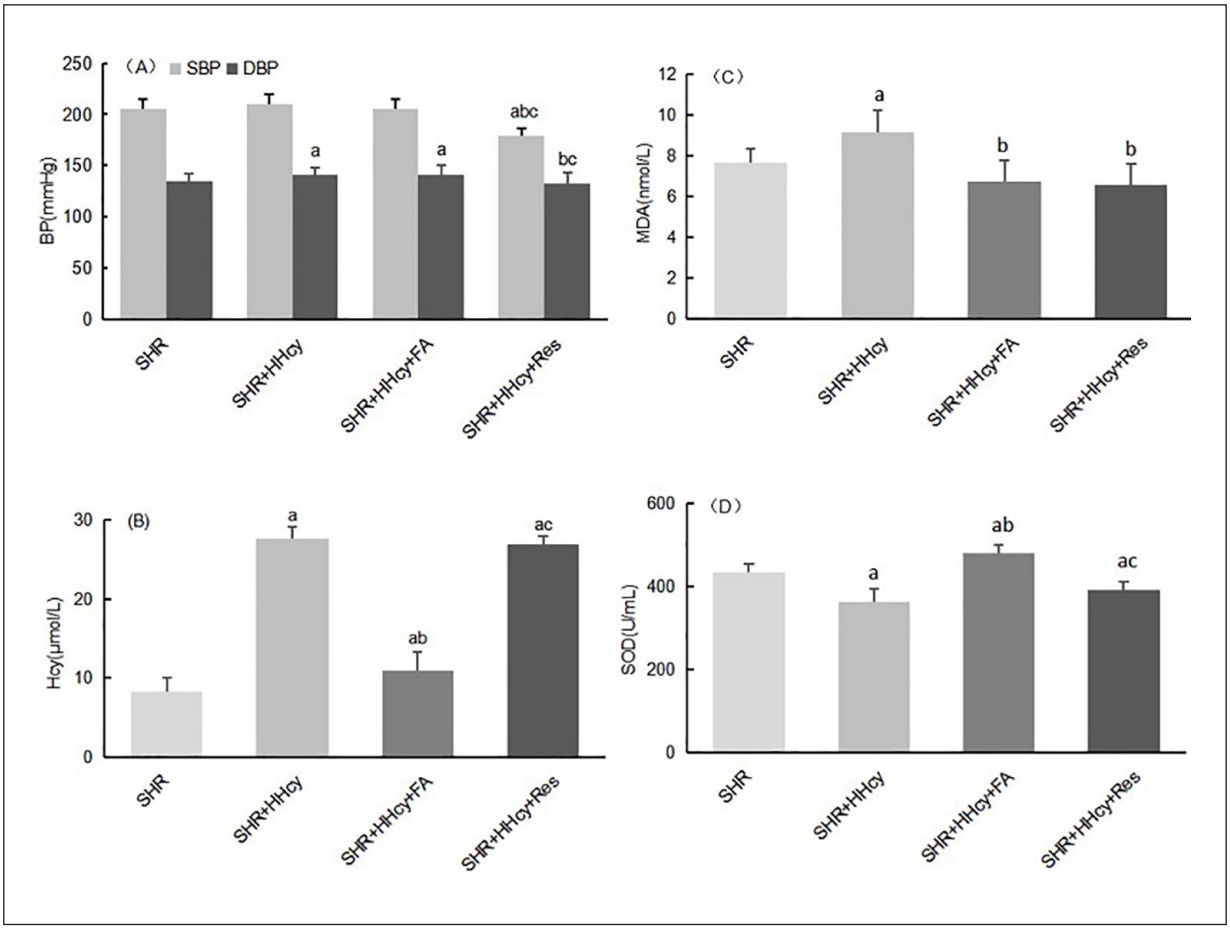

Figure 2. Levels of SBP, DBP, Hcy, MDA, and SOD in the spontaneously hypertensive rat (SHR) group, the SHR + HHcy group, the SHR + HHcy + folic acid (FA) group, and the SHR + HHcy + resveratrol (Res) group. (a) Comparison of BP between the four groups. (b) Comparison of Hcy levels between the four groups. (c) Comparison of MDA levels between the four groups. (d) Comparison of SOD activity between the four groups. Values represent means ± SD.
[a]$p < 0.05$ versus SHR group, [b]$p < 0.05$ versus SHR + HHcy group, [c]$p < 0.05$ versus SHR + HHcy + FA group, $n = 6$.

Statistical analysis

Statistical analysis was performed using one-way analysis of variance and LSD test. The results were expressed as mean ± standard deviation ($\bar{x} \pm s$). All statistical analyses were performed using SPSS 22.0 software, and $p < 0.05$ was considered statistically significant.

Results

Resveratrol-reduced BP of SHRs combined with HHcy

SBP levels were significantly lower in the SHR + HHcy + Res group than all the SHR group, the SHR + HHcy group, and the SHR + HHcy + FA group ($p < 0.05$); DBP levels were also significantly lower in the SHR + HHcy + Res group than both the SHR + HHcy group and the SHR + HHcy + FA group ($p < 0.05$). In addition, compared with the SHR group, DBP levels increased significantly in the SHR + HHcy group and the SHR + HHcy + FA group ($p < 0.05$) (Figure 2(a)).

Folic acid reduced Hcy levels in SHRs combined with HHcy

The levels of Hcy in the SHR + HHcy group, the SHR + HHcy + FA group, and the SHR + HHcy + Res group were significantly higher than those in the SHR group ($p < 0.05$). On the contrary, Hcy levels in the SHR + HHcy + FA group were significantly lower than those in both the SHR + HHcy group and the SHR + HHcy + Res group ($p < 0.05$) (Figure 2(b)).

Resveratrol and folic acid decreased MDA levels in SHRs combined with HHcy

MDA levels were significantly increased in the SHR + HHcy group compared with the SHR group ($p < 0.05$). On the contrary, MDA levels were significantly decreased in both the SHR + HHcy + FA group and the SHR + HHcy + Res group, compared with the SHR + HHcy group ($p < 0.05$). However, there was no significant difference in MDA levels between the SHR + HHcy + FA group and the SHR + HHcy + Res group ($p > 0.05$) (Figure 2(c)).

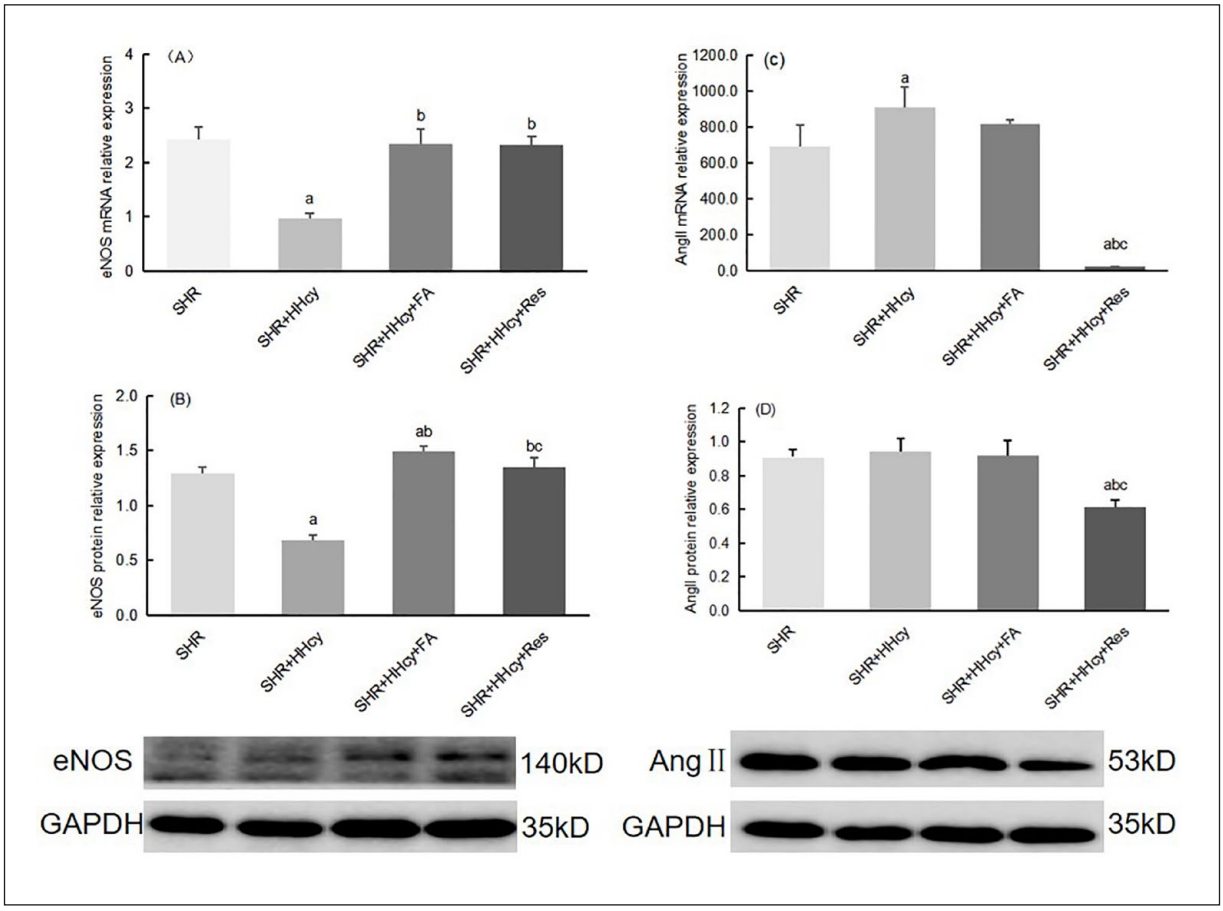

Figure 3. The relative expression levels of eNOS and angiotensin II (Ang II) by quantitative real-time polymerase chain reaction analysis and western blot analysis after normalized to GAPDH in the spontaneously hypertensive rat (SHR) group, the SHR + HHcy group, the SHR + HHcy + folic acid (FA) group, and the SHR + HHcy + resveratrol (Res) group. (a) eNOS mRNA relative expression levels. (b) The protein relative expression levels and representative images of eNOS. (c) Ang II mRNA relative expression levels. (d) The protein relative expression levels and representative images of Ang II. Values represent means ± SD.
[a]$p < 0.05$ versus SHR group, [b]$p < 0.05$ versus SHR + HHcy group, [c]$p < 0.05$ versus SHR + HHcy + FA group, $n = 6$.

Folic acid promoted SOD activity in SHRs combined with HHcy

Compared with the SHR group, SOD activity was significantly decreased in the SHR + HHcy group and the SHR + HHcy + Res group ($p < 0.05$). On the contrary, SOD activity was significantly increased in the SHR + HHcy + FA group than that in the SHR group, the SHR + HHcy group and the SHR + HHcy + Res group ($p < 0.05$) (Figure 2(d)).

Resveratrol and folic acid promoted eNOS expression in SHRs combined with HHcy

Compared with the SHR group, the mRNA transcription and protein expression levels of eNOS in the SHR + HHcy group were significantly decreased ($p < 0.05$). However, compared with SHR + HHcy group, the mRNA transcription and protein expression levels of eNOS were significantly increased in both the SHR + HHcy + FA group and the SHR + HHcy + Res group ($p < 0.05$). In addition, the protein expression level of eNOS was also significantly higher in the SHR + HHcy + FA group than the SHR + HHcy + Res group ($p < 0.05$) (Figure 3(a) and (b)).

Resveratrol inhibited Ang II expression in SHRs combined with HHcy

The mRNA transcription and protein expression levels of Ang II in the SHR + HHcy + Res group were significantly lower than those in the SHR group, the SHR + HHcy group and the SHR + HHcy + FA group ($p < 0.05$). However, Ang II mRNA transcription and protein expression levels were not significantly reduced in the SHR + HHcy + FA group compared with the SHR + HHcy group ($p > 0.05$). (Figure 3(c) and (d)).

Discussion

By establishing a rat model of hypertension complicated with HHcy and subsequently administering folic acid or resveratrol via gavage, we observed that resveratrol effectively

attenuated the excessive elevation of BP in SHRs combined with HHcy. Resveratrol exhibited superior antihypertensive efficacy compared to folic acid, potentially attributed to its ability to inhibit angiotensin II expression.

Epidemiological studies have found that the incidence of cardiovascular and cerebrovascular diseases is significantly increased in patients with hypertension combined with HHcy,[2] and folic acid supplementation significantly reduces Hcy levels[5] and inhibits the incidence of cardiovascular and cerebrovascular diseases.[18] In terms of HHcy synergistically aggravating the vascular complications of hypertension, some studies have suggested that HHcy may be related to promoting BP elevation,[19] increasing oxidative stress[3] in the body, and reducing gas signal NO-induced vasodilation function.[20] Our study also found that HHcy promoted DBP elevation, aggravated the level of circulating oxidative stress, and inhibited eNOS expression of the arterial tissue in SHRs. Although folic acid significantly inhibited HHcy and exhibited the opposite effect of HHcy, it did not reduce HHcy-induced high DBP. The reason might be that folic acid did not completely eliminate the effect of HHcy, or that folic acid only partially reversed the adverse factors related to HHcy. On the contrary, resveratrol had a significant inhibitory effect on BP, and not by reducing HHcy.

Studies have found that the occurrence and development of hypertension are related to high oxidative stress,[21,22] and both folic acid and resveratrol have significant anti-oxidative stress effects.[10–12] As we all know, the levels of oxidative stress in the body are dynamically regulated by two factors: oxidant and antioxidant molecules. MDA is a lipid peroxide, which is formed by oxygen free radicals attacking polyunsaturated fatty acids in biofilms. Therefore, MDA levels are often used to reflect the body's ability to cause oxidative stress. Oppositely, SOD is one of the products of the body's anti-oxidative stress molecular pathway. And SOD activity reflects the body's ability to scavenge oxygen free radicals and represents the partial level of anti-oxidative stress in the body. Therefore, analyzing the dynamic changes of SOD and MDA will help to evaluate the levels of oxidative stress. We found that both folic acid and resveratrol significantly reduced MDA levels, and folic acid was superior to resveratrol in increasing SOD activity. It can be seen that the superior antihypertensive effect of resveratrol is not simply dependent on the ability of anti-oxidative stress, there may be other reasons.

The eNOS, as a metabolic enzyme of arginine and proline, is widely distributed in the heart and the vascular tissues, and its important function is to synthesize and release NO.[23] NO is a vascular relaxation factor, which can regulate blood flow, reduce the proliferation of vascular smooth muscle cells, inhibit the adhesion and aggregation of platelets and white blood cells, and protect vascular endothelial cells.[24] We retrieved PubMed for articles related to HHcy, folic acid, resveratrol, and eNOS in the past 5 years and concluded that HHcy induces high oxidative stress and promotes eNOS uncoupling; and folic acid prevents ROS and reduces eNOS uncoupling; resveratrol increases eNOS levels, thereby promoting NO production.[25–27] We also found that HHcy decreased eNOS expression; in contrast, both folate and resveratrol promoted eNOS expression in SHRs combined with HHcy. Moreover, folic acid was superior to resveratrol in inducing eNOS protein expression. This might be due to the fact that folic acid significantly increased SOD activity and partially reversed the high oxidative stress response induced by HHcy, thus playing a dual protective effect on eNOS. However, this protective effect was not sufficient to lower the BP.

Ang II, as the most important component of the angiotensin system (RAS), is a strong vasoconstricting substance and is widely expressed in the cardiovascular system.[28] In addition, Ang II is also a growth factor that can cause cardiac hypertrophy through Ang II receptors (AT1, AT2), and affects the biological behavior of vascular tissue, such as proliferation, differentiation, and apoptosis.[29] We also retrieved PubMed for articles about HHcy, folic acid, resveratrol, and Ang II in the past 5 years and found that HHcy directly interacts and activates the angiotensin II type I receptor (AT1) to aggravate vascular injury[30]; folic acid improves vascular function by regulating HHcy[31]; and resveratrol can inhibit vasoconstrictor molecules such as Ang II.[32,33] Our experiment found that HHcy and folic acid had no significant effect on the final expression of Ang II. However, resveratrol significantly reduced Ang II expression of rat arterial tissue in SHRs combined with HHcy, and the inhibitory effect was significantly better than folic acid. Therefore, the superiority of resveratrol over folic acid in lowering BP might be related to the low expression of Ang II in SHRs combined with HHcy.

Potential strengths and limitations of the study are as follows: (1) We investigated the effects of folic acid and resveratrol on BP in SHRs combined with HHcy. This might be a potentially important report on the comparative use of resveratrol and folic acid to lower BP in SHRs given HHcy. This study is helpful to guide the daily nutrient intake for patients with hypertension and HHcy, and to provide a theoretical basis for antihypertensive adjuvant drugs. (2) We established a rat model of hypertension combined with HHcy, and then folic acid or resveratrol was administered to the model rats by gavage. In terms of animal drug dosage, we borrowed from previous experimental models without special experimental verification, which is the limitation of our experiment. (3) We selected MDA versus SOD, and eNOS versus Ang II, as the target molecules, and compared the changes of these indicators before and after folic acid and resveratrol intervention. The results are helpful to guide our future mechanism research.

Conclusion

Both resveratrol and folic acid reduced the levels of circulating oxidative stress and increased the eNOS expression of arterial tissue in SHRs combined with HHcy. In addition, folic acid significantly reduced Hcy levels and promoted SOD activity, while resveratrol significantly reduced BP levels and the relative expression of Ang II. To sum up, resveratrol exhibited superior antihypertensive efficacy compared to folic acid, potentially attributed to its ability to inhibit angiotensin II expression.

Animal ethics consent

Animal care and experimental protocol for this study were approved by the Committee on the Use of Live Animals in Teaching and Research of Jinan Huaiyin People's Hospital. (ethical code kyks-070101).

Animal welfare

The present study followed international, national, and/or institutional guidelines for humane animal treatment and complied with relevant legislation.

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Author contributions

Chunbo Gong designed the study and interpreted the data. Yi Lu acquired, analyzed, and interpreted the data. Yi Lu and Lihua Zhang wrote the article and Chunli Wang revised the article.

Data statement

The study is reported in accordance with ARRIVE guidelines. Additionally, I solemnly declare that the research data and experimental materials are true and reliable. The raw data supporting the conclusions of this article will be made available by the corresponding author.

Declaration of conflicting interests

The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

Ethics approval

Ethical approval for this study was obtained from the committee on the Use of Live Animals in Teaching and Research of Jinan Huaiyin People's Hospital (ethical code kyks-070101).

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