

Original Research Article

Grape seed proanthocyanidin alleviates left ventricular remodeling by regulating systolic pressure, oxidative stress and vasoactive substances in spontaneously hypertensive rats

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Abstract

Purpose: To study the effect of grape seed proanthocyanidin (GSP) on left ventricular remodeling in spontaneously hypertensive rats, and the underlying mechanism.

Methods: Spontaneously hypertensive rats were randomly divided into two groups, with 12 rats per group. One group was injected with GSP. Systolic pressure in each group was measured at baseline (before treatment), and at 3, 6 and 9 weeks after treatment. Whole heart index and left heart index were calculated. Histopathological changes in myocardium and left ventricular posterior wall thickness of rats in each group were measured. The cross-sectional area of myocardial cells and myocardial collagen volume fraction were assessed. The levels of malondialdehyde (MDA), superoxide dismutase (SOD), catalase (CAT), nitric oxide (NO), renin, angiotensin II (Ang II), endothelin-1 (ET-1) and endothelial nitric oxide synthase (eNOS) in each group of rats were assayed.

Results: Compared with the spontaneous hypertension group, whole heart mass index, left heart mass index, left ventricular posterior wall thickness, cross-sectional area of myocardial cells, myocardial collagen volume fraction, and levels of MDA, renin, Ang II and ET-1 in GSP group were significantly decreased, while the expression levels of SOD, CAT, NO and eNOS were markedly increased in GSP group ($p < 0.05$). Cell hypertrophy in the GSP group was significantly mitigated, when compared with that in the spontaneous hypertension group.

Conclusion: GSP mitigates left ventricular remodeling in rats by reducing systolic blood pressure, improving antioxidant capacity and regulating levels of vasoactive substances, thus suggesting its potential for the management of hypertension.

Keywords: Grape seed, Proanthocyanidin, Spontaneous hypertension, Left ventricular remodeling, Cell hypertrophy

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INTRODUCTION

Primary hypertension is a global disease caused by genetic and environmental factors. It is a

major risk factor for cardiovascular and cerebrovascular diseases, and a major cause of disability and death in patients. Due to changes in lifestyle and increases in aging populations,

the incidence of hypertension in China is gradually increasing, even in the younger generation [1]. The heart is one of the frequently-damaged target organs due to hypertension.

Ventricular remodeling is an important pathological process in the development of hypertension and centripetal heart failure, and an independent risk factor for cardiovascular events, especially myocardial infarction and stroke [2]. At present, early detection of hypertension and prompt treatment are used to prevent and reverse lesions in the heart, brain and kidney. Calcium antagonists, diuretics, angiotensin-converting enzyme inhibitors and other drugs are usually used in clinics for treatment of hypertension. However, the overall efficacy of these treatments is not satisfactory, and they do not simultaneously act on the multiple mechanisms involved in left ventricular remodeling in hypertension [3]. Therefore, it is important to identify newer and safer drugs that can act on multiple mechanisms associated with left ventricular remodeling in hypertension.

Grape seed proanthocyanidin (GSP), a flavonoid with special molecular structure extracted from grape seeds, is recognized as a natural antioxidant. It has been reported that GSP mitigated myocardial injury caused by chemotherapy drugs [4]. However, there are limited reports on its effect on remodeling of left ventricle in rats with spontaneous hypertension. In this study, the out-turn of GSP on left ventricular remodeling in rats with spontaneous hypertension, and the mechanism involved, were investigated.

EXPERIMENTAL

Animals

Twenty-four healthy male rats with spontaneous hypertension, and 12 control rats of the same age were purchased from Shanghai Nanfang Model Biotechnology Co. Ltd [production license SCXK (Shanghai) 2018-0002]. The rats were aged 10 weeks, with mean body weight of 212 ± 18 g.

Animal grouping

The rats were adaptively fed for 7 days at laboratory temperature of 25 ± 4 °C and humidity of 52 ± 14 % under 12-h light/12-h dark photoperiod. The spontaneously hypertensive rats were randomly divided into two groups, with 12 rats in each group. Rats in one group were intraperitoneally injected with GSP at a dose of 200 mg/kg, while rats in the other group received

normal saline in place of GSP via the same route. In addition, 12 healthy rats in the control group were given normal saline intravenously. This research was approved by the Animal Ethical Committee of Affiliated Hospital of Hubei University of Arts and Science (approval no. HUAS2020012), and conducted according to "Principles of Laboratory Animal Care" (NIH publication no. 85-23, revised 1985) [5].

Assay of biomarkers

The rats were immobilized and kept calm, and the systolic blood pressure was measured with animal blood pressure monitor at baseline, and after 3, 6 and 9 weeks of treatments.

After 4 weeks, rats in each group were anesthetized, and their hearts were surgically removed, cleaned, blotted with filter paper, and weighed. The atrium, right ventricle and vascular tissue were discarded, while the left heart was blotted with filter paper. Whole heart mass index and the left heart ventricle mass index were calculated.

Paraffin sections of myocardial tissue of left ventricle were prepared using routine histological procedures. Pathological changes in myocardial tissue of rats in each group were determined using hematoxylin and eosin (H&E) staining. The left ventricle was photographed, and the left ventricular posterior wall thickness was measured in each group of rats. The cross-sectional area of myocardial cells and myocardial collagen volume fraction were calculated.

Malondialdehyde (MDA) levels in each group of rats were measured using thiobarbituric acid method. The level of superoxide dismutase (SOD) was determined with oxidase method, while catalase (CAT) levels in each group were assayed with ammonium molybdate method.

Nitric oxide (NO) levels were determined using nitrate reductase method. Renin and angiotensin II (Ang II) levels in each group of rats were measured with ELISA, while protein expression levels of ET-1 and eNOS in each group of rats were determined with Western blot assay.

Statistical analysis

Measurement data in each group are expressed as mean \pm standard deviation (SD). Single factor multivariate mean comparison was used for comparison among multiple groups, while independent sample *t*-test was used for comparison between two groups. All statistical analyses were done with SPSS 21.0 software

package. Values of $p < 0.05$ were considered as indicative of statistically significant differences.

RESULTS

Changes in systolic blood pressure in rats

Before treatment, systolic blood pressure was markedly raised in the spontaneous hypertension and GSP groups, relative to control ($p < 0.05$), but there was no significant difference in systolic blood pressure between the spontaneous hypertension group and the GSP group. From 3 weeks after treatment, blood pressure of rats in the spontaneous hypertension group was markedly increased, relative to control group ($p < 0.05$), while systolic blood pressure of rats in the GSP group was significantly decreased, relative to spontaneous hypertension group ($p < 0.05$). Moreover, systolic blood pressure of rats in the spontaneous hypertension group was gradually increased, while that of rats in the GSP group was gradually decreased with time ($p < 0.05$). These results are shown in Table 1.

Table 1: Systolic blood pressure of rats (mm Hg)

Group	Before	3 weeks	6 weeks	9 weeks
Control	107.35 ±5.25	108.75 ±5.62	109.97 ±6.52	110.49 ±5.46
Spontaneous hypertension	151.37 ±6.15	153.45 ±6.39	159.74 ±5.49	164.53 ±5.28
GSP	152.22 ±6.41	143.43 ±5.47	131.26 ±5.18	118.67 ±6.63
<i>F</i>	222.69	193.55	222.65	300.47
<i>P</i> -value		<0.001		

Results are presented as mean ± SD

Changes in heart indices

As shown in Table 2, compared with control group, whole heart mass index and left heart mass index of rats in the spontaneous hypertension group were markedly increased, while whole heart mass index and left heart mass index in GSP group were significantly decreased, relative to the spontaneous hypertension group ($p < 0.05$).

Table 2: Changes in heart indexes in the 3 groups

Group	Whole heart mass index	Left heart mass index
Control	3.07±0.05	2.44±0.10
Spontaneous hypertension	3.51±0.11	2.83±0.10
GSP	3.11±0.07	2.51±0.08
<i>F</i>	109.29	58.95
<i>P</i> -value		<0.001

Results are presented as mean ± SD

Histopathological changes in myocardium of rats

Myocardial cells in the control group were orderly arranged and uniform in shape. In contrast, myocardial cells in the spontaneous hypertension group were disordered and significantly enlarged. However, cell hypertrophy in the GSP group was markedly decreased, when compared with cells in the spontaneous hypertension group. These results are shown in Figure 1.

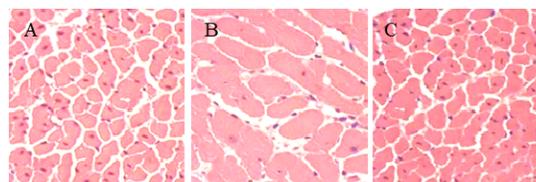


Figure 1: Histopathological changes in myocardium in each group of rats. A: control group; B: spontaneous hypertension group; C: GSP group

Left ventricular posterior wall thickness, myocardial cell cross-section area, and myocardial collagen volume fraction of rats

Table 3 shows that, compared with the control group, left ventricular posterior wall thickness, cross-sectional area of myocardial cells and myocardial collagen volume fraction were markedly increased ($p < 0.05$). However, left ventricular posterior wall thickness, cross-sectional area of myocardial cells and myocardial collagen volume fraction were significantly decreased, relative to the spontaneous hypertension group.

Table 3: Thickness of left ventricular posterior wall, myocardial cell cross section area and myocardial collagen volume fraction in the 3 groups of rats

Group	Left ventricular posterior wall thickness (mm)	Cross-sectional area of myocardial cells (μm^2)	Myocardial collagen volume fraction (%)
Control	3.26±0.11	454.99±18.18	2.71±0.23
Spontaneous hypertension	4.88±0.13	699.93±16.18	4.71±0.30
GSP	3.58±0.13	481.85±16.47	3.18±0.27
<i>F</i>	577.36	752.29	182.44
<i>P</i> -value	<0.001	<0.001	<0.001

Results are presented as mean ± SD

MDA, SOD and CAT levels of rats

In the spontaneous hypertension group, MDA level was significantly increased, relative to control group, while SOD and CAT levels were markedly decreased. However, compared with the spontaneous hypertension group, MDA level in the GSP group was significantly decreased, while SOD and CAT levels were markedly increased ($p < 0.05$; Table 4).

Table 4: MDA, SOD and CAT levels in rats

Group	MDA (nmol/mg)	SOD (U/mg)	CAT (U/mg)
Control	2.57±0.75	184.05±29.78	1.49±0.22
Spontaneous hypertension	4.35±0.45	113.07±21.41	1.05±0.09
GSP	2.75±0.77	165.54±23.81	1.39±0.09
F	25.45	25.52	29.65
P-value	<0.001	<0.001	<0.001

Results are presented as mean ± SD

NO, renin and Ang II levels

The level of NO in spontaneous hypertension group was markedly reduced, when compared with control, while levels of renin and Ang II were markedly increased. However, relative to spontaneous hypertension group, NO level in the GSP group was markedly increased, while renin and Ang II levels were significantly decreased ($p < 0.05$). These results are shown in Table 5.

Table 5: Levels of NO, renin and Ang II levels in rats

Group	NO (μmol/g)	Renin (mU/mL)	Ang II (pg/mL)
Control	62.23±8.76	146.14±14.49	15.90±0.43
Spontaneous hypertension	41.12±5.16	183.97±8.69	30.60±2.16
GSP	58.25±5.03	150.60±13.45	18.07±1.01
F	35.20	32.99	386.13
P-value	<0.001	<0.001	<0.001

Expression levels of ET-1 and eNOS

The expression level of ET-1 in spontaneously hypertensive rats was markedly raised, relative to controls, while that of eNOS was markedly decreased ($p < 0.05$). However, relative to the spontaneous hypertension group, the expression level of ET-1 in the GSP group was markedly decreased, while eNOS expression was significantly up-regulated ($p < 0.05$). These results are shown in Figure 2.



Figure 2: Expression levels of ET-1 and eNOS of rats

DISCUSSION

Hypertension is among the major chronic ailments seen in clinical practice, and it is also a high-risk factor for lesions in target organs such as heart, brain and kidney. In hypertension, hypertrophy of cardiomyocytes and fibrosis of myocardial interstitium lead to ventricular remodeling. Studies have shown that myocardial fibrosis increases myocardial stiffness and decreases diastolic compliance, leading to hypertension and heart failure [6]. With improvements in medical science and technology, great progress has been made in the study of the pathogenesis of hypertension and related drug therapy. However, not much progress has been achieved in effectively controlling hypertension-induced damage such as myocardial infarction, heart failure and stroke. Therefore, it is important to identify new drugs that can act on multiple pathways involved in the pathogenesis of hypertension, and improve or even reverse organ injury.

Proanthocyanidin (GSP) is a polyphenol extracted from grape seeds. It has many pharmacological attributes such as anti-oxidant, anti-tumor, anti-inflammatory and anti-aging properties. It has been found that GSP plays an important protective role against myocardial injury caused by ischemia reperfusion and chemotherapy drugs. In addition, GSP is used as an antioxidant for protection against cardiovascular diseases [7]. Spontaneously hypertensive rats were bred by Okamoto *et al* in 1963. Spontaneously hypertensive rats have high incidence of hypertension, high incidence of cardiovascular disease and high degree of sensitivity to antihypertensive drugs, and the pathogenesis of hypertension in these rats is similar to that of human essential hypertension [8]. Spontaneously hypertensive rats can be used as animal model for studying the pathogenesis of human essential hypertension, and for selecting antihypertensive drugs.

This study investigated the effect of GSP on remodeling of left ventricle in rats with

spontaneous hypertension, and the underlying mechanism. The pathogenesis of left ventricular remodeling in hypertension is relatively complex, and it is believed to be closely related to oxidative stress and vasoactive substances [9]. It has been reported that continuously increased blood pressure is one of the important reasons for left ventricular remodeling. Prolonged high blood pressure increases the synthesis of myocardial proteins, resulting in hypertrophy of cardiomyocytes and left ventricular remodeling [10].

The results of this study showed that GSP significantly reduced the level of systolic blood pressure in spontaneously hypertensive rats. Oxidative stress refers to the imbalance between oxidative and antioxidant systems of the body, leading to increase in the production of oxygen free radicals or decrease in radical scavenging. These changes lead to oxidative damage. Studies have shown that when hypertension occurs, oxygen free radicals are significantly increased, thereby damaging endothelial cells [11]. Malondialdehyde (MDA) is the final product of lipid peroxidation, and its level is significantly related to the degree of cell damage and oxidative stress. Superoxide dismutase (SOD) and CAT are endogenous antioxidant enzymes which remove excessive hydrogen peroxide and protect cells from toxic radicals [12]. The results of this study showed that GSP improved antioxidant capacity, and thus mitigated left ventricular remodeling. It is likely that GSP suppressed the concentration of ROS in cardiomyocytes and mitigated pathological hypertrophy and interstitial fibrosis of cardiomyocytes [13].

Some researchers have found that vasoactive substances may be directly involved in left ventricular remodeling [14]. It is known that ET-1 and Ang II are important growth-promoting factors of vasoactive substances which are involved in enhancement of left ventricular hypertrophy. Endothelial nitric oxide synthase (eNOS) is present in endothelial cells. It catalyzes the production of NO from arginine, and it relaxes vascular smooth muscle, decreases blood pressure and relieves pressure load, thereby alleviating left ventricular remodeling in hypertension [15-17]. The results of this study indicate that GSP decreases the levels of renin, Ang II and ET-1, and increases the levels of NO and eNOS, thereby alleviating left ventricular remodeling in hypertension.

CONCLUSION

Proanthocyanidin (GSP) suppresses left ventri-

cular remodeling in rats by reducing systolic blood pressure, improving antioxidant capacity and regulating the levels of vasoactive substances. Thus, GSP has the potential for managing some cardiovascular conditions.

DECLARATIONS

Conflict of interest

No conflict of interest is 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. Xu Zhang designed the study, supervised the data collection, and analyzed the data. Jianfei Liu interpreted the data and prepared the manuscript for publication. Shujuan Song supervised the data collection, analyzed the data and reviewed the draft of the manuscript.

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