Study on effect of Baisha Green tea extract on blood pressure in treating spontaneous hypertension rats

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ABSTRACT

Objective: To explore the effect and Mechanisms of Baisha Greentea extract on blood pressure in treating spontaneous hypertension rats. Methods: The 60 primary hypertensive rats (SHR) were randomly divided into hypertension of control group, amlodipine besylate group and the high, medium and low dose group of green tea extract of five groups, with twelve rats in each group. All treatments were given through oral, and the control group with distilled water. Each measurement of rat blood pressure was detected in the morning every week. After 7 weeks treatment, blood was collected from the rat abdominal aorta for determination of serum nitric oxide (NO) and superoxide dismutase (SOD) activity and level of plasma angiotensin II. At the same time, heart tissue was embedded in 10% neutral buffered formaldehyde fixation, paraffin and sectioned, And then observe the changes of histopathological after HE staining. Results: Compared with the SHR control group, Systolic and diastolic blood pressure of essential hypertension in high dose group and middle dose group and Amlodipine group had significantly reduced. At the same time, the NO and SOD contents of Baisha Greentea extract in high dose group (200 mg•kg⁻¹•d⁻¹) and medium dose group (100 mg•kg⁻¹•d⁻¹) and Amlodipine group (0.5 mg•kg⁻¹•d⁻¹) had significantly increased. Conclusion: Long-term ingestion of Baisha Greentea extract could significantly elevated serum NO and SOD levels of SHR, reduce the level of plasma angiotensin II to common level, while reversed the hypertensive rat myocardial remodeling effectively.

1. Introduction

The incidence of people "three high" disease gradually increased when modern living standards gradually improved. essential hypertension as one of the diseases in "three high", what mainly for repeated systemic arterial blood pressure continues to rise and spasticity that the development of the course, often involving the other organs such as the heart, brain, kidney and other diseases and can make it happen, so people's health and lives have been serious harm . Since the latest relevant information on the survey, high blood pressure will be in developing countries, rising 39.3% rate; The prevention and treatment of hypertension is imminent that mortality rate will reach 79% of the total deaths until 2020 [1]. Patients are mostly chemotherapy hypertension currently mainly due to the adverse effects of chemical drugs, limiting its widespread clinical use and long-term. a major issue of research is Developing high efficiency and low side effects of antihypertensive drugs. With the social development and progress, tea has become a necessity of people's lives, but also the social spiritual civilization jewel , thus tea culture occupied an important part in chinese traditional culture. As the tea contains a lot of medicinal value, so there are some prospects for the development of hypertension prevention, treatment. Many of our research buck tea found that it
relates to the main component-based granules in most current patent. For example rhizome buck tea, etc[2]. study Meicha of HanLing Yao and "Jiwei buck tea" of patent etc[3]. With antihypertensive effect as mainly Ile and a-aminobutyric acid (GABA) tea etc[4]. But the real tea extract under the antihypertensive effects was less reported.

Baisha Greentea is Camellia camellia rue mesh plant, the main active ingredient is polyphenols. Studies have shown that tea polyphenols with a variety of pharmacological activities, such as the prevention of cardiovascular disease, lower blood pressure, blood sugar and blood lipid[5]. In recent years, studies have shown that daily consumption of green tea can lower blood pressure and improve endothelial function in humans, a variety of pharmacologically active prevention of cardiovascular disease and other effects[6]. These are just preliminary exploration, and subsequent reports less.Green tea reduce blood pressure levels and NO and SOD angiotensin II (Ang II) in primary rat model of hypertension in this study that the period of green tea to provide some references for further research with content relationship.

2. Materials and Methods

2.1. Materials

2.1.1. Test sample preparation of aqueous extract of green tea
Go to Hainan green tea (Hainan State Farms white sand Tea Co., Ltd. Production batch: 2015040418) 500 g, respectively, plus 2 000 mL boiling water 3 times 0.5-1.0 h, until relatively clear TCM decoction. Liquid vacuum filtration, concentrated under reduced pressure to a viscous (50 °C), vacuum dried and pulverized to obtain extract powder. With 0.9% NaCl solution was formulated as 200 mg×kg-1, 100 mg×kg-1 drug solution and 50 mg×kg-1 when administered. Doses of green tea extract administration group with reference to the value of commercially available green tea in the human dose for conversion, the whole test dose of 2 times the amount of increment[7]. Amlodipine tablets available in Pharmaceutical of hu yuan of Shanghai Co., Ltd. Production batch: 20150092, Configured to liquid when administered concentration 0.5 mg×kg-1.

2.1.2. Animal Essencial hypertension (SHR) rats
60, male, Cleaning stage, body weight: (220 ± 10) g, Beijing Vital River Laboratory Animal Technology Co., Ltd. Animal license number: SCXK(Jing)2006-0009.

2.1.3 Reagents
0.9% Sodium Chloride Injection, Hainan Hua Tuo Tianya Pharmaceutical Co., Ltd. Production batch: 150818-1. Kits of Nitric Oxide (NO), superoxide dismutase (SOD) and angiotensin II, Institute of Biological Engineering from Nanjing Jiancheng.

2.1.4 instrument
BP-100A automatic noninvasive blood pressure measurement system from chengdu Thai meng Co., Ltd. Microscope of Olympus from Olympus optical microscope of Japanese company. HH-6 digital temperature water bath from Union Institute of Jiangsu Province Jintan Instruments. 722 UV spectrophotometer from Beijing Chen Xi Yong Chuang Co., Ltd. Low-temperature low-speed centrifuge of Eppendorf from Germany Eppendorf China Ltd.

2.2 Method

2.2.1 Animal groups and each group method of administration
All purchases and acclimated to feeding one week after the blood pressure measurement, normal saline and other daily operations, and acclimated to its operation. The 60 primary hypertensive rats (SHR) were randomly divided into hypertension of control group, amlodipine besylate group and the high, medium and low dose group of green tea extract of five groups, with twelve rats in each group. Three dose groups of Green tea extract respectively will gavage by rat at a concentration of 200 mg×kg-1•d-1,100 mg×kg-1•d-1,50 mg×kg-1•d-1. Yet amlodipine besylate group will gavage by rat at 0.5 mg×kg-1•d-1. Hypertension control group gavage a dose of 1 mL/100 g of 0.9% NaCl solution. It is weighing once a week During the administration, and as adjusted for changes in body weight dose by seven weeks. All rats were fed with normal diet and water ad libitum.

2.2.2 Blood pressure measurement
We are measurement of arterial pressure in rats of each group's awake that it is indirect measurement method by BP-100A automatic noninvasive blood pressure measurement system. Let rats adapt to the environment Before the official measurement week training load of 1 to 2 times a day, and blood pressure was measured after two hours drench. Rats have been placed before the first load in advance to set a good (37 ± 1 °C) electric incubator after preheating 15 min. Then the rats were placed invasive measurement of blood pressure measurement system in the cage, until stable blood pressure curve measured systolic and diastolic rat tail artery, repeated measured three times and averaged.

2.2.3 Serum samples were collected and measured
The animals were fasted for 12 h, but can not help but water and weighed after the last administration in Experimental administration of 7 weeks. Abdominal aortic blood samples were taken at 5 mL centrifuge tube and centrifuged to prepare serum with 3% sodium pentobarbital anesthesia. Take the prepared serum, respectively nitrate reductase and xanthine oxidase serum NO and SOD content.

2.2.4 Determination of angiotensin II (Ang II) content
The animals were taken blood to the abdominal aorta of the previously prepared containing 20 g/L sodium ethylenediaminetetraacetate (EDTA) in cryovials, then freeze the blood in the tube moved into 1.5 mL centrifuge tubes and Into the low-temperature low-speed Eppendorf centrifuged 15 min, then using competitive enzyme-linked immunosorbent assay Ang II content.

2.2.5 Collect samples of animal heart
Take heart tissue with saline wash the blood, while placed in 10% neutral formalin fixed that collected of rat abdominal aortic blood when the rat death. It is HE staining pathological and light microscope and tissue diagnosis by pathological changes of the pathology specialist.

2.3. Statistical method

Experimental data applications Excel software for processing what are used mean ± standard deviation (Mean ± SD) represent, and using statistical software SPSS 20.0 t test between sets of data, P value calculated, P<0.05 was statistically significant.

3. Result

3.1. Effect of green tea extract on blood pressure

Blood pressure (systolic and diastolic) in each group (SHR) rats substantially at the same level, there is no significant difference before the experiment, The treatment group had certain level of decline in blood pressure after 7 weeks except blood pressure of control group, and there are differences in hypertension control group, with statistical significance(P<0.01) by Table 1 & 2.

3.2. Effect of green tea extract on the weight of the heart and kidneys of SHR

Each rat heart and kidney mass administration group showed reduced that compared with control group after Seven weeks. And green tea extract high dose group and the amlodipine group was significantly reduced tendency in heart and kidney mass, Having significant (Respectively, P<0.01 and P<0.05). Green tea extract low-dose group and the control group with hypertension was decreasing trend. But there are small differences, not statistically significant (P>0.05). Administration of green tea and amlodipine certain period of time to be able to on ventricular remodeling in hypertensive rats have a certain role, Table 3.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Dose(mg/kg)</th>
<th>Heart Weight (g)</th>
<th>Kidney weights (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>1.52±0.39</td>
<td>2.33±0.22</td>
<td></td>
</tr>
<tr>
<td>Amlodipine</td>
<td>0.5</td>
<td>1.27±0.04&quot;</td>
<td>2.03±0.03&quot;</td>
</tr>
<tr>
<td>Green tea extract</td>
<td>50</td>
<td>1.38±0.34</td>
<td>2.24±0.43</td>
</tr>
<tr>
<td>Green tea extract</td>
<td>100</td>
<td>1.29±0.05&quot;</td>
<td>2.07±0.16&quot;</td>
</tr>
<tr>
<td>Green tea extract</td>
<td>200</td>
<td>1.25±0.10&quot;</td>
<td>2.02±0.07&quot;</td>
</tr>
</tbody>
</table>

Note: Compared with the control group with hypertension *P<0.05, "P<0.01.

3.3. Effect of green tea extract on serum nitric oxide (NO) and superoxide dismutase (SOD) content

Each treatment group hypertensive rat serum nitric oxide (NO) and superoxide dismutase (SOD) and the content of high blood pressure compared to the control group, were significantly higher, and the high dose group the effect is significant, with statistical significance after 7 weeks (Respectively, P<0.01 and P<0.05). Amlodipine group and low dose group and the control group, while having significantly increased, but the difference between small, not statistically significant(P>0.05), Table 4.

<table>
<thead>
<tr>
<th>Groups</th>
<th>NO(µmol/mL)</th>
<th>SOD(U/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>33.08±4.73</td>
<td>65.36±7.15</td>
</tr>
<tr>
<td>Amlodipine</td>
<td>27.55±6.56</td>
<td>62.56±8.62</td>
</tr>
<tr>
<td>Green tea extract</td>
<td>100</td>
<td>33.08±4.73</td>
</tr>
<tr>
<td>Green tea extract</td>
<td>200</td>
<td>37.81±4.97&quot;</td>
</tr>
</tbody>
</table>

Note: Compared with the control group with hypertension *P<0.05, "P<0.01.
3.4. Effect of green tea extract on plasma angiotensin II (Ang II) content

The treatment group significant difference except Angiotensin (Ang II) green tea extract low-dose group were content with statistical significance after 7 weeks when compared with hypertension control group (Respectively, \( P<0.01 \) and \( P<0.05 \)). But also the low dose group than the control group, but no significant difference \( (P>0.05) \). Ang II content with the green tea extract reduced the increase was dose, The experiments showed that after a period of drinking green tea can reduce the amount of angiotensin II.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Dose (mg/kg)</th>
<th>Ang II (mg/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>0</td>
<td>1.46±0.05</td>
</tr>
<tr>
<td>Amlodipine</td>
<td>0.5</td>
<td>1.27±0.03</td>
</tr>
<tr>
<td>Green tea extract</td>
<td>50</td>
<td>1.38±0.06</td>
</tr>
<tr>
<td>Green tea extract</td>
<td>100</td>
<td>1.30±0.03</td>
</tr>
<tr>
<td>Green tea extract</td>
<td>200</td>
<td>1.28±0.02</td>
</tr>
</tbody>
</table>

Note: Compared with the control group with hypertension \( P<0.05, \) \( P<0.01 \).

3.5. Morphological changes of heart

Shows from Figure 1, myocardial HE staining light microscopy, high blood pressure in the control group most visible disorder cardiac fibroblasts, and occasionally see the muscle fibers break, thicker, cardiomyocyte hypertrophy. Green tea high dose group and positive drug amlodipine myocardial fibers arranged in neat, uniform dyeing, stripes clear, uniform distribution of myocardial cells, no significant increase in the cells. Green tea, the low-dose group is insignificant, a small part of the visible region of thicker muscle fibers, but also disorganized, occasionally there is breakage.

![Figure 1. Effect of green tea extract on SHR Myocardial cell morphology.](Image)

Blood pressure is the main regulator of NO synthesis of endothelial nitric oxide synthase and a substance release of vasoactive. In a state of hypertension may be relaxation of vascular smooth muscle cells and blood pressure and inhibiting smooth muscle cell proliferation plays an important role in cardiac and vascular remodeling[12]. Because nitric oxide synthase in blood pressure caused by decreased serum NO degradation is weak, so the content is also reduced accordingly[13]. Superoxide dismutase (SOD) is the body's natural antioxidant enzymes and free radicals can maintain homeostasis that the body's cells to avoid damage. SOD significantly reduced the body under pathological conditions of hypertension[14]. The experimental results show that from each treatment group were serum NO and SOD in rats increased, Wherein the green tea extract high dose group compared with the control of hypertension, it was statistically significant \( (P<0.01 \) and \( P<0.05 \)). The positive antihypertensive drug amlodipine group and low dose group, while also able to lower blood pressure, but the effect is not significant \( (P>0.05) \).

Angiotensin II (Ang II) is very important in the RAS effector molecule having adjusted to maintain blood volume and blood pressure, but also on chronic inflammation is considered to play a participatory role of left ventricular remodeling of cellular processes important to stimulate product, it is One formed in the myocardium and is involved in the occurrence of hypertension, leaving the development of target organ damage factor in the renin - angiotensin system plays to promote cell proliferation, thus promoting protein synthesis induced myocardial hypertrophy, an important role in increasing the glomerular pressure leads to kidney dysfunction, etc. Through its effects receptor vasoconstriction, sympathetic activation, aldosterone secretion, sodium retention, etc[15]. Therefore, to
reduce the generation of Ang II, reduce blood pressure, weakened left ventricle reconstruction play a role. Green tea extract each treatment group and positive drug amlodipine group decreased Ang II content decreased blood pressure in SHR SHR, where green tea high dose group and amlodipine quite effective from this study. This Show Hainan green tea with amlodipine mean inhibition of the angiotensin-converting enzyme activity, so their role in lowering blood pressure.

Essential hypertension lead to cardiac hypertrophy, heart failure and thus, arrhythmias, myocardial ischemia or stroke and other diseases, a great threat to their life and health. The results suggest that, along with blood pressure, green tea extract administration group were also significantly decreased heart weight. Morphology cardiac fibroblasts each treatment group were better than the control group with hypertension. Green tea with high-dose group and positive drug amlodipine group as well on both cardiac cell morphology is insignificant. Long-term consumption of green tea can be seen on hypertension myocardial fibrosis and some improvement. Its cardiac structure has a protective effect, reducing the extent of myocardial injury, reversing ventricular remodeling.

Green tea as one of the most local characteristics of Hainan in "Hainan specialty". Green tea has become currently a hot research at home and abroad. It was discussed in terms of lowering blood pressure in this study, I believe that with further research, green tea will better benefit of mankind that Effective and safe drugs for heart and cerebrovascular disease foundation.

References