Effect of green tea on angiotensin II level and myocardial microstructure in spontaneous hypertensive rats

Y. R. Liang¹, J. Y. Xu¹, X. Y. Luo², X. Q. Zheng³, Q. L. Sun¹, S. C. Ma¹ and J. L. Lu¹*

¹Zhejiang University Tea Research Institute, Hangzhou 310029, China.
²Tea Research Institute, Academy of Agricultural Science of Guizhou Province, Guiyang 550006, China.
³Key Laboratory of Horticultural Plants Growth, Development and Biotechnology, Agricultural Ministry of China, Hangzhou 310058, China.

Accepted 28 July, 2010

Effects of green tea on blood pressure, level of plasma angiotensin II (Ang II) and ultrastructure of left ventricular tissue in spontaneous hypertensive rats (SHR) were investigated. Intragastric administration at dosages 0.2 and 1.0 g kg⁻¹ d⁻¹ green tea significantly decreased blood pressure and plasma Ang II level, accompanying the improvement of ultra structures of left ventricular mitochondrion and myofibrillae. It is considered that the effect of green tea on hypertension might be related to its inhibition of angiotensin converting enzyme.

Key words: Green tea, hypertension, spontaneous hypertensive rats, angiotensin, ultra structure, left ventricular, mitochondrion, myofibril.

INTRODUCTION

Hypertension (HT > = 140/90 mm Hg) is one of the leading causes of disability and death from stroke, heart attack and kidney failure. Hypertensive heart disease is considered to be the first cause of death associated with high blood pressure and is actually a group of disorders that include heart failure, ischemic heart disease and left ventricular hypertrophy. It is reported that age-adjusted prevalence of hypertension is 52% for men and 34% for women in urban Japanese population (Hasegawa, 2010) and one-fifth of the adult Danish population is found to be hypertensive (Kronborg et al., 2009). Approximately 65 million adults in the United States have hypertension (Fields et al., 2004). Worldwide, approximately 972 million adults were diagnosed with hypertension in 2000 and by 2025 the number of cases is predicted to increase by about 60% to a total of 1.56 billion (Ivanov et al., 2005). Diet and lifestyle have been reported to have a substantial impact on hypertension. It was reported that alterations in baroreflex and chemoreflex pathways are involved in occurrence of hypertension (Xie et al., 1990). Arterial baroreceptors are reset to a higher pressure in hypertensive patients, which seems to be mediated by a central action of angiotensin II (Lohmeier, 2001). Angiotensin II (Ang II), a potent vasoconstrictor of all blood vessels, is an indirect production of angiotensinogen catalyzed by renin and angiotensin converting enzyme (ACE) in turn (Lazartigues et al., 2007; Kurita et al., 2010). An overactive renin-angiotensin system leads to vasoconstriction and retention of sodium and water, and increase in blood volume then leads to hypertension (Manrique et al., 2009).

Green tea is reported to have many health benefits (Kim et al., 2007; Hamden et al., 2009; Wu et al., 2009). It is reported that green tea and its catechins decrease blood pressure (Yokozawa et al., 1994) and effectively inhibit cardiac hypertrophy in mice and rats (Li et al., 2006; Sheng et al., 2007). There is little information on physiological and anatomical mechanism of effects of green tea on hypertension. Spontaneous hypertensive...
rats (SHR) is frequently used as animal model of genetic hypertension that develops compensated hypertrophy to heart failure with aging, similar to humans (Bing et al., 2002). The aim of present study was to reveal the effects of green tea on angiotensin II level and changes in myocardial ultrastructures in SHR.

MATERIALS AND METHODS

Green tea supplied by Guizhou Tea Research Institute (Guiyang, China) was ground and sifted through 100-mesh sifter. Spontaneous hypertensive rats and Wistar-Kyoto rats (WKY) (13 weeks old with a body weight of 330 - 350 g) were purchased from the Experimental Animals Center of the Zhejiang Chinese Medical University (Hangzhou, China). The rats were placed in metabolic cages and kept in an animal room at ambient temperature 23 ± 1°C and humidity 55 ± 5%, with a 12 h light/dark cycle and an air exhaust cycle (10 min/h). Rats were fed with standard feed supplied by the Experimental Animals Center of the Zhejiang Chinese Medical University (Hangzhou, China) and water ad libitum for one week adaptation period before treatments.

Animal treatments

Twenty four SHR were randomly divided into three groups (n = 8 in each): control group (distilled water), low dosage group (green tea dosage 0.2 g kg⁻¹ based on body weight) and high dosage group (green tea dosage 1.0 g kg⁻¹). The tea powder was dissolved in 3 mL distilled water and fed by intragastric administration. The control group was gavaged with only 3 mL distilled water. The treatment period was 30 successive days. For comparison of myocardial microstructure, a group of 8 WKY rats was fed with the standard feed and water for the same period. The surgical and experimental procedures were consistent with Guide for the Care and Use of Laboratory Animals published by the US National Institutes of Health (NIH Publication no. 85 - 23, 1996) and were approved by the Ethics Committee for Animal Experimentation of the Zhejiang Chinese Medical University.

Systolic blood pressure measurement

Systolic blood pressure (SBP) for each conscious rat was determined by a tail-pulse pick-up method and recorded with a ZH-HX-Z Automatic Sphygmomonograph (Zhenghua Bio-Instruments Ltd, Huaibei City, China) according to method by Wang et al. (2008). The SBP values expressed in the paper were the mean of 3 consecutive determinations.

Determination of angiotensin II

For the determination of angiotensin II (Ang II) in plasma, blood samples were collected in chilled tubes containing ethylenediaminetetraacetic acid (EDTA, 20 g L⁻¹) at pH 5.5 so as to inhibit any possible enzymatic generation and degradation of Ang II. The collected blood samples were placed into micro-centrifuge tubes (1.5 mL) and centrifuged at 3000 x g for 15 min. The plasma samples collected were then stored at –20°C until subsequent analysis. Determination of Ang II was performed by enzyme-linked competitive immunoassay using a commercial Kit (SPI Bio, Berlin Group, France), following the procedure recommended by the manufacturer.

Transmission electron microscopy

In seven rats from each group, left ventricular tissues were obtained at the time of death and fixed in 2.5% glutaraldehyde overnight at 4°C, and then sliced into 1 mm³ pieces. The glutaraldehyde solution was poured out and the tissues were washed with 0.1 M phosphate buffer (pH 7.0) for three times and then re-fixed in 1% (v/v) osmium tetroxide (OsO₄) solution for 2 h and washed using with 0.1 M phosphate buffer as before. The embedding with epoxy resin and ultra-cut sectioning with Reichert-Jung ultra-cut 701701 Microtome (Reichert-Jung Co., Heidelberg, Germany) were made according to methods described by Du et al. (2008). The ultrastructures were examined and photographed with a JEM-1230 microscope (JEOL Ltd. Akishima, Tokyo, Japan).

Statistical analysis

Results are expressed as means ±SEM, compared with controls. The data were statistically analyzed using one-way ANOVA, followed by the Statistical Analysis System (SAS Institute, Cary, NC, USA). Values of P < 0.05 were considered to indicate statistical significance.

RESULTS AND DISCUSSION

Effect of green tea on systolic blood pressure of spontaneous hypertensive rats

As shown in Figure 1, there was no significant difference in SBP values between 3 groups of SHR before test. At the end of 30-day test, SBP values in treatments of both green tea dosages were significantly lower than control group though all the 3 groups increased significantly, compared to the initial values. It shows that green tea had suppressive effect on blood pressure of SHR.

Effect of green tea on levels of plasma angiotensin II

Levels of plasma Ang II in green tea groups treated at both dosages were significantly lower than that in control group. The Ang II level decreased in a dose-dependent (Table 1). Renin-angiotensin system plays a critical role in the regulation of blood pressure. Ang II is regarded as the leading mediator of clinical systemic hypertension and it plays a role in the restructuring arterial walls in both atherosclerosis (Peng et al., 2010) and hypertension (Kurita et al., 2010). Various studies have shown that either an increased Ang II level by its relevant metabolism or an impaired response of Ang II production to dietary salt intake are associated with a salt-sensitive component of blood pressure regulation (Declue et al., 1978; Ivanov et al., 2005; Crowley et al., 2006; Papparella et al., 2008). Green tea extract and its catechins are reported to effectively inhibit smooth muscle cell collagen gel contraction (Ivanov et al., 2005), reactive oxygen species production (Papparella et al., 2008) and C-reaction protein secretion (Peng et al., 2010) induced by ANG II. The present study suggests that suppressive effect of green tea on blood pressure might be mediated through its regulation of Ang II.
Figure 1. Effect of green tea on SBP of SHR, the columns with different alphabetic letters were significantly different at ρ = 0.05 (n = 8).

Table 1. Changes in plasma Ang II (mg L⁻¹).

<table>
<thead>
<tr>
<th>Green tea dosage (g kg⁻¹d⁻¹)</th>
<th>Ang II¹</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>1.48 ± 0.03 a</td>
</tr>
<tr>
<td>0.2</td>
<td>1.39 ± 0.04 b</td>
</tr>
<tr>
<td>1.0</td>
<td>1.30 ± 0.03 c</td>
</tr>
</tbody>
</table>

¹Data (mean ± standard deviation) with different alphabetic letters were significantly different at ρ = 0.05 (n = 8).

Effect of green tea on myocardial ultra structure of left ventricular tissue

TEM observation showed that there were significant differences in ultrastructures of left ventricular tissue between WKY rats and SHR Figure 2. The myofibrillae were lined up without disruption, and the structures of mitochondria, myofilaments and myotomes were intact and regular in WKY rats Figure 2A1, A2. However, in the control group of SHR rats (without green tea administration), the myofibrillae were partially broken, the myofilaments were partially dissolved, and some mitochondria showed obvious vacuolization and degeneration Figure 2B1, B2. These phenomena were observed in ventricular hypertrophy and left ventricular remodeling in rats (Wang et al., 2008). Following administration with green tea 0.2,1.0 g kg⁻¹d⁻¹ respectively, the morphology of the mitochondria and myofibrillae was significantly improved in dose-dependent manner ((Figure 2C1, C2). The mitochondrion vacuolization was not observed in the group of 1.0 g kg⁻¹d⁻¹ green tea dosage (Figure 2D). This suggests that green tea has positive effect on the myocardial ultra structure of left ventricular tissue. The result is consistent with the finding which was observed in combination treatment of rats with green tea extracts and Ang II (Papparella et al., 2008).

Left ventricular remodeling, characterized by changes in size, shape and function of left ventricular, occurs in several clinical conditions, including hypertension, chronic heart failure (Frigerio and Roubina, 2005; Wang et al., 2008). Ang II is formed locally within the myocardium and it is known to be an important stimulus...
to the cellular processes responsible for left ventricular remodeling (Sadoshima et al., 1993). Angiotensin converting enzyme is responsible for the biosynthesis of vasoconstrictor Ang II (Lazartigues et al., 2007) and the degradation of vasodilator bradykinin (Imig, 2004). Therefore, inhibition of ACE results in the decreased formation of Ang II and decreased mediation of bradykinin, leading to a decrease in SBP (Imig, 2004) and an attenuation of the remodeling process (James and Udelson, 2004). The present study shows that green tea decreases Ang II and improves ultra structures of left ventricular myocardium and myofibrillae, accompanying with reduction in blood pressure in SHR rats. It suggests that the effect of green tea on hypertension might be related to its inhibition of angiotensin converting enzyme.

ACKNOWLEDGEMENT

This study was financially supported by the Science and Technology Department of Guizhou Province, China (Project no. 2008-6015).

REFERENCES


