Effects of Qindan Capsule (芩丹胶囊) on Blood Pressure, Endothelin, Calcitonin Gene-related Peptide and Angiotensin-II in Spontaneous Hypertensive Rats*

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ABSTRACT
Objective: To observe the hypotensive effects of Qindan Capsule (芩丹胶囊, QC) on spontaneous hypertensive rats (SHR) and its effect on the contents of endothelin (ET), calcitonin gene-related peptide (CGRP) and angiotensin-II (Ang-II) in plasma and vascular tissues, and to investigate the possible mechanism of QC in lowering blood pressure. Methods: Forty SHRs were divided into 5 groups: the high dosage QC group (QCHD, 750 mg/(kg·d)), the low dosage QC group (QCLD, 150 mg/(kg·d)), the Niuhuang Jiangya Pill group (牛黄降压丸, NJP, 200 mg/(kg·d)), the Captopril group (15 mg/(kg·d)) and the model group, 8 in each group. Meanwhile, a normal control group consisting of 8 Wistar Kyoto (WKY) rats was set up also. All the rats were administered with medicine through gastrogavage. Systolic blood pressure (SBP), level of ET, CGRP and Ang-II in plasma and Ang-II in tissues of mesenteric artery were detected in all the rats after 12 weeks of treatment. Results: The level of SBP after treatment in the QCHD group was lower than that in the model group (P < 0.01), but with no significant difference as compared with that in the Captopril group and the NJP group (P > 0.05). After treatment, the plasma level of ET was lower and CGRP higher than those in the model group (both P < 0.05), and also higher than those in the NJP and Captopril group (both P < 0.05). As for the content of Ang-II, in mesenteric arterial tissues, it was lower in the QCHD group than that in the model group (P < 0.05), but in plasma, it showed no significant difference between the two groups (P > 0.05). Conclusion: QC has a satisfactory hypotensive action on SHR rats, and its mechanism may be associated with the regulation on plasma vasoactive peptide and regional renin-angiotensin system.

KEY WORDS Qindan Capsule, spontaneous hypertensive rat, endothelin, calcitonin gene-related peptide, angiotensin-II

Qindan Capsule (芩丹胶囊, QC), a compound preparation of traditional Chinese medicine prescribed clinically for the treatment of hypertension, possesses the actions of clearing away heat to calm Gan (肝) and removing blood stasis to activate the collaterals and is always applied in treating patients with hypertension of Gan-yang heat-excessive type accompanied with blood stasis. In order to further explore its mechanism in reducing blood pressure, spontaneous hypertensive rats (SHR) were chosen to observe the hypotensive effect of QC and its effect on contents of endothelin (ET), calcitonin gene-related peptide (CGRP) and angiotensin-II (Ang-II) in plasma and Ang-II in mesenteric arterial tissues.

METHODS

Experimental Animals
Forty male SHRs, 14 weeks old, weighing 260 ± 20 g and 8 male Wistar Kyoto (WKY) rats, 14 weeks old, weighing 280 ± 20 g, were all purchased from Shanghai Slac Laboratory Animal Co. Ltd., China.

Drugs
QC, consisting of Radix Scutellariae, Radix Salviae Miltiorrhizae, Rhizaoma Coptidis, Ramulus Uncariae Cum Uncis, Rhizoma Chuanxiong, Lumbricus, Herba Leonuri, and Herba Taxilli, etc. was provided by Pharmaceutical Department, Qilu Hospital Affiliated to Shandong University, each gram containing 2.7 g of crude drugs. Niuhuang Jiangya Pill (牛黄降压丸, NJP), 0.4 g/pill, was the product of Darentang Pharmaceutical Co. Ltd., Tianjin, batch number E373004. Captopril, 25 mg/tablet, was the product of Xinhua Pharmaceutical Co. Ltd., Shandong, batch number: 043274.

Apparatus and Reagents
Rat blood pressure measurer type RBP-I was purchased from Institute of Clinical Medicine, China-Japan Friendship Hospital. Hypothermal ultracentrifuge type

*Supported by Administration of Traditional Chinese Medicine of Shandong Province (No. 1-60)
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Biofuge-stratos was the product of Kendro Co., Germany, and γ - radioimmunity counter type GC911 was product of the General Science & Technology Company, University of Science and Technology of China. The commercial test kits for ET, CGRP, and Ang-Ⅱ were purchased from Institute of Radioimmunity, the General Hospital of PLA.

Grouping and Treatment of Animals

The forty SHRs were separately raised at constant temperature of (22 ± 2) °C and humidity of (55 ± 5)%, and were randomly divided into five groups, 8 in each group, namely: the QCHD group treated with high dosage (750 mg/kg per day) of QC, equivalent to 15 folds of the dosage for human adult used in clinical practice; the QCLD group treated with low dosage (150 mg/kg per day) of QC, equivalent to 3 folds; the NJP group treated with NJP 200 mg/kg per day, equivalent to 15 folds; the Captopril group treated with Captopril 15 mg/kg per day, equivalent to 20 folds; and the model group was only fed with equal volume of normal saline instead. Besides, eight WKY rats were enlisted in the normal control group and fed also with saline.

All the treatments were administered by gastrogavage once a day for 12 successive weeks. After the last administration, all rats were fasted with free access to water for 24 h, and anesthetized with intraperitoneal injection of 3% pentobarbital (30 mg/kg), their blood sample was drawn out for ET, CGRP and Ang-Ⅱ determination, and then they were sacrificed to separate the mesenteric artery for measuring Ang-Ⅱ.

Measurement of SBP

Systolic blood pressure (SBP) of rats was measured once a week in the first 3 weeks of experiment (before treatment, the 1st and the 2nd weeks after treatment), and then measured fortnightly till the end of the experiment, i.e. 8 times in total. This was proceeded by connecting the rat blood pressure measurer to the rats following the method of tail-cuff, with the rats’ tail heated interruptedly for maintaining suitable temperature under the condition that the rats were kept calm with the pulsatory signal of caudal artery displayed steadily when measuring. The mean value of three successive measuring (the difference of values between them should be within 5 mm Hg), was regarded as the measured value of SBP.

Determination of Levels of ET, CGRP and Ang-Ⅱ in Plasma and Contents of Ang-Ⅱ in Tissues of Mesenteric Artery

Blood sample (6 ml) drawn out from rats’ common carotid artery was put immediately into anticoagulant tube containing enzyme inhibitor, centrifuged at 3 000 r/min in 4 °C for 10 min to get the supernatant for ET, CGRP and Ang-Ⅱ determination adopting radioimmunoassay (RIA) according to the manual of the kits.

Rats’ mesenteric artery was separated from peripheral connective tissues and made into homogenate, which was centrifuged to get the supernatant to determine Ang-Ⅱ also by RIA.

Statistical Analysis

All data were expressed as mean ± standard deviation, and statistic analysis was performed with SPSS 10.0 software. ANOVA was adopted in analyzing the data, and P < 0.05 was considered statistically significant.

RESULTS

Comparison of SBP before and after Treatment

See Figure 1. From the second week of treatment, SBP was significantly lower in the normal control group and the treated groups (QCHD, QCLD, NJP and Captopril) than that in the model group (all P < 0.01), and the lowering in the QCHD group was greater than that in the QCLD group, with a significant difference from the 6th to the 10th week (P < 0.05 or P < 0.01). By the end of the 12th week, except that in the QCLD group, SBP got significantly lower in all the treated groups than that in the model group (P < 0.01), even approaching to that in the normal control group, but no significant difference was shown in comparison among these three treated groups (P > 0.05).

Figure 1.   Comparison of SBP before and after Treatment

Comparison of Levels of ET, CGRP and Ang-Ⅱ in Plasma and Ang-Ⅱ in Tissues of Mesenteric Artery

See Table 1. Compared with the normal control group, the levels of plasma ET and Ang-Ⅱ in tissues of mesenteric artery in the model group were significantly higher (P < 0.01), and level of plasma CGRP significantly