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Effect of combination of valsartan with benazepril on blood pressure and left ventricular hypertrophy in SHR

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KEY WORDS hypertension; benazepril; valsartan; inbred SHR rats; combination drug therapy; left ventricular hypertrophy

ABSTRACT

AIM: To evaluate the antihypertensive efficacy of angiotensin converting enzyme inhibitor (ACEI) benazepril in combination with AT_1 receptor antagonist valsartan and their effect on left ventricular hypertrophy, renin angiotensin aldosterone system (RAAS) and endoxin in spontaneously hypertensive rat (SHR). **METHODS**: WKY control group ($n = 6$) and other 4 groups consisted of 24 SHR (14-week-old, male, $n = 6$): SHR control group, benazepril group, valsartan group, and combination drug therapy group. Systolic blood pressure (SBP) of SHR was measured at the beginning and at the end of 2, 4, 6, and 8 wk of drug intervention. Morphometric determination, renin activities, angiotensin Ⅱ (Ang Ⅱ), endoxin, and ATPase activity analysis were performed at the end of 8 week of drug intervention. **RESULTS**: SBP, ratio of left ventricular mass (LVM), body weight (BW) (LVM/BW), and transverse diameter of myocardial cell (TDM) of SHR were remarkably decreased after drug intervention, and this decrease was most remarkable in the combination drug therapy group. Renin activities of plasma and myocardium were remarkably increased in drug intervention groups. The levels of Ang Ⅱ in plasma and myocardium were remarkably increased in valsartan group, decreased in benazepril group and combination drug therapy group. Na^+-K^+-ATPase activities in myocardium were remarkably increased and the level of endoxin in myocardium were remarkably decreased as SBP decreased after drug intervention. **CONCLUSION**: Both benazepril and valsartan can decrease SBP of SHR, and cause regression of ventricular hypertrophy. The efficacy of combination drug therapy group is most remarkable among all groups and avoids the side effects of induction of high Ang Ⅱ levels in plasma and myocardium caused by long-term use of valsartan alone.

INTRODUCTION

Renin angiotensin aldosterone system (RAAS) plays a key role in maintaining normal blood pressure, liquids, and electrolyte balance. Meanwhile, it also affects the pathophysiology of hypertension. Benazepril is an angiotensin converting enzyme inhibitor (ACEI) and has an excellent antihypertensive effect by blocking the formation of angiotensin Ⅱ (Ang Ⅱ). It is extensively used to treat hypertension[1,2]. Valsartan is an Ang Ⅱ receptor 1 (AT_1) antagonist and has an antihypertensive effect by blocking the action of Ang Ⅱ on AT_1 receptor[3]. Levels of plasma Ang Ⅱ are remarkably increased when valsartan alone is used to treat hypertension. High concentration of Ang Ⅱ can competitively combine with AT_1 receptor to weaken long-term efficacy of valsartan although high concentration Ang Ⅱ may combine with AT_2 receptor on myocardium and vascular smooth cell to induce cell apoptosis. This research combined valsartan with benazepril to evaluate their antihypertensive efficacy as a combination drug therapy and their effects on left ventricular hypertrophy, RAAS, and endoxin in spontaneously hypertensive rat (SHR).

MATERIALS AND METHODS

Animals Twenty-four SHR (Grade Ⅱ, Certificate № 02-37-2), male, fourteen weeks old, weighing 210 - 250 g; Sex and age matched WKY rats (Grade Ⅱ, Certificate № 02-37-1), weighing 205 - 263 g; were purchased from Shanghai Institute of Hypertension Research (China).

Reagents Radioimmunoassay kits with reagents of plasma renin activity (PRA) and Ang Ⅱ were purchased from Beijing Northern Biological Technological

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Institute (China). Radioimmunoassay kit with reagent of endoxin was purchased from Radioimmune Institute of Tongji University (China). The kit with reagent of ATPase activity was purchased from Nanjing Jiancheng Biological Engineering Institute (China). Valsartan and benazepril were provided by Beijing NOVARTIS Pharmaceutical, Ltd (China).

Grouping Six WKY rats were constituted as a normal control group (Group A): fed with 0.9 % NaCl 10 mL·kg^{-1}·d^{-1} for 8 wk. Twenty-four SHR were randomly divided into four groups, six rats in each group. SHR control group (Group B): fed with 0.9 % NaCl 10 mL·kg^{-1}·d^{-1}; Benazepril group (Group C): fed with benazepril 1 mg·kg^{-1}·d^{-1}; Valsartan group (Group D): fed with valsartan 8 mg·kg^{-1}·d^{-1}; Combination drug therapy group (Group E): fed with valsartan 8 mg·kg^{-1}·d^{-1} and benazepril 1 mg·kg^{-1}·d^{-1}. The experimental drugs were given by gavage in 0.9 % NaCl 10 mL at 8 AM. The experiment continued for 8 wk. All rats were fed with standard rat chow and tap water *ad lib* during experiment.

Measurement of systolic blood pressure (SBP) SBP was measured using tail-cuff technique (MRB-Ⅲ A computer control sphygmomanometer for rat, Shanghai Institute of Hypertension Research, China) at the beginning and at the end of 2, 4, 6, and 8 wk of treatment.

Morphometric determination and renin activities, Ang Ⅱ, endoxin, and ATPase activity analysis After weighing the animal and measuring the blood pressure at the end of the experiment, rats were anesthetized by 2 % thiopental 40 mg·kg^{-1} celiac injection. The blood from carotid was put into anticoagulation test tubes having cooled aprotinin and edetic acid and centrifuged at 1200 × *g* at 4 ℃ for 10 min. The supernatant was preserved at −20 ℃ to detect PRA and Ang Ⅱ. The heart was quickly removed and perfused with 0.9 % NaCl liquid through the aorta. Moisture content of the heart was absorbed with filter paper. The weight of left ventricle and ventricular septum served as left ventricular mass (LVM). Ratio of LVM and body weight (BW) was calculated as the index of left ventricular hypertrophy. The left ventricular myocardial tissue 0.5 cm × 0.5 cm × 0.5 cm was taken out and placed in glass bottle with 10% formalin. Left ventricular myocardium was processed and embedded in paraffin for transverse sectioning (6 − 10 μm) and stained with haematoxylin/eosin (HE). Transverse diameter of myocardial cell (TDM) was examined with optic microscope. Twenty myocardial cells were randomly examined in each slice and average value of TDM were calculated. The partial left ventricular myocardium was mixed with acetic acid 0.5 mol·L^{-1} and boiled for 15 min. After cooling, the homogenates were centrifuged at 1200 × *g*, 4 ℃ for 20 min. The supernatant was examined for Ang Ⅱ. The rest of the ventricular myocardium was mixed with cooled aprotinin (W:V = 1:10) and homogenated. The homogenates were centrifuged at 1200 × *g* for 20 min at 4 ℃. The supernatant was examined for renin activities, Na^+-K^+-ATPase activities and endoxin.

Estimating renin, concentration of Ang Ⅱ, endoxin, and ATPase The renin, Ang Ⅱ, and endoxin in myocardial tissues and plasma were assayed with radioimmunoassay. ATPase activities were determined by chromometry. The content of protein in myocardial tissues was determined by protein-dye binding method[4].

Statistic analysis All data were shown as $\bar{x} \pm s$, and test by Student-Newman-Keul's test (ANOVA). Interrelation of two factors was adopted with simple beeline correlation analysis.

RESULTS

Effects of drug intervention on systolic blood pressure in SHR The level of SBP in SHR control group was remarkably higher than that in WKY control group. The level of SBP after drug intervention was remarkably lower than that before drug intervention in benazepril, valsartan, and combination of benazepril with valsartan group and antihypertensive effect of combination of benazepril with valsartan was most remarkable (Tab 1).

Effects of drug intervention on renin activities and levels of Ang Ⅱ in plasma and myocardial tissues in SHR As shown in Tab 2, the renin activities and Ang Ⅱ in plasma and myocardial tissue were remarkably higher in group B than in group A. After drug intervention, renin activities in plasma and myocardial tissue in group C, D, E were remarkably higher than in group A, B; the levels of Ang Ⅱ in plasma and myocardial tissue in group D were remarkably higher than in group B; the levels of plasma Ang Ⅱ in group C were slightly lower than in group B, but the levels of Ang Ⅱ in myocardial tissue in group C, E were remarkably lower than in group B.

Tab 1. Effects of drug intervention on SBP (mmHg) in SHR. $n = 6$. $\bar{x} \pm s$. $^cP < 0.01$ *vs* group A. $^fP < 0.01$ *vs* group B. $^hP < 0.05$ *vs* group C, D, respectively. $^lP < 0.01$ *vs* before treatment.

Group	Before	2 wk	4 wk	6 wk	8 wk
WKY (A)	103 ± 6	102 ± 7	105 ± 7	104 ± 6	105 ± 8
SHR (B)	174 ± 12^c	177 ± 10^c	181 ± 10^c	183 ± 8^c	183 ± 6^c
Benazepril (C)	174 ± 12^c	149 ± 9^{cfl}	143 ± 8^{cfl}	141 ± 8^{cfl}	140 ± 8^{cfl}
Valsartan (D)	174 ± 10^c	148 ± 8^{cfl}	141 ± 7^{cfl}	138 ± 6^{cfl}	138 ± 7^{cfl}
B plus V (E)	174 ± 11^c	144 ± 7^{cfl}	130 ± 8^{cfhl}	128 ± 5^{cfhl}	128 ± 5^{cfhl}

Tab 2. Effects of drug intervention on renin activities and levels of Ang Ⅱ in plasma and myocardial tissues in SHR. $n = 6$. $\bar{x} \pm s$. $^bP < 0.05$, $^cP < 0.01$ *vs* group A. $^eP < 0.05$ *vs* group B.

Group	Renin activities Plasma/ ng·L^{-1}	Renin activities Heart/ pg·g^{-1}	Ang Ⅱ Plasma/ ng·L^{-1}	Ang Ⅱ Heart/ ng·g^{-1}
WKY (A)	2.7 ± 0.7	1.4 ± 0.4	589 ± 128	190 ± 18
SHR (B)	3.1 ± 0.8^b	2.1 ± 0.4^b	732 ± 135^b	271 ± 40^b
Benazepril (C)	4.2 ± 0.6^{ce}	2.9 ± 0.8^{ce}	630 ± 83	211 ± 32^e
Valsartan (D)	4.6 ± 0.7^{ce}	3.0 ± 0.8^{ce}	957 ± 138^{be}	332 ± 56^{be}
B plus V (E)	4.6 ± 0.8^{ce}	3.4 ± 0.6^{ce}	704 ± 138	216 ± 46^e

Effects of drug intervention on activities of Na^+-K^+-ATPase and level of endoxin in myocardial tissues in SHR Myocardial tissue activities of Na^+-K^+-ATPase in group B were remarkably lower than in group A; the level of endoxin in group B was remarkably higher than in group A. After drug intervention, myocardial tissue activities of Na^+-K^+-ATPase rose remarkably and level of endoxin decreased remarkably.

Tab 3. Effects of drug intervention on activities of Na^+-K^+-ATPase and level of endoxin in myocardial tissues in SHR. $n = 6$. $\bar{x} \pm s$. $^bP < 0.05$ *vs* group A. $^eP < 0.05$ *vs* group B.

Group	Na^+-K^+-ATPase /μmol·mg^{-1}(protein)·h^{-1}	Endoxin /ng·g^{-1}
WKY (A)	3.4 ± 0.6	0.20 ± 0.13
SHR (B)	2.0 ± 0.6^b	0.47 ± 0.16^b
Benazepril (C)	3.2 ± 0.9^e	0.31 ± 0.17^e
Valsartan (D)	2.9 ± 0.5^e	0.35 ± 0.13^e
B plus V (E)	3.8 ± 0.6^e	0.26 ± 0.15^e

Effects of drug intervention on LVM/BW and TDM in SHR LVM/BW in group B was remarkably higher than in group A, TDM in group B was remarkably more than in group A. After drug intervention, LVM/BW and TDM in group C, D, and E remarkably decreased as decrease of SBP. Effect of group E was very significant. Significant positive correlation between SBP and LVM/BW, TDM were observed. By correlation analysis, r was 0.6771, 0.6812, respectively, $P < 0.01$.

Tab 4. Effects of drug intervention on LVM/BW and TDM in SHR. $n = 6$. $\bar{x} \pm s$. $^bP < 0.05$ *vs* group A. $^eP < 0.05$ *vs* group B.

Group	LVM/BW/mg·g^{-1}	TDM/μm
WKY (A)	2.12 ± 0.19	12.6 ± 0.6
SHR (B)	3.60 ± 0.24^b	15.7 ± 0.6^b
Benazepril (C)	2.61 ± 0.22^e	13.9 ± 0.5^e
Valsartan (D)	2.66 ± 0.21^e	14.0 ± 0.6^e
B plus V (E)	2.45 ± 0.19^e	13.4 ± 0.5^e

DISCUSSION

RAAS plays a key role in maintaining normal blood pressure, liquids and electrolyte balance. Ang Ⅱ induces effects such as contracting vascular smooth cells, increasing aldosterone secretion, promoting vascular and myocardial cell growth by combining with AT_1 receptor[5,6]. Therefore, Ang Ⅱ takes part in the pathophysiological process of hypertension. ACEI has antihypertensive effects, regresses myocardial hypertrophy by inhibiting ACE activities and decreasing Ang Ⅱ levels in plasma and tissues[1]. But ACEI can not effect the formed Ang Ⅱ. AT_1 receptor antagonists have antihypertensive effects by inhibiting Ang Ⅱ combination with AT_1 receptor. Studies show that levels of Ang Ⅱ in plasma and myocardial tissue significantly rise after AT_1 receptor antagonist is used. It was thought that high concentration of Ang Ⅱ might combine with AT_2 receptor on myocardial cell to induce cell apoptosis and lead to re-

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gression in myocardial hypertrophy[7]. But, at high concentrations Ang Ⅱ can compete with AT_1 receptor antagonist to combine with AT_1 receptor, therefore, efficacy of AT_1 receptor antagonist is weakened. This effect may be evident in patients who undertake long-term AT_1 antagonist therapy when the drug dose is decreased. Therefore, it is important to search for an optimal treatment in order to improve the long-term prognosis of hypertensive patients.

In our study it was observed that benazepril, valsartan, benazepril plus valsartan all had significant antihypertensive effects in SHR. This effect was most remarkable in the combination drug therapy group. It was found that renin activities in the plasma and myocardial tissue were significantly raised in the three drug intervention groups. The level of Ang Ⅱ in the plasma and myocardial tissue significantly rose in valsartan group, decreased in benazepril group and the combination drug therapy group. The regression in myocardial hypertrophy was observed in three drug intervention groups (LVM/BW and TDM were observed to decrease) and was most remarkable in the combination drug therapy group. The regression in myocardial hypertrophy was positive correlation with decrease of SBP. The result was in consistence with the literature[8]. The antihypertensive efficacy of the combination drug therapy was excellent, and its effect on circulating and tissue RAAS balance was insignificant. Combination drug therapy can decrease high levels of Ang Ⅱ caused by the use of AT_1 receptor antagonist alone and thus can avoid side effect caused by high levels of Ang Ⅱ.

Endoxin is an inhibitor of Na^+-K^+-ATPase. Previous research implicates its part in the process of hypertensive pathophysiology and myocardial ischemic-reperfusion injury[9,10]. The present study found that the level of endoxin in the myocardial tissue in SHR was significantly raised and activities of Na^+-K^+-ATPase were significantly decreased in myocardial tissue. The level of endoxin in myocardial tissue significantly decreased and activities of Na^+-K^+-ATPase were significantly raised as blood pressure decreased after drug intervention. This shows that endoxin may take part in the pathogenesis of hypertension. Meanwhile, it also shows that antihypertensive efficacy of benazeprial and valsartan maybe related with the fact that these drugs inhibit endoxin synthesis and secretion in the myocardial and vascular tissue except for effect on RAAS.

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Valsartan 与 benazepril 联合应用对自发性高血压大鼠血压和左心室肥厚的影响

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关键词 高血压；苯那普利钠；缬沙坦；近交 SHR 大鼠；联合药物疗法；左心室肥厚

目的：评价血管紧张素转换酶抑制剂苯那普利钠和 AT_1 受体拮抗剂缬沙坦联合应用对自发性高血压大鼠(SHR)的降压疗效及其逆转心肌肥厚作用和对肾素-血管紧张素-醛固酮系统(RAAS)、内洋地黄素水平的影响. **方法**：24 只 14 周龄雄性 SHR 随机分成空白对照组、Benazepril 组、Valsartan 组和 Benazepril + Valsartan 组，另设 WKY 正常对照组. 分别于药物干预前、药物干预后 2、4、6、8 周末测定大鼠 SBP；于药物干预后 8 周末检测心肌组织和血浆肾素活性、血管紧张素 Ⅱ 浓度、心肌组织 Na^+-K^+-ATP 酶活性和内洋地黄素水平，并行心肌组织形态学检查. **结果**：药物干预各组 SHR 动脉收缩压(SBP)水平明显下降，尤以联合用药组 SBP 下降最显著；药物干预各组血浆和心肌组织肾素活性均明显升高；Benazepril 组和 Benazepril + Valsartan 组血浆和心肌组织 Ang Ⅱ 水平降低，而 Valsartan 组血浆和心肌组织 Ang Ⅱ 水平则明显升高；随 SBP 水平的降低，心肌组织 Na^+-K^+-ATP 酶活性明显升高，而内洋地黄素水平则明显下降；药物干预各组 LVM/BW、TDM 均明显减低，尤以联合用药组改变最为显著. **结论**：ACEI Benazepril 和 AT_1 拮抗剂 Valsartan 均有明显的降低 SHR 的 SBP 作用，能明显逆转左室肥厚；联合用药效果最为显著，并能有效防止单一 AT_1 拮抗剂所致血浆和心肌组织 Ang Ⅱ 水平的升高的副作用.

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