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Regression of cardiac hypertrophy and myosin isoenzyme patterns by losartan and captopril in renovascular hypertensive rats

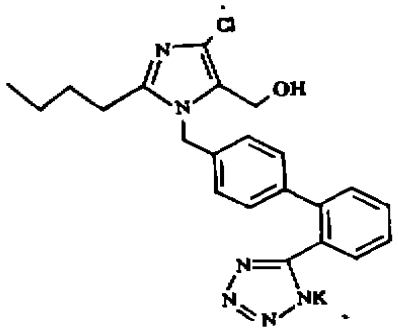
LING Qi, GUO Zhao-Gui[1], SU Zhi, GUO Xun
(Research Section of Pharmacology, Hu'nan Medical University, Changsha 410078, China)

ABSTRACT To test the effect of losartan and captopril on cardiac hypertrophy and myosin isoenzyme, two-kidney, one-clip (2K1C) renovascular hypertensive rats (RHR) were used. Eight weeks after the onset of hypertension, losartan 5 mg·kg^{-1}·d^{-1} and captopril 50 mg·kg^{-1}·d^{-1} were administered *po* to 2 groups of RHR, respectively for 8 wk. The results showed that captopril significantly decreased the cardiac mass (607±169 mg *vs* 1029±132 mg) and total protein content (120±38 mg *vs* 198±31 mg), concomitant with significant decrease of arterial blood pressure (BP) (15.4±5.2 kPa *vs* 28.5±4.9 kPa). Losartan also induced a significant decrease in cardiac mass (671±116 mg *vs* 1029±132 mg) and protein content (142±29 mg *vs* 198±31 mg), as well as significantly lowered the BP (15.2±2.1 kPa *vs* 28.5±4.9 kPa). It is important to be note that both drugs normalized the shift of myosin isoenzyme in RHR. These results indicated that both drugs, having potent antihypertensive effects, can effectively reverse the cardiac hypertrophy and abnormal distribution of myosin isoenzyme patterns.

KEY WORDS losartan, angiotensin receptors; captopril; renovascular hypertension; heart hypertrophy; myosin ATPase

The renin-angiotensin system (RAS) plays an important role in the development of renovascular hypertension and cardiac hypertrophy[1,2]. It has been well documented that captopril, an angiotensin converting enzyme inhibitor, reversed the cardiac hypertrophy and lowered the arterial blood pressure (BP), as well as improved the survival of patients. Losartan (DuP 753), a newly produced, orally active, nonpeptide specific angiotension II receptor 1 (Ang II-1) antagonist, could inhibited the vasopressor response to Ang II and reduced BP in renal and spontaneously hypertensive rats[3,4]. However, until recently its chronic therapeutic efficacy in cardiac hypertrophy has not been precisely evaluated.

Cardiac myosin isoenzyme, existing in 3 forms (V_1, V_2, and V_3), is not only a sensitive biochemical index of hypertensive cardiac hypertrophy, but also an important determinant of cardiac performances[5]. The action of antihypertensive treatment with blockers of the RAS on the isoenzyme patterns of cardiac myosin is, as yet, unknown. The present study was carried out to determine the effects of losartan and captopril on cardiac



Losartan potassium (Dup 753, MK 954)
2-*n*-butyl-4-chloro-1-{[2'-(1*H*-tetrazol-5-yl)[1,1'-biphenyl]-4-yl]methyl}-1*H*-imidazole-5-methanol, monopotassium salt

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[1] Correspondence should be addressed to Prof GUO Zhao-Gui.

hypertrophy and myosin isoenzyme patterns.

MATERIALS AND METHODS

Rat model preparation Wistar rats, ♂, weighing 179±s 18 g (Animal Center of Hunan Medical University), were used. Renovascular hypertension was produced by 2K1C method[6], in which the left renal artery was occluded about 50—70 % by a silver clip with an ID of 0.3 mm. Control rats were submitted to a sham-operation (sham). Before and after surgery, BP was measured once a week by tail cuff method (Buffington 101A, USA). Rats, with BP greater than 20.0 kPa within 3 wk after clipping, were considered hypertensive[6].

Determination of cardiac mass and protein content Rats were killed, and their hearts removed immediately. The ventricles were trimmed off atria and visible blood vessels. The left and right ventricles, washed with ice-cold NaCl 0.9%, were separated and weighed, independently. The left ventricular weight (LVW) and ratio of left to right ventricular weight (LVW/RVW) and ventricular weight to body weight (VW/BW) were determined. Thereafter, the left ventricle was frozen in liquid nitrogen for determination of protein content and myosin isoenzymes.

The amount of left ventricular protein was determined spectrophotometrically by Lowry's method. Bovine serum albumin (Sigma, USA) was used as standard reference.

Analysis of left ventricular myosin isoenzyme patterns The extraction of myosin isoenzyme was done according to that of Mercadier's, *et al*[7]. Left ventricular samples, weighing 100 mg, were homogenized with 5 vol of fresh modified Guba's solution at 4 ℃ and centrifuged by 30 000×g, at 0—4 ℃ for 20 min. The supernatant, mixed with equal volume of cold glycerol, was stored at −20 ℃. Polyacrylamide gel electrophoresis, consisting of acrylamide 3.8% (Fluka, Switzerland) and N, N'-methylene bis acrylamide 0.12% (Fluka, Switzerland), was performed on vertical gels in a Pharmacia apparatus (LKB-2197, Pharmacia Corporation, Sweden). The temperature of the electrophoresis buffer was controlled at 2—4 ℃ and the gel was run at a constant voltage of 11—14 V·cm^{-1} for 28 h. After fixation, staining and destaining, the gel was scanned in a TLC scanner (CS-930 TLC scanner, Shimadzu, Japan) with λex 450 nm and λem 550 nm. The scanning curve and the content percentage of V_1, V_2, and V_3 myosin isoenzymes were shown automatically.

Experiment protocol The sham-operated rats and RHR were randomly divided into 5 groups (n=6—7 each) according to their body weight and BP at 8 wk after the onset of hypertension. 1) Sham-operated group (sham): served as normal control. 2) losartan-treated group (LOS): Losartan (Du Pont Merck Pharmaceutical Company), 5 mg·kg^{-1} was given po to the RHR qd for 8 wk. 3) Captopril-treated group (CAP): captopril (from Squibb), 25 mg·kg^{-1} was administered po to RHR bid for 8 wk. 4) Hypertrophy 8 wk group (H8WK): served as pretreatment control. 5) Hypertrophy 16 wk group (H16WK): as non-treatment control.

Statistical analysis Results were expressed as $\bar{x}$ ±s, and tested by ANOVA. $P<0.05$ was considered statistically significant.

RESULTS

Changes of left ventricular mass and protein content After captopril treatment, the left ventricular protein content decreased significantly, the LVW and VW/BW ratio were normalized, concomitant with a significant decrease of BP. Similar to captopril, losartan induced nearly the same degree of decrease in protein content and left ventricular mass. Both losartan and captopril, 2 different types of RAS blockers, had similar efficacy in lowering the BP and reversing the cardiac hypertrophy in RHR, but the dose of captopril required was 10-fold higher than that of losartan (Tab 1, Fig 1).

Changes in myosin isoenzyme patterns A marked change in myosin isoenzyme patterns was observed in RHR. The content percentage of V_1 and V_3 in sham at 16 wk was 40.4± 5.5%, and 25.3±3.7%, respectively (Fig 2, Fig 3). In the H16WK group, a significant shift from V_1 to V_3 was observed as compared to sham. The V3 content percentage was significantly increased to 40.1±5.6% at 8 wk

Tab 1. Effect of captopril (50 mg·kg^{-1}·d^{-1} *po* for 8 wk) and losartan (5 mg·kg^{-1}·d^{-1} *po* for 8 wk) on blood pressure and cardiac mass. $n=6-7$, $\bar{x}\pm s$. $^{a}P>0.05$, $^{b}P<0.01$ *vs* hypertrophy 16 wk (H16WK); $^{c}P>0.05$, $^{d}P<0.01$ *vs* sham.

	BP/kPa	BW/g	LVW/mg	LVW/RVW	VW/BW, mg·g^{-1}
sham	15.4±1.1	333±40	707±77	2.9±0.3	2.87±0.26
LOS	15.2±2.1^{ad}	337±48^{ad}	671±116^{ad}	2.8±0.4^{ad}	2.71±0.26^{ad}
CAP	15.4±5.2^{ad}	306±46^{ad}	607±169^{ad}	2.8±0.3^{ad}	2.67±0.34^{ad}
H8WK	28.3±3.4	276±41	801±74	5.0±0.4	3.72±0.44
H16WK	28.5±4.9^{f}	290±56^{d}	1 029±132^{f}	4.7±0.5^{f}	4.25±0.52^{f}

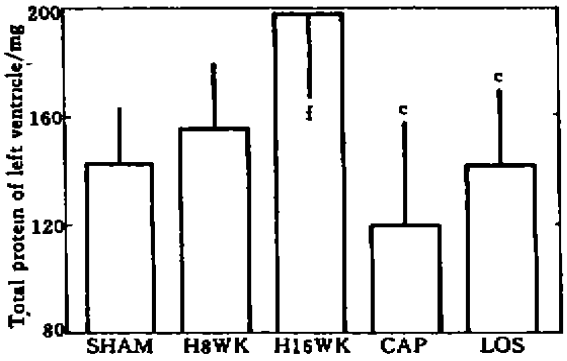

Fig 1. Effect of losartan (5 mg·kg^{-1}·d^{-1} *po* for 8 wk) and captopril (50 mg·kg^{-1}·d^{-1} *po* for 8 wk) on myocardial protein content in RHR. SHAM: sham-operated; H16WK: hypertrophy 16 wk; CAP: captopril-treated; LOS: losartan-treated. $n=6-7$, $\bar{x}\pm s$. $^{c}P<0.01$ *vs* H16wk, $^{f}P<0.01$ *vs* sham.

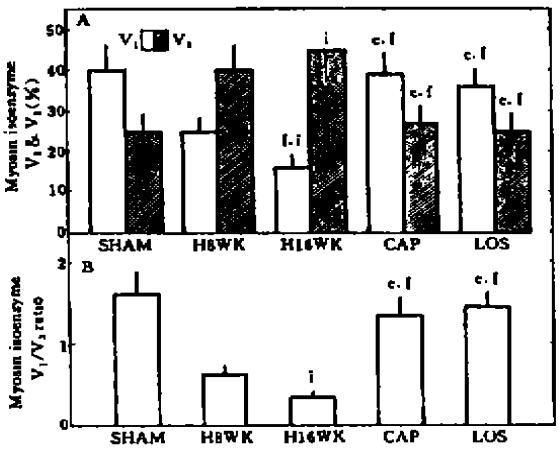

Fig 2. Changes in myosin isoenzyme patterns V_1 and V_3(A) and V_1/V_3 ratio (B) before and after losartan 5 mg·kg^{-1}·d^{-1} and captopril 50 mg·kg^{-1}·d^{-1} *po* for 8 wk treatment in RHR. SHAM: sham-operated; H8WK: hypertrophy 8 wk; H16 WK: hypertrophy 16 wk; CAP: captopril-treated; LOS: Losartan-treated. $n=6-7$, $\bar{x}\pm s$. $^{c}P<0.01$ *vs* H16WK; $^{f}P<0.01$ *vs* H8WK; $^{i}P<0.01$ *vs* sham.

and 45.0±8.0% at 16 wk. Concomitantly, the percentage of V_1 decreased dramatically to 24.9±2.9% at hypertrophy of 8 wk and 15.7 ±2.9% at hypertrophy of 16 wk. Losartan or captopril treatment induced a shift of isoenzyme pattern in opposite direction, that is, a shift of myosin V_3 toward V_1, thus resulting in a remarkable increase in the ratio of V_1/V_3 *vs* that of the H16WK and H8WK groups (Fig 2, Fig 3).

DISCUSSION

Our present study showed that losartan could reduce the total protein content of the hypertrophied rat hearts and induce almost complete regression of the increased heart weight, concomitant with a decrease of BP. A similar efficacy in lowering BP and reversing cardiac hypertrophy was also observed in captopril-treated RHR. It appeared that both vasopressor and myocardial growth response to Ang II in RHR might be mediated primarily by Ang II-1 receptor subtype since losartan, which was defined as a selective antagonist of Ang II-1 receptor, was as effective as an ACE inhibitor. These results were also supported

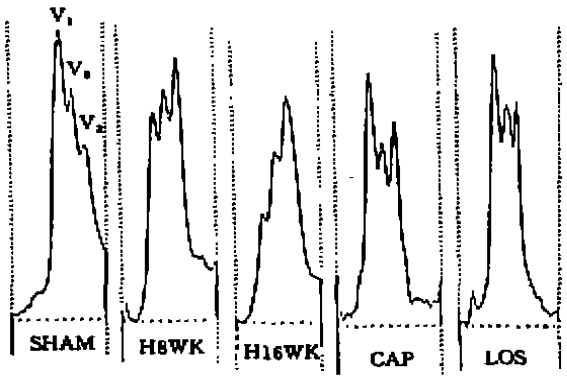

Fig 3. Scanning chromatography of myosin isoenzyme patterns V_1, V_2, and V_3 after polyacrylamide gel electrophorsis in rats. SHAM: sham-operated; H8WK: hypertrophy 8 wk; H16WK: hypertrophy 16 wk; CAP: captopril-treated; LOS: losartan-treated.

by Qing, *et al*[8].

In this study, we found that the dose of losartan, in lowering BP and decreasing myocardial mass, was only one-tenth of that of captopril. It was most likely that losartan was more potent than captopril in blocking the influence of the RAS in RHR[4]. The possible explanations were as follows 1) losartan blocked the effect of Ang II at its receptor sites, irrespective of the source of Ang II[3], and captopril can not completely block the formation of Ang II in the vascular wall and heart[9,10]. 2) losartan is a predrug; its active metabolite EXP 3174 was also a selective Ang II-1 receptor antagonist[11]. 3) Only Ang II-1 receptor existed in the blood vessels and about 50% Ang II-1 receptor were present in the heart[3,12], further suggesting that both vasopressor and myocardial growth response to Ang II in RHR might be mediated primarily by this subtype.

The changes in the relative amounts of 3 cardiac myosin heavy chain (MHC) isoenzymes were believed to be responsible for the altered cardiac performances in various pathophysiological conditions[5]. V_1 has the highest ATPase activity, while V_3 has the lowest ATPase activity. The population of the isoenzyme is decisive for ATPase activity, shortening velocity, and economy of tension generation[5]. The depressed contractility in hypertrophic myocardium is associated with decreased myosin ATPase activity[5,13], which was reflected by a decrease in the ratio of myosin V_1/V_3. In agreement with other studies[14], our present data showed that there was a significant shift of myosin isoenzyme V_1 to V_3 in RHR. It suggested that qualitative and quantitative changes occurred in the biosynthesis of myosin in cardiac hypertrophy. The results confirmed that both losartan and captopril were able to reverse this abnormal isoenzyme pattern of cardiac hypertrophy in RHR. But it was unknown whether the shift of V_3 toward V_1 by losartan and captopril was a consequence of the decrease of heart afterload, and/or directly resulted from blockade of AII in cardiac myocytes. Some reports showed that MHC gene regulation during hemodynamic overload may not be induced by intrinsic factors, such as hormones, catecholamine, but by direct local response to increased load[15].

Taken together, the present study showed that both losartan and captopril could effectively reverse the cardiac hypertrophy and normalize the distribution of myosin isoenzyme patterns in RHR. It is also reasonable to postulate that the cardiac hypertrophy in RHR may be primarily mediated by AII-1 receptor.

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Losartan 和卡托普利逆转肾血管性高血压大鼠心肌肥厚及心肌肌球蛋白 ATP 酶同功酶谱

凌 琦，郭兆贵，苏 志，郭 迅 R965.2
(湖南医科大学药理研究室，长沙410078，中国)

摘要 Losartan (5 mg·kg^{-1}·d^{-1} *po*)和卡托普利(50 mg·kg^{-1}·d^{-1} *po*)治疗肾性高血压大鼠 8 周均能在降压的同时降低左室重量34.8%～41.0%，减少左室总蛋白量28.3%～39.4%，使迁移的心肌凝蛋白 ATP 酶同功酶谱回复正常. 表明两药能有效逆转肾性高血压大鼠心肌肥厚及心肌凝蛋白同功酶谱，通过改变心肌凝蛋白 ATP 酶活性水平改善心肌收缩功能.

关键词 losartan；血管紧张素受体；卡托普利；肾血管性高血压；心肌肥厚；肌球蛋白腺苷三磷酸酶