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Captopril Fails to Reverse Hypertrophy of the Left Ventricle Induced by Aortic Insufficiency in Rabbits

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Summary

Angiotensin converting enzyme (ACE) inhibition has been reported to induce regression of hypertrophy in several models of hemodynamic pressure overload. The aim of the present study was to determine whether the ACE inhibitor captopril can reduce hypertrophy of the left ventricle induced by a chronic volume overload and modify collagen composition of the hypertrophied myocardium. Rabbits with four months lasting aortic insufficiency were divided into two groups: treated with captopril (20 mg/kg/day) for five weeks and treated with placebo. The respective control groups were represented by sham-operated animals. Aortic insufficiency induced a decrease of diastolic pressure, an increase of systolic and pulse pressure, hypertrophy of the left and right ventricle, and an increase of hydroxyproline content in the left ventricle without a change of hydroxyproline concentrations in either ventricle. Captopril treatment further enhanced pulse pressure by decreasing diastolic blood pressure. Hypertrophy of the left ventricle, hydroxyproline content and concentration in both ventricles were unaffected by captopril treatment. It is concluded that ACE inhibition did not reverse the left ventricular hypertrophy developed as a result of overload induced by aortic insufficiency. We suggest that mechanisms different from activation of the renin-angiotensin system may play a decisive role in the maintenance of hypertrophy in this particular model of volume hemodynamic overload.

Key words

Aortic insufficiency • Hypertrophy regression • Fibrosis • Cardiac hypertrophy

Introduction

There is a host of evidence that ACE inhibition is able to modify the process of adaptive myocardial growth (Šimko 1994, 1996, Pelouch *et al.* 1997, Šimko and Šimko 1999, 2000, Šimko *et al.* 2000a). ACE-inhibitors and calcium channel blockers are probably the most effective drugs in achieving regression of hypertrophy (Schlaich and Schmieder 1998). The heart regressed by ACE inhibition seems to be of more physiological nature than the hypertrophied myocardium due to increased capillary density (Unger *et al.* 1992), improved diastolic function and restoration of energy production (Gohlke *et al.*1994).

It was reported that the response of the renin-angiotensin system (RAS) could differ during the

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development of hypertrophy and in the maintenance of already developed hypertrophy. For example, an ACE inhibitor, quinalapril, was not able to prevent the development of LV hypertrophy, however, it was successful in achieving regression of cardiac hypertrophy when the administration started 6 weeks after banding of the thoracic aorta (Kromer and Rieger 1988). In the aortic banding model of renal hypertension, the ACE inhibitor ramipril was shown to prevent the development or induce regression of developed left ventricular hypertrophy in both antihypertensive and non-antihypertensive doses (Linz *et al.* 1995). In the same model, however, losartan, the angiotensin II type I receptor antagonist, had merely a slight effect on prevention but a strong effect on regression of concentric hypertrophy of the left ventricle. The authors suggested that Ang II may be more important in the maintenance of left ventricular hypertrophy than in its development (Linz *et al.* 1995). These and many other experiments suggested that the effect of ACE inhibition may vary with respect to the period of hypertrophic growth in a particular model of hemodynamic overload.

In the light of the above facts, it appears to be necessary to investigate the effect of ACE inhibition both in prevention and regression studies in order to conceive the importance of RAS in the hypertrophic process of the myocardium. In our previous work with aortic insufficiency in rabbits (Šimko *et al.*1997), we showed that four weeks of ACE inhibition with captopril did not prevent left ventricular hypertrophy development. The aim of the present work was to show whether captopril is able to induce regression of developed left ventricular hypertrophy and collagen remodeling.

Methods

Animals and experimental groups

All rabbits (55) were males of the Chinchilla strain, with average body weight of about 3000 g and fed a standard pellet mixture. The number of animals are given in Table 1.

Six groups of rabbits were investigated:

1) Control (C) - age matched animals were followed after a sham operation for four months without any treatment.

2) Aortic insufficiency (I) - after induction of a volume overload, the animals were followed up for four months without any treatment. The next four groups were followed up for additional five weeks:

3) Control + placebo (Cp) - four months after the sham operation, placebo was given twice daily 0.5 ml intramuscularly.

4) Control + captopril (Cc) - four months after the sham operation, captopril 10 mg/kg b.w. intramuscularly in 0.5 ml distilled water was given twice daily (total dose: 20 mg/kg b.w./day).

5) Aortic insufficiency + placebo (Ip) - four months after surgery, distilled water was given twice daily 0.5 ml intramuscularly.

6) Aortic insufficiency + captopril (Ic) - four months after surgery, captopril 10 mg/kg b.w. intramuscularly in 0.5 ml distilled water was given twice daily (total dose - 20 mg/kg b.w./day).

Aortic insufficiency was induced by perforation of the aortic valve *via* the right carotid artery by a hollow metal perforator. This technique was described in detail elsewhere (Fízeľ and Fízeľová 1971).

Hemodynamic measurements

Aortic pressures were measured by a tip catheter manometer (Statham DB P23 GB) introduced into the aorta through the left carotid artery and recorded on an oscillographic recorder Mark VII, type WR 3101, Graphtec Corp., USA. The measurements were performed under thiopental anesthesia (35 mg/kg.b.w.).

Heart weight

Immediately after the animals had been sacrificed, the heart was immersed into a cold (4 °C) Krebs-Henseleit solution, the left ventricle, septum, right ventricle and atria of the heart were detached and the weight of the free left ventricle (without septum) and of the right ventricle was quickly determined. The weight ratios of free left ventricle and right ventricle to the body weight of the respective animal were calculated.

Hydroxyproline measurements

Transmural samples of myocardial tissue were taken from the free left and right ventricle of control and experimental animals. The dry mass was digested in 6 M HCl for 16 h at 105 °C. 4-hydroxyproline (HYP) concentration (taken as an index of cardiac collagen) was expressed as mg/g dry weight, collagen content was expressed as mg HYP/ventricle (Pelouch *et al.* 1997).

Statistical analysis

The results are expressed as means ± S.E.M.. Differences between groups were evaluated by ANOVA and Duncan's multiple range test. Differences were considered statistically significant at the level of $p < 0.05$.

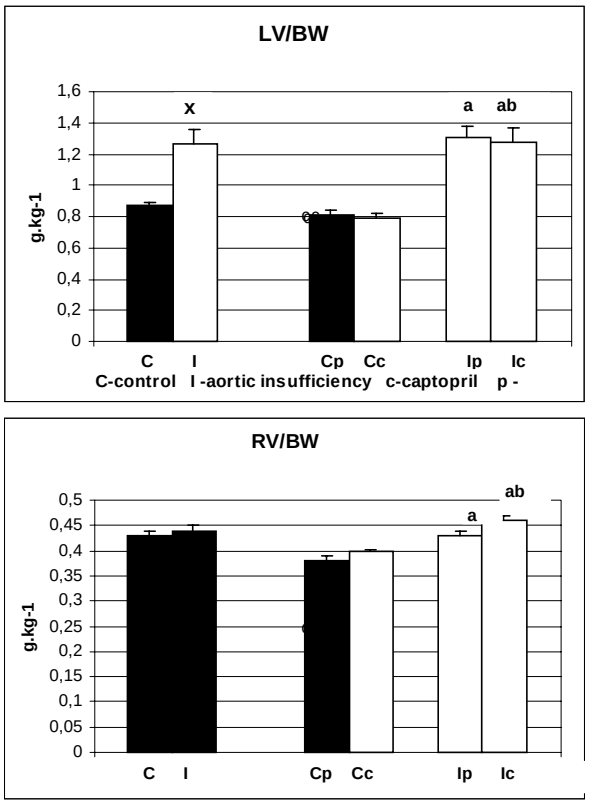

Fig. 1. *Relative weight of the left and right ventricle; number of animals: control (C) = 9, aortic insufficiency (I) = 9, control+placebo (Cp) = 11, control+captopril (Cc) =11, aortic insufficiency+placebo (Ip) = 8, aortic insufficciency+captopril (Ic) = 7; a - different from Cp (p≤ O.O5), ab - different from Cc (p≤ 0.05), x - different from previous group (p≤ 0.05).*

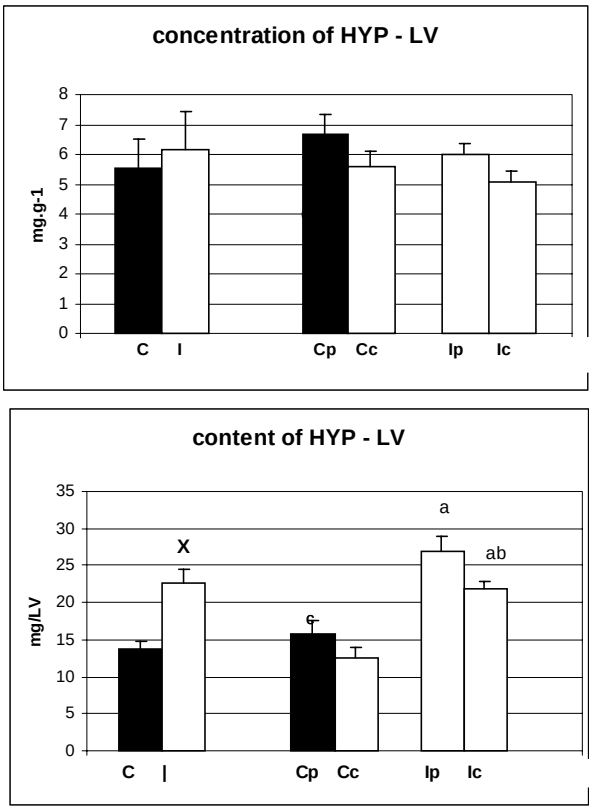

Fig. 2. *Concentration and content of hydroxyproline in the left ventricle (n=6 in each group); a - different from Cp (p ≤ 0.05), ab - different from Cc (p ≤ 0.05), x - different from previous group (p ≤ 0.05).*

Results

The relative weights of the LV and of RV were increased in aortic insufficiency + placebo (Ip) *vs.* control + placebo group (Cp) ($p<0.05$) and captopril did not significantly influence the extent of hypertrophy (Fig.1).

Systolic pressure in the aorta was higher in Ip *vs.* Cp ($p<0.05$) and captopril decreased it only in the controls but not in aortic insufficiency. The diastolic pressure was lower in Ip *vs*. Cp and captopril decreased diastolic pressure both in the controls ($p<0.05$) and in aortic insufficiency ($p<0.05$). Pulse pressure was enhanced in Ip *vs.* Cp ($p<0.05$) and captopril further increased it (Ic *vs.* Ip, $p<0.05$) (Table1).

Hydroxyproline content in the LV was enhanced in Ip *vs.* Cp ($p<0.05$) and captopril did not change it (Fig. 2).

Hydroxyproline concentration in the LV (Fig. 2), and hydroxyproline content and concentration in the RV (Fig. 3) were not changed either by hemodynamic overload or by the captopril treatment.

Discussion

Fízeľ and Fízeľová (1971) differentiated four periods of left ventricular hypertrophic growth on the experimental model of aortic insufficiency in rabbits:
1. developing hypertrophy (one month after induction of aortic insufficiency),
2. developed hypertrophy (4th-6th month of overload),
3. spontaneous regression of hypertrophy (7th-10th month), and
4. the period of heart failure (10th-14th month).

Table 1. Body weight, heart weight and aortic pressures.

	Control n=9	Aort. insuf. n=9	Control+placebo n=11	Control+captopril n=11	Aort. insuf.+placebo n=8	Aort. insuf.+captopril n=7
Body weight (g)	3287+84	3330+114	3086+89	2899+49	3558+164 ^{o}	3281+120$^{\#}$
Left ventricle weight (g)	2.88+0.184	4.204+0.485 *	2.412+0.103	2.271+0.073	4.472+0.208^{o}	4.164+0.232$^{\#}$
Right ventricle weight (g)	1.431+0.102	1.454+0.105	1.172+0.064	1.144+0.027	1.476+0.064^{o}	1.459+0.091$^{\#}$
Heart weight/body weight (g/kg)	2.11+0.04	2.64+0.08*	1.90+0.03	1.88+0.02	2.81+0.05^{o}	2.68+0.06$^{\#}$
Systolic pressure (mmHg)	118.9+1.7	120.9+1.4	111.2+1.1	100.7 +1.0*	123.3+2.3^{o}	118.3+2.2$^{\#}$
Diastolic pressure (mmHg)	95.6+1.3	90.0+0.9	89.2+1.0	77.6+1.0 *	82.5+2.2^{o}	71.2+1.0 $^{\#}$ *
Pulse pressure (mmHg)	22.7+0.7	31.0+0.9 *	22.0+0.4	22.8+0.6	40.7+0.9^{o}	47.2+1.7$^{\#}$ *

*Values are means ± S.E.M., ^{o}different from control+placebo group ($p \leq 0.05$), $^{\#}$different from control+captopril group ($p \leq 0.05$), * different from previous group ($p \leq 0.05$)*

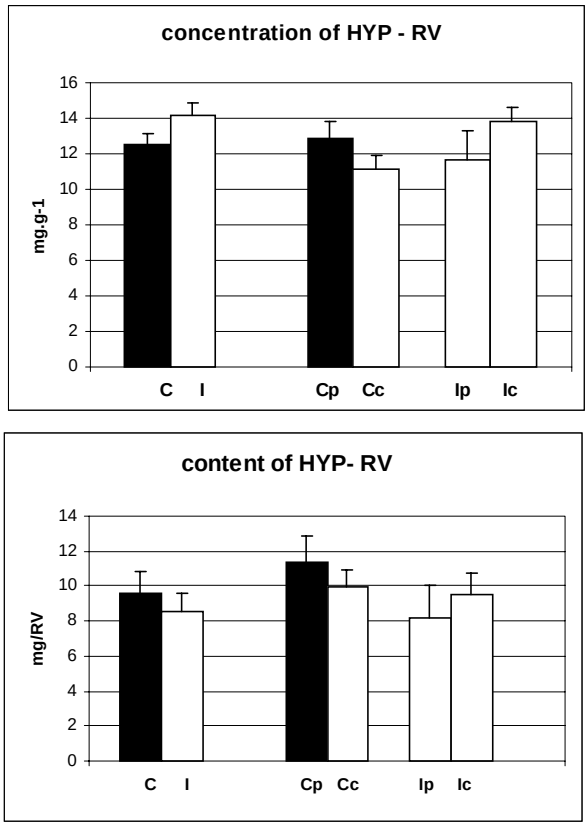

Fig. 3. *Concentration and content of hydroxyproline in the right ventricle (n=6 in each group).*

During developed hypertrophy, the period also investigated in this work, the weight and relative weight of the left ventricle more than doubled, the concentration of left ventricular contractile proteins increased, but the content of fibrotic tissue was near the control values (Fízeľ *et al.* 1984). Similarly, in several other models of volume hemodynamic overload, such as chronic anemia (Bartošová *et al.* 1969), atrial septal defect (Marino *et al.* 1985), arteriovenos fistula (Weber *et al.* 1990a) or hyperthyroidism (Holubarsch *et al.* 1983), hypertrophy of the LV was not associated with fibrotic tissue enhancement.

The lack of fibrosis development and the failure of captopril to reverse hypertrophy of the LV in our experiment are in contrast with the findings in various types of pressure hemodynamic overload. In SHR (Linz *et al.* 1995, Brilla *et al.* 1996), renovascular hypertension (Weber *et al.* 1990b), and NO deficient hypertension (Pecháňová *et al.* 1997), left ventricular hypertrophy was accompanied by increased collagen concentration and ACE inhibitors induced regression of the hypertrophic mass and/or attenuation of fibrosis (Linz *et al.* 1995, Brilla *et al.* 1996, Weber *et al*. 1990, Bernátová *et al.* 2000). Information concerning the volume-overloaded heart is rather scarce. Enalapril did not prevent hypertrophy of the LV or RV when volume overload was

provoked by aortocaval shunt or by chronic administration of minoxidil. On the other hand, when LVH had already developed, enalapril was able to reverse it (Ruzicka *et al.* 1994). In our previous work, captopril did not prevent hypertrophic growth of the left ventricle during four weeks of aortic insufficiency (Šimko *et al.* 1997) and regression of developed excentric hypertrophy was not achieved in the same model of volume overload in this experiment. The failure of captopril to alter hypertrophic growth in aortic insufficiency may plausibly be accounted for as follows:

– First, in most models of pressure-overloaded myocardium, hypertrophic growth of the LV is related to myocardial fibrosis development. Extra cellular matrix protein growth seems to be more dependent on activation of the systemic and/or tissue RAS than on hemodynamic changes (Weber *et al.* 1993, Weber *et al.* 1995). Since no LV fibrosis was observed in the hypertrophied LV in this experiment, RAS is not supposed to play a significant role in the hypertrophic process in this model of volume hemodynamic overload. Thus, inhibition of ACE is not to be expected to provide any substantial effect resulting in reversion of hypertrophy.

– Second, specific hemodynamic alterations in aortic insufficiency may also play a role: they are characterized by a systolic blood pressure increase and a diastolic pressure decrease, resulting in enhancement of pulse pressure (Fízeľ *et al.* 1984, Šimko *et al.* 1997). It is now widely accepted that pulse pressure is an independent cardiovascular risk factor (Darne *et al.* 1989). Pulse pressure is considered to be the major mechanical factor contributing to the onset of cardiac hypertrophy and a more reliable predictor of hypertrophy development than the value of systolic or diastolic pressure itself (Pannier *et al.* 1989). Indeed, we previously showed on this particular model of LVH that the weight of the LV correlated well with the value of pulse pressure (Šimko *et al.* 1997). On the other hand, the selective reduction of pulse pressure was associated with structural improvement of cerebral and mesenteric arteries in animal experiments (Christensen 1991) and seems to be one of the new important goals of antihypertensive therapy. In this experiment, captopril decreased diastolic blood pressure but the pulse pressure was even increased. Thus, the unloading effect of decreased diastolic pressure could have been counterbalanced by enhancement of blood pressure amplitude, and this might have contributed to the maintenance of LVH.

We conclude that the hemodynamic overload in aortic insufficiency induced hypertrophy of the left ventricle and increased the hydroxyproline content in left ventricule. However, hydroxyproline concentration was not elevated. Captopril was neither able to induce hypertrophy regression of the left ventricle nor to change the collagen amount in either ventricle. We conclude therefore that mechanisms other than activation of renin-angiotensin system are decisive in the maintenance of hypertrophy in this particular model of volume hemodynamic overload.

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Reprint requests

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