

Effect of Captopril on the Development of Left Ventricular Hypertrophy in Rabbits With Aortic Insufficiency

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Summary

We investigated the effect of captopril on the growth of the left ventricle in an experimental model of aortic insufficiency. Four groups of rabbits were studied 28 days after experimental intervention: 1. control, 2. control with captopril (10 mg/kg/day), 3. aortic insufficiency, 4. aortic insufficiency with captopril (10 mg/kg/day). Aortic insufficiency induced hypertrophic growth of the left ventricle demonstrated by increased weight and ribonucleic acid (RNA) concentration. Administration of captopril only slightly attenuated the weight increase of the left ventricle and the increase in concentration of left ventricular RNA. However, captopril reduced the concentration of left ventricular deoxyribonucleic acid (DNA) both in the control and even more in the group with aortic insufficiency. The chronic haemodynamic overload enhanced mitochondrial respiration in the left ventricle which was not influenced by captopril. We conclude that captopril in the dose 10 mg/kg/day did not prevent hypertrophy of the left ventricle but reduced left ventricular DNA concentration.

Key words

Aortic insufficiency – Captopril – Cardiac hypertrophy – Nucleic acids

Introduction

Myocardial hypertrophy of the left ventricle (LV), although an adaptive-compensatory mechanism, involves the risk of increased cardiovascular morbidity and mortality. It is generally believed that prevention of hypertrophic growth or regression of hypertrophy diminish the increased cardiovascular risk brought about by left ventricular hypertrophy (LVH). Thus the prevention or regression of LVH are considered to be of primary therapeutic importance (Šimko 1994).

In several types of haemodynamic overload, the circulating renin-angiotensin system (RAS) and also the local-tissue RAS were demonstrated to play an important role in the process of heart remodelling (for review see Pelouch *et al.* 1994, Weber *et al.* 1995, Šimko 1996). It was shown that chronic administration of the angiotensin converting enzyme (ACE) inhibitor,

fosinopril, induced regression of left ventricular hypertrophy (LVH) and improved cardiac function and survival of rats with stenosis of the ascending aorta, (Weinberg *et al.* 1994). Both the ACE inhibitor, enalapril, and the angiotensin II receptor antagonist, losartan, reversed LVH provoked by an aorto-caval shunt or minoxidil (Ruzicka *et al.* 1993). Antihypertensive doses of captopril (Petkov *et al.* 1988) or ramipril (Rhaleb *et al.* 1994) were found to prevent the development of LVH in rats with aortic coarctation. Captopril completely prevented LVH in the model of NO-deficient hypertension in rats (Bernátová *et al.* 1996). In hypertensive patients, regression of LVH was achieved by treatment with different ACE inhibitors (Lièvre *et al.* 1995, Kohno *et al.* 1995).

Despite this boom of information on the effects of ACE inhibitors in the heart with a

haemodynamic overload there are only few data concerning the possible effect of ACE inhibition on hypertrophic growth in the volume overload caused by aortic insufficiency (Bonow 1994). Thus the aim of this study was to assess whether four-week captopril administration is able to prevent hypertrophy development of the left ventricle in rabbits with aortic insufficiency.

Material and Methods

Animals and experimental groups

The experiments were performed on male Chinchilla rabbits, with an average body weight of about 3000 g (Table 1) which were fed a standard pellet mixture. The animals were examined 28 days after experimental intervention.

Four groups of rabbits were examined: 1. control (C) (n=11) – sham-operated animals + saline (the sham operation was performed by insertion of a catheter into the right carotid artery without aortic valve perforation, 0.5 ml saline was given twice daily intramuscularly) 2. control + captopril (Cc) (n=12) – 5 mg/kg body weight (b.w.) in 0.5 ml saline) captopril was administered i.m. to sham-operated animals twice a day; 3. aortic insufficiency (A) (n=14) (0.5 ml saline was given twice daily intramuscularly); 4. aortic insufficiency + captopril (Ac) (n=16) – captopril was injected twice daily in a single dose of 5 mg/kg b.w. in 0.5 ml saline). Saline or captopril administration started 24 h after the operation.

Aortic insufficiency was induced by perforation of the aortic valve *via* the right carotid artery with a hollow metal perforator employing the technique described in details elsewhere (Fízel and Fízel'ova 1969, 1971, Šimko 1995). Fízel and Fízel'ova (1969, 1971) divided the process of adaptation in this model into four periods: developing hypertrophy, developed hypertrophy, spontaneous regression of hypertrophy, and heart failure. In the present experiment we studied the period of developing hypertrophy.

Haemodynamic measurements

Aortic pressures were measured by a catheter with an electric transducer (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 anaesthesia (35 mg/kg. b.w.).

Heart weight

Immediately after the animals had been sacrificed, the heart was submerged into a cold (4 °C) solution (0.225 mol.l⁻¹ manitol, 0.075 mol.l⁻¹ sucrose, 0.2 mmol.l⁻¹ EDTA, pH 7.4), various anatomical parts

of the heart were detached and rapidly weighed. The ratios of the weight of individual parts to the body weight were calculated in each animal.

Nucleic acid levels

The RNA concentration was determined using the single step method of RNA isolation by acid guanidinium thiocyanate-phenol-chloroform extraction (Chomczynski and Sacchi 1987). The DNA concentration was estimated using Davidson method (1976).

Respiration and phosphorylation of left ventricular mitochondrial fraction

The sample from the basal part of the LV was cut into small slices and 1–2 g of tissue was used for mitochondria isolation according to Palmer *et al.* (1977). The concentration of mitochondrial protein was estimated according to Lowry *et al.* (1951).

The mitochondrial oxygen consumption and phosphorylation activity were determined polarographically by means of Clark's oxygen electrode according to Rouslin and Millard (1981) in a reaction medium containing glutamate/maleate as substrate. The ADP concentration was determined by means of a spectrophotometer at a wavelength of 259 nm using the molar extinction coefficient 15.4 x 10³. The oxygram was evaluated using the following parameters: a) State 3 – oxygen consumption after addition of ADP; b) State 4 – oxygen consumption without ADP; c) RCI – respiratory control index (relative number) – state 3/state 4; d) OPR (oxidative phosphorylation rate) – nmol ATP produced during state 3 x mg protein⁻¹ x min⁻¹.

Specific activity of mitochondrial cytochrome c oxidase was determined according to Muscatello and Carafolli (1969).

Statistical analysis

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.

Results

Aortic pressures

Systolic and diastolic pressures in the aorta were not changed either in aortic insufficiency (A) or aortic insufficiency combined with captopril (Ac) vs. controls. Pulse pressure was increased in A vs. C (by 88 %, p<0.05) and captopril did not affect this change (Table 1).

Table 1. Body weight, heart weight and aortic pressures

	Control	Control + captopril	Aortic insufficiency	Aortic insufficiency + captopril
Body weight before (g)	2981±96.1	3178±183	3121±61.8	3246±72.7
Body weight after (g)	3073±76.8	3181±112	3207±63.06	3234±54.5
Heart weight (g/kg b.w.)	2.158±0.093	1.965±0.069	2.913±0.122*	2.680±0.128*
Systolic aortic pressure (mm Hg)	123.8±5.95	127.5±5.18	136.3±10.5	125.6±5.17
Diastolic aortic pressure (mm Hg)	102.5±5.09	105.0±5.38	96.3±11.5	89.4±5.87
Pulse aortic pressure (mm Hg)	21.3±1.6	22.5±1.8	40.0±3.3*	36.1±2.0*

Values are means ± S.E.M., * different from control group ($p < 0.05$)

Weight of various heart parts related to body weight

The relative weight of the LV was increased in A by 35% as compared with the controls (from 1.406 ± 0.223 to 1.896 ± 0.261 g/kg b.w., $p < 0.05$), the weight of the right ventricle (RV) increased by 28% (from 0.424 ± 0.068 to 0.541 ± 0.108 g/kg b.w., $p < 0.05$),

and the weight of the whole heart increased by 35% ($p < 0.05$). Captopril did not prevent the growth of either the LV, RV or of the whole heart (Fig. 1, Table 1). The body weight of rabbits did not change in any group in comparison with the controls (C) (Table 1).

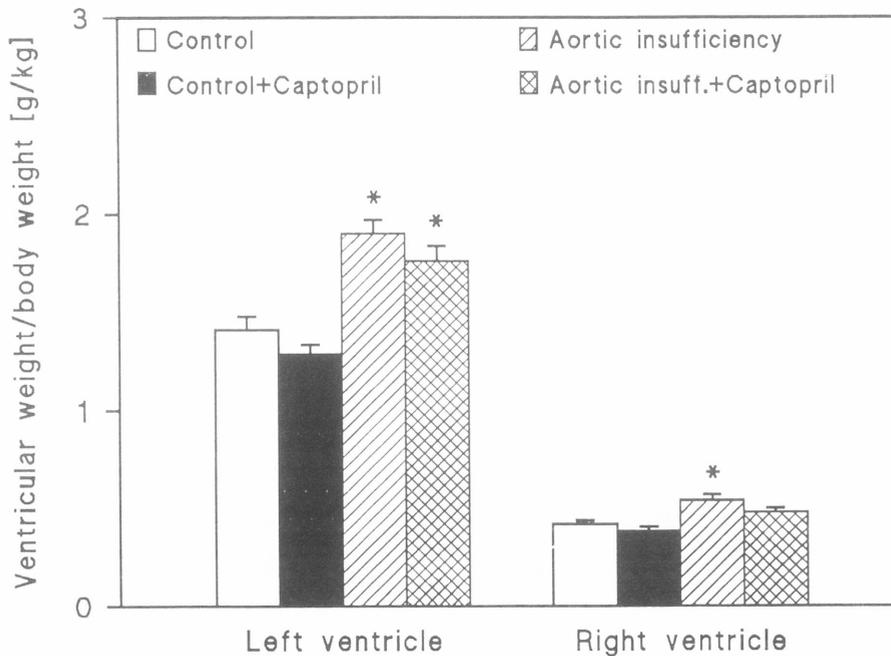


Fig. 1. Left and right ventricular weights (mg/kg b.w.). Values are mean ± S.E.M, b.w. – body weight, * values different from control group, $p < 0.05$, $n_C = 11$, $n_{Cc} = 12$, $n_A = 14$, $n_{Ac} = 16$.

Nucleic acids

RNA concentration in the LV was increased in A vs. C (by 68%, $p < 0.05$). Captopril did not diminish RNA concentration in the hypertrophied LV (Fig. 2).

Left ventricular DNA concentration was not changed by aortic insufficiency. However, captopril decreased DNA concentration of the LV in both Cc vs C (by 19%, $p < 0.05$) and Ac vs C (by 30%, $p < 0.05$) (Fig. 2).

Mitochondrial bioenergetic parameters

OPR and state 3 were increased in Cc vs. C ($p < 0.05$). Aortic insufficiency stimulated OPR

($p < 0.05$), state 3 ($p < 0.05$), cytochrome c oxidase activity ($p < 0.05$) and captopril did not influence these changes (Table 2).

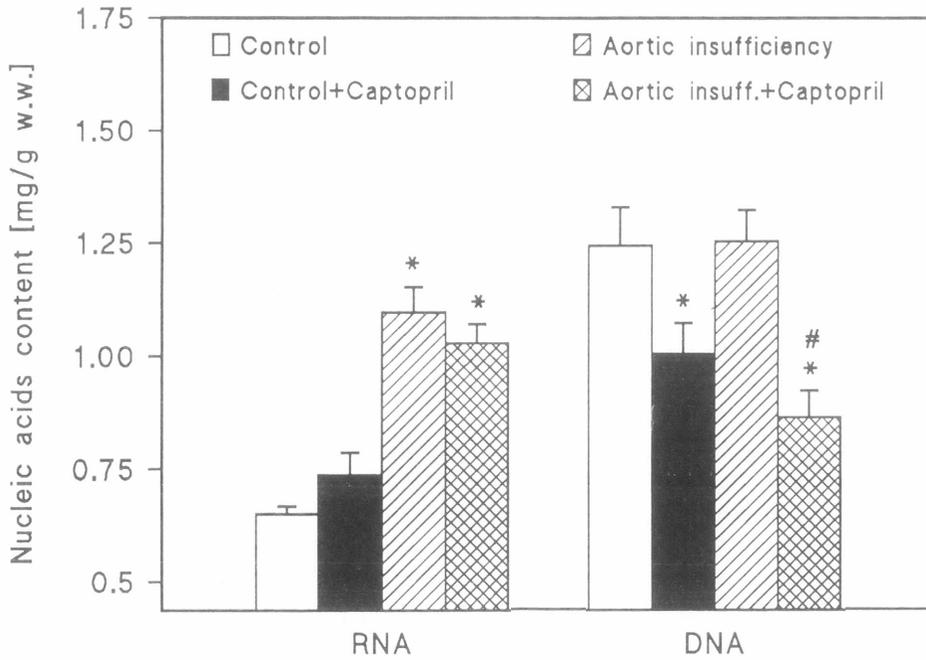


Fig. 2. Left ventricular RNA and DNA concentration (mg/g w.w.). Values are mean \pm S.E.M, w.w. – wet weight, * values different from control group (C), $p < 0.05$, # values different from untreated group (A), $p < 0.05$, $n = 7$ in each experimental group.

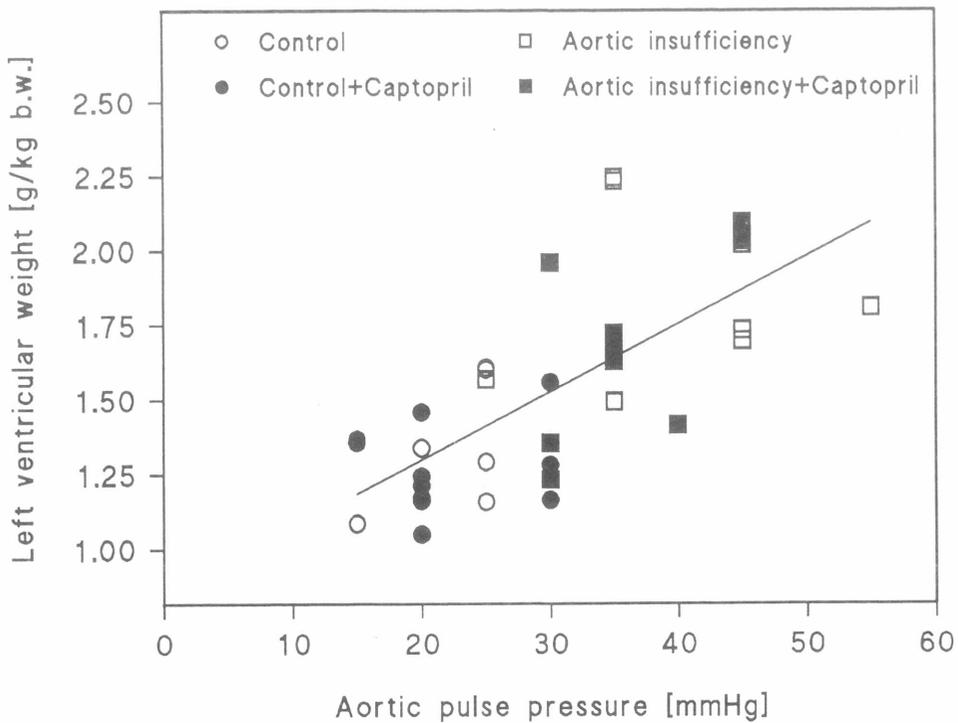


Fig. 3. Correlation of the left ventricular weight and pulse aortic pressure. $y = 0.0228x + 0.843$, $r = 0.696$, $p < 0.05$, $n_C = 8$, $n_{Cc} = 10$, $n_A = 8$, $n_{Ac} = 9$.

Table 2. Bioenergetic parameters of the mitochondrial fraction of the left ventricle

	Control	Control + captopril	Aortic insufficiency	Aortic insufficiency + captopril
State 3 (ngatom O/ min.mg)	172±13.7	229±10.6*	247±12*	255±13*
State 4 (ngatom O/ min.mg)	43.1±4.46	45.4±2.9	51.9±1.3	53.6±2.5
Respiratory control index (State 3/State 4)	4.60±0.20	5.03±0.35	5.13±0.21	5.07±0.30
OPR (nmol ATP/min.mg)	409±30	566±30*	624±25*	581±27.9*
Cytochrome oxidase (ngatom O/ min.mg)	1496±630	1643±32.5	1863±80.7*	1801±78.0*

Values are means ± S.E.M., * different from control group ($p < 0.05$)

Discussion

The present study has demonstrated that a volume overload by aortic insufficiency resulted in the development of left ventricular hypertrophy and enhanced RNA concentrations. Administration of ACE-inhibitor captopril (10 mg/kg/day) from the beginning of the overload did not prevent the development of hypertrophy and the enhancement of RNA concentrations, but, it decreased DNA concentration.

Compared with the information available on the prevention of pressure induced hypertrophy, literary data on the prevention of hypertrophy induced by volume overload are rather scarce. Our results partly confirm some results but contradict others. In agreement with our results, Ruzicka *et al.* (1993) found that enalapril did not prevent hypertrophy of either the left or right ventricle induced by a volume overload caused by minoxidil or an aorto-caval shunt. On the other hand, in the rat model of aortic insufficiency (Gay 1990), two-month captopril treatment mitigated the development of hypertrophy of both left and right ventricles and improved left ventricular function. The fact that captopril only insignificantly attenuated hypertrophy development in our experiment may be ascribed to the following factors: First, the dose of captopril used in the rat model (Gay 1990) was ten times higher than in our experiment. Second, species differences may play a role. The third and probably the most important factor may be the model of aortic insufficiency and the duration of the adaptive process investigated in the individual experiments. The RAS was found to play a different role not only in various types of haemodynamic overload but also at different

periods of the same model (Ruzicka *et al.* 1993, 1994). No data are available on the natural history of the above mentioned rat aortic insufficiency model, yet. In any case, different time periods were investigated (two months after aortic valve perforation in the rat model in comparison to four weeks in our rabbit model).

The most interesting result of the present study is that captopril decreased left ventricular DNA concentration. As we did not perform a protein profile analysis of the hypertrophied myocardium, the processes underlying changes of DNA concentration can only be assumed. Angiotensin II is a potent growth promoter and ACE inhibition is capable of preventing its proliferative effect (Pelouch *et al.* 1994). As cardiomyocytes are not supposed to divide in the myocardium of adult individuals, the reduced DNA content might be due to proliferation of other non-myocyte cells. Fibrocytes represent the majority of myocardial cells. Several authors have emphasized that the RAS plays a substantial role in the growth and turnover of connective tissue of the heart (Pelouch and Jirmář 1993, Weber *et al.* 1995). Moreover, it has been shown that fibrosis of the hypertrophied left or right ventricle can be reduced by chronic administration of ACE inhibitors (Kolář *et al.* 1993, Pelouch *et al.* 1993, 1994). It thus seems reasonable to suppose that the reduction of DNA concentration in the left ventricle observed in our experiment may be accounted for primarily by fibrotic cell and interstitial tissue reduction. It is difficult to decide whether it was the interaction of captopril with circulating or with local ACE (Šimko and Šimko 1996) that determined the reduction of DNA concentration. As the pulse pressure, which was revealed to correlate closely with left ventricular weight in all the investigated groups

(Fig. 3), was not influenced by captopril, the inhibition of local rather than of systemic RAS might be responsible for the changes revealed in the composition of the LV.

To establish whether captopril can influence the energy metabolism of the hypertrophied heart, we investigated bioenergetic parameters in the mitochondrial fraction of the LV. The mechanism of captopril-induced stimulation of mitochondrial respiration in the control group is not clear. It might have some relation to the presence of SH groups in the captopril molecule, which is suggested to have antioxidant properties and is supposed to participate in the positive energetic captopril effect observed under ischaemic conditions (Juggi *et al.* 1993) and in chronic heart failure (Schultheiss *et al.* 1990). Aortic insufficiency, in agreement with our previous experiments on this model (Fízel *et al.* 1986), stimulated the activity of mitochondria. This stimulation suggests that the haemodynamic overload increased the demands on energy-producing

mechanisms of the LV. The lack of effect of captopril on mitochondrial activity of the hypertrophied LV may be associated with the failure of captopril to affect the extent of LVH.

In conclusion, captopril in the dose 10 mg/kg/day did not prevent the development of the heart hypertrophy during the first four weeks of aortic insufficiency. However, captopril significantly reduced DNA concentration of the LV. Whether this alteration of the DNA concentration, with a potential decrease of left ventricular fibrosis, might improve left ventricular function and even postpone heart failure in our particular model of aortic insufficiency remains to be elucidated.

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