Original Article

Effects of the Prolonged Inhibition of the Angiotensin-Converting Enzyme on the Morphological and Functional Characteristics of Left Ventricular Hypertrophy in Rats with Persistent Pressure Overload

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Objective

To assess the effects of lisinopril (L) on mortality (M) rate and congestive heart failure (CHF), and the characteristics of geometrical myocardial remodeling and left ventricular function in rats with supravalvular aortic stenosis (SAS).

Methods

Some Wistar rats underwent SAS or the simulated surgery (CG, n=10). After 6 weeks, the animals were randomized to receive lisinopril (LG, n=30) or no treatment (SG, n=73) for 15 weeks. Cardiac remodeling was assessed in the sixth and 21st weeks after the surgical procedures through concomitant echocardiographic, hemodynamic, and morphological studies.

Results

The M were 53.9% and 16.7% in SG and LG, respectively; the incidence of CHF was 44.8% and 20%, in SG and LG, respectively, (P<0.05). At the end of the experiment, the values of LV systolic pressure in SG and LG were equivalent and significantly greater than those in CG;(P<0.05) and did not differ from those observed 6 weeks after the surgical procedures. The values of LV diastolic pressure in SG were greater than those in LG;(P<0.05), and both were greater than those in CG;(P<0.05). The same behavior was observed with the following variables: E/A ratio; mass index; sectional area of the myocytes; and LV hydroxyproline content. Left ventricular shortening percentage was similar in CG and LG;(P>0.05), and both were greater than those in SG;(P<0.05). Similar results were obtained with the values of the positive and negative first derivate of LV pressure.

Conclusion

In rats with SAS, the treatment with L reduced M rate and ICC and had beneficial effects on geometrical myocardial remodeling and left ventricular function.

Key words

cardiac hypertrophy, arterial hypertension, angiotensin-converting enzyme inhibitors, lisinopril, ventricular function

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Left ventricular hypertrophy (LVH) is an adaptive response of the heart to sustained work overload. Data in the literature [1] have shown that the load conditions and neurohumoral systems, among which the renin-angiotensin-aldosterone system (RAAS) stands out, modulate the characteristics of left ventricular hypertrophy that develop in the presence of chronic pressure overload. Clinical studies [2,3] have shown that angiotensin-converting enzyme inhibitors (ACEI) improve the survival of patients with heart failure, delay heart decompensation in patients with asymptomatic left ventricular dysfunction, and attenuate the progression of left ventricular dilation and dysfunction after myocardial infarction. Experimental studies [4-6] have shown that ACEIs attenuate ventricular remodeling, improve survival, and delay the progression of left ventricular dysfunction in rats with myocardial infarction and hamsters with cardiomyopathy. Angiotensin-converting enzyme inhibitors have also been proved to decrease left ventricular hypertrophy in human beings [7] and in experimental animals with chronic pressure overload [8,9].

The favorable effects of ACEI on survival and regression of left ventricular hypertrophy in heart failure and arterial hypertension are frequently attributed to interference with the RAAS, resulting in a reduction in blood pressure and peripheral vascular resistance with a consequent reduction in cardiac overload [10]. However, several studies [10-12] have reported that the angiotensin-converting enzyme (ACE) and the myocardial tissue RAAS are activated in animal models of heart failure and cardiac hypertrophy. Although the efficacy of the ACEI in prolonging survival, delaying the development of ventricular dysfunction, and reversing cardiac hypertrophy is widely recognized, it has been difficult to dissociate the hemodynamic effects of those agents on systemic blood pressure from the effects of the myocardial tissue ACE blockade. Therefore, the use of ACEI in arterial hypertension, acute myocardial infarction, and cardiomyopathy is clearly justified, because those drugs block RAAS activity, reduce cardiac load, and reverse left ventricular hypertrophy. The validity of that strategy is controversial when the increase in cardiac load is relatively fixed, as in the case of aortic valvular stenosis. In that circumstance, the reduction in left ventricular hypertrophy may be seen as a loss of the adaptive mechanism that can result in maladjustment of the afterload, and dilation and impairment of left ventricular function. Alternati-

vely, because left ventricular hypertrophy is a predictive factor of cardiovascular morbidity and mortality independent of other conditions [13], the limitation of the left ventricular hypertrophic response may be associated with beneficial effects not related to the reduction in hemodynamic load. Controversy still exists in the literature [8,9,12] about whether in the long run the influence of the ACEI on left ventricular hypertrophy in the presence of persistent pressure overload (PPO), such as aortic stenosis, results in beneficial or adverse effects on survival, incidence of heart failure, and left ventricular function. If the activation of the cardiac tissue RAAS contributes to the development of left ventricular hypertrophy and subsequent myocardial failure [14-16], the chronic administration of ACEI should decrease the extension of the hypertrophic response and favorably modify the ventricular function in rats with established left ventricular hypertrophy even in the presence of severe PPO.

This study aimed at assessing the long-term effects of lisinopril on mortality, incidence of heart failure, myocardial remodeling, and left ventricular systolic and diastolic functions in rats with compensated left ventricular hypertrophy in the presence of PPO caused by supravalvular aortic stenosis.

Methods

In this experiment, left ventricular hypertrophy was caused by persistent pressure overload caused by ascending aortic banding in male Wistar rats, weighing between 70 and 90 g, which were anesthetized with sodium pentobarbital (50 mg/kg – IP), and then mechanically ventilated with 100% oxygen at 30 cps. After a median sternotomy, the ascending aorta was dissected and isolated with a 0.60-mm inner diameter metallic clip placed in the aorta immediately after the pericardial sac emergence. The thoracic cavity was closed, and the sternum, the muscle layers, and the skin were reconstructed in planes. Another group of animals underwent simulated surgery, in which all the procedures of aortic banding were performed, except for placement of the metallic clip.

After the surgical procedure, the rats were placed in plastic boxes for recovery, with free access to food preparation and water, 12-hour lightness/darkness cycles, and controlled environmental temperature (21 to 24ºC).

Six weeks after the surgical procedures, all rats that had undergone simulated surgery (n=20) or aortic banding (n=103) underwent echocardiography. Then, 10 rats that had undergone simulated surgery and 10 rats with aortic banding were randomized for in vivo hemodynamic assessment, being later sacrificed for morphological study. Their results represent the baseline conditions before the beginning of the observation period. The remaining rats with aortic stenosis were randomized to receive lisinopril or no treatment for 15 consecutive weeks. Three experimental groups were constituted as follows: control group (CG; n=10), comprising rats that had undergone simulated surgery; aortic stenosis group (SG; n=63), comprising rats with aortic banding that had not undergone treatment; and lisinopril group (LG; n=30), comprising rats with aortic stenosis that received lisinopril orally (20 mg/kg/day) dissolved into the water of the cages. The control of the dose ingested was performed daily by measuring the water volume drunk by each animal. That dose has antiproliferative and antihypertensive effects [17]. In the period from 6 to 21 weeks, the animals were assessed daily and the deaths recorded. Twenty-one weeks after the surgical procedures, all animals underwent new echocardiographic, hemodynamic, and morphological assessments, including measurement of the left ventricular hydroxyproline content, and, at the end of follow-up, the presence of heart failure was assessed. The clinical signs of heart failure were tachypnea, edema, ascites, pleural or pericardial effusion, or both. The morphological signs of heart failure were the presence of left atrial thrombus and water content in the lungs and liver. The criteria for diagnosing heart failure were as follows: animals with one clinical sign; one clinical sign + morphological sign; and 2 or more morphological signs. Figure 1 is a schematic representation of the experimental protocol used.

For the echocardiographic and hemodynamic assessments, the animals were anesthetized with ketamine (50 mg/kg) and xylazine (1 mg/kg) administered intramuscularly. A Sonos 2000 echocardiographic device (Hewlett-Packard) equipped with a 7.5-MHz transducer was used for obtaining the 2-dimensional image of the smaller left ventricular axis immediately below the mitral valve plane. M-mode recordings of the cardiac structures were performed with paper transportation velocity of 100 mm/s. A curve of transmitral diastolic flow velocity in the apical 4-chamber plane was obtained by positioning the volume sample of pulsatile Doppler immediately below the ventricular face of the leaflets of the mitral valve. Recordings allowing adequate evaluations of the morphological and functional left ventricular parameters were obtained in all animals. Later, the tracings were manually calibrated with the aid of a pachymeter (reading accuracy, 0.1 mm) and using at least 3 consecutive cardiac cycles. This methodology has already been standardized in our laboratory and has reproducibility and variability comparable to those reported in the literature [18,19]. After the echocardiography, an incision was made in the median line of the upper abdomen, and the cardiac apex was palpated. A hypodermal needle (25x9) was connected to a Stathan P23ID transducer filled with saline solution, and introduced into the left ventricular cavity through the apex. Approximately 1minute was allowed for hemodynamic stabilization, after which recordings of the ventricular pressure and its first temporal derivate were obtained. A Gould Windograf polygraph, model 40–9800–20, was used for recordings, at a paper transportation velocity of 100 mm/s. Figure 2 shows the tracings representing the recordings obtained.

After blood pressure recording, the animals were sacrificed, their chests opened, and their hearts were rapidly removed. The atria were extirpated, and the ventricular musculature was divided into right ventricular smooth wall and left ventricular muscular mass, including the septum. Those structures were separately weighed. The left ventricular tip was used for measuring the hydroxyproline content, according to the technique described by

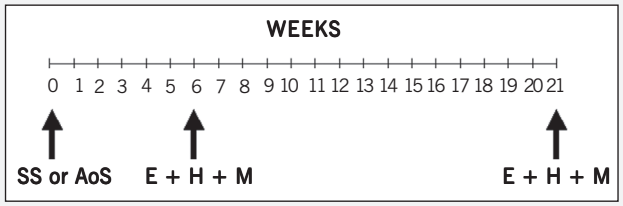

Fig. 1 - Schematic representation of the experimental protocol. SS: simulated surgery; AoS: aortic stenosis; E, H, M: echocardiographic, hemodynamic, and morphological assessments, respectively.

Switzer [20]. A fragment of the musculature of the left ventricular free wall was fixed in 10% formalin and embedded in paraffin, and, then, cut into 4-mm histological sections that were stained with hematoxylin-eosin and analyzed under optical microscopy (40x magnification) coupled to a video camera. With the aid of a microcomputer equipped with an image analysis program (IMAGE-PRO PLUS 3.0, Media Cybernetics, Silverspring Maryland, USA), the sectional area of the myocytes was measured and used as an indicator of cell size. In each animal, at least 50 myocytes transversely cut with a clearly central nucleus were measured. Fragments of the lungs and liver were weighed and then put to dehydrate at 70°C for 96 hours, and, then, weighed again, enabling the determination of the water content of those organs.

In the assessments performed 6 and 21 weeks after the surgical procedures, the following variables were analyzed: body weight (BW, g); heart rate (HR, bpm); diastolic thickness of the left ventricular posterior wall (DTPW, mm); left ventricular diastolic diameter (LVDD, mm); shortening percentage (ΔD %); ratio between the peak velocities of the early (E) and late (A) transmitral flow (E/A ratio); left ventricular systolic (LVSP, mmHg) and end-diastolic (LVEDP, mmHg) pressures; positive (+dP/dt, mmHg/s) and negative (-dP/dt, mmHg/s) first derivate of left ventricular pressure; left ventricular mass index (LVMI, mg/g), corresponding to the weight of the left ventricular muscular mass divided by body weight; left ventricular hydroxyproline content (LVHP, mg%); sectional area of left ventricular myocytes (SA, μm^2); water content of the lungs (H_2O Lu, %) and liver (H_2O Li, %).

The results obtained in CG and SG in the evaluation performed 6 weeks after the simulated surgery or aortic banding were compared for assessing the degree of hypertrophy, the severity of pressure overload, and the left ventricular function before starting the treatment with lisinopril. The results obtained in CG, SG, and LG 21 weeks after the surgical procedures were compared for assessing the effects of the treatment with lisinopril on the severity of left ventricular pressure overload, morphology, and function. In each group, the results obtained 6 and 21 weeks after surgery were compared for characterizing the temporal evolution of the variables analyzed. Considering that the animals with heart failure are known to have impaired left ventricular function, in this study, the analysis of the echocardiographic, hemodynamic and morphological data obtained 6 and 21 weeks after surgery refer only to those animals with no evidence of heart decompensation at the end of follow-up.

All values are expressed as mean (x) ± standard deviation (sd). The analyses related to the frequency of death and heart failure at the end of the experiment were performed with the chi-square (χ^2) test. The analyses of the echocardiographic, hemodynamic, and morphological variables assessed 6 and 21 weeks after the surgical procedures were performed with univariate ANOVA, an entirely casual model, complemented with the Bonferroni multiple comparisons test. The study of the sectional area of the myocytes and left ventricular hydroxyproline content was performed with the univariate ANOVA, an entirely casual model, complemented with the Tukey test for the comparisons between all pairs of means. The significance level adopted was 5%.

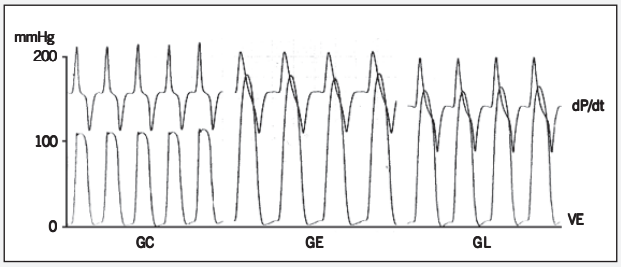

Fig. 2 - Tracings representing the hemodynamic recordings obtained comparing CG, SG and LG (control, stenosis and lisinopril groups).

Results

No statistically significant difference was observed in the body weights of the animals at the time they were included in the study (CG: 74 ± 6 vs SG: 76 ± 5 vs 73 ± 7 g; P>0.05). In the assessments performed 6 weeks after the surgery, neither clinical nor morphological signs of heart decompensation were found in CG and SG. In rats with established left ventricular hypertrophy, the administration of the ACEI, lisinopril, for 15 weeks reduced the frequency of deaths and heart failure. During the follow-up period, neither death nor heart failure was observed in CG. In SG (n=63), 34 rats died (53.9%) and, of the 27 survivors, 13 (44.8%) had signs of cardiac decompensation at the end of the experiment. In LG (n=30), 5 rats (44.8%) died, and, of the 25 survivors, only 5 (20%) had heart failure, P<0.05 (fig. 3). These results corres-

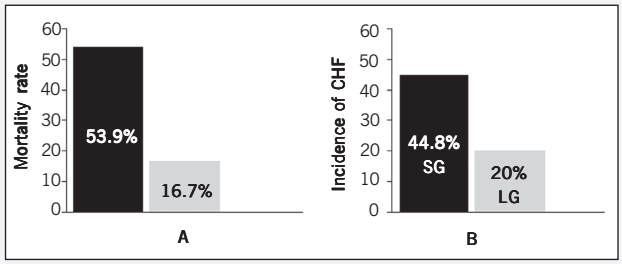

Fig. 3 - A: mortality rates (M); B: incidence of heart failure (HF) in the group with aortic stenosis (SG) and the group treated with lisinopril (LG).

Table I - Left ventricular echocardiographic variables obtained 6 and 21 weeks after simulated surgery or aortic stenosis

Variables	Groups	6 weeks	21 weeks
DTPW (mm)	CG	1.54 ± 0.12	1.52 ± 0.10
	SG	1.81 ± 0.12*	1.91 ± 0.20*
	LG	1.83 ± 0.13*	1.85 ± 0.18*
LVDD (mm)	CG	8.2 ± 0.2	8.2 ± 0.2
	SG	7.8 ± 0.3	9.8 ± 0.3*+
	LG	8.0 ± 0.3	8.7 ± 0.3#
EDPP/DDVE	CG	0.21 ± 0.02	0.18 ± 0.01
	SG	0.29 ± 0.03*	0.25 ± 0.02*
	LG	0.31 ± 0.03*	0.23 ± 0.03*
ΔD (%)	CG	49 ± 5	52 ± 3
	SG	59 ± 6*	44 ± 5*+
	LG	58 ± 5*	51 ± 5#+
E/A	CG	1.64 ± 0.27	1.72 ± 0.33
	SG	2.07 ± 1.05	6.71 ± 2.08*+
	LG	2.11 ± 1.08	4.23 ± 2.38*#+

Values expressed as mean ± standard deviation; CG, SG, and LG - control, aortic stenosis, and lisinopril groups, respectively; DTPW - diastolic thickness of the posterior wall; LVDD - left ventricular diastolic diameter; ΔD(%) - percentage of left ventricular systolic shortening; E/A - ratio between the peak velocities of early (E) and late (A) diastolic flow through the mitral valve. *P<0.05 vs CG; # P<0.05 vs SG; + P<0.05 vs 6 weeks.

pond to 69% and 55% reductions in the mortality rate and heart failure, respectively. On autopsy, neither clinical nor morphological previously established signs of heart failure were found.

Table I depicts the results of the echocardiographic assessment, showing that 6 weeks after the surgical procedures, the rats with AoS had established concentric left ventricular hypertrophy. The DTPW and DTPW/LVDD values in SG and LG are equivalent and significantly greater than those in CG. The LVDD and E/A ratio values were similar in the 3 groups. These results show that before treatment, the animals in SG and LG had equivalent degrees of left ventricular concentric hypertrophy and preserved diastolic function. The analyses of ΔD% showed that the rats in SG and LG had hyperdynamic left ventricle. At the end of the experimental period, the DTPW and DTPW/LVDD values in SG and LG were similar and significantly greater than those in CG, and those values were equivalent to those observed 6 weeks after the surgical procedures. The analyses related to the parameters of ventricular function (ΔD% and E/A) showed that the treatment with lisinopril preserved the systolic function and attenuated the impairment in left ventricular diastolic function. The ΔD% values were significantly smaller in SG, while the E/A ratio values showed significant increases with a restrictive pattern in SG and LG, but the impairment was significantly smaller in the group treated with lisinopril.

Table II shows the results of the hemodynamic assessments performed 6 and 21 weeks after the surgical procedures, in which the HR values were equivalent in the 3 groups. The left ventricular systolic pressure (LVSP) values were also similar in the SG and LG animals and significantly greater than those in the CG animals. The treatment with lisinopril did not change the values in the rats with aortic banding, evidencing the presence of equivalent PPO in SG and LG during the 15 weeks of follow-up. Six weeks after the surgical procedures, the LVEDP values were significantly higher in the animals undergoing aortic banding. At the end of follow-up (21 weeks), the values of that variable did not change in CG, but showed significant increases in SG and LG, although the values in the group treated were significantly smaller. The +dP/dt and –dP/dt measurements performed 6 weeks after the simulated surgery or aortic banding showed no significant differences in the indices of myocardial contractility. On the other hand, 21 weeks after surgery, the +dP/dt and –dP/dt values were significantly smaller only in SG. These results showed that the treatment with lisinopril for 15 consecutive weeks had a beneficial effect on myocardial contractility and relaxation.

Table III shows the results obtained in the morphological assessments performed 6 and 21 weeks after the surgical procedures. Before treatment, the body weights (BW) of the CG and SG animals were equivalent. At the end of the experiment, the BW values of the rats treated with lisinopril (LG) were significantly lower than those in CG and SG, which were equivalent. Six weeks after inducing AoS, the left ventricular mass index (LVMI) value in SG was significantly greater than that in CG, corresponding to a 34.7% increase in the left ventricular muscular mass. At the end of the experiment, it was evident that the LVMI values in SG and LG were greater than those in CG, those in SG being greater than those in LG ($P<0.05$). Compared with the increase of LVMI in the CG group, the magnitudes of increase of LVMI were 68% in SG and 36% in LG (fig. 4). The measurements of the sectional area (AS) and left ventricular hydroxyproline content (LVHP) showed that even in the presence of persistent pressure overload, the treatment with lisinopril also limited the enlargement of the dimensions of the myocytes and accumulation of myocardial collagen. The water content of the lungs and liver did not significantly differ in the 3 groups. In addition to the lack of clinical signs, we are certain that, in the 3 groups, no animal whose ventricular function was assessed at the end of the experiment had heart failure.

Discussion

In the animal models of left ventricular hypertrophy triggered by pressure overload, such as aortic stenosis and arterial hypertension, ventricular remodeling consists of an increase in the thickness of the wall, an increase in the diameter of the myocytes, and changes in the myocardial interstitial components, which most of the time include accumulation of myocardial collagen [21-23]. Data in the literature [21] have shown that spontaneously hypertensive rats and those undergoing banding of the thoracic aorta have initially normal contractile function and ventricular performance, which evolve to progressive depression of the contractile status, heart

Table II - Hemodynamic variables obtained 6 and 21 weeks after simulated surgery or aortic stenosis

Variables	Groups	6 weeks	21 weeks
HR	CG	279 ± 15	283 ± 14
(bpm)	SG	289 ± 20	288 ± 18
	LG	-	291 ± 20
LVSP	CG	105 ± 8	108 ± 6
(mmHg)	SG	198 ± 14*	195 ± 17*
	LG	-	188 ± 12*
LVEDP	GC	4 ± 1	4 ± 2
(mmHg)	CG	8 ± 2*	15 ± 3*+
	SG	-	8 ± 3*#
+dP/dt	CG	5980 ± 484	5810 ± 320
(mmHg/s)	SG	5729 ± 519	3998 ± 550*+
	LG	-	5474 ± 470#
-dP/dt	CG	2970 ± 222	2950 ± 245
(mmHg/s)	SG	3148 ± 355	2358 ± 300*+
	LG	-	3110 ± 399#

Values expressed as mean ± standard deviation; HR - heart rate; LVSP - left ventricular systolic pressure; LVEDP - left ventricular end-diastolic pressure; +dP/dt e -dP/dt - positive and negative first temporal derivate of left ventricular pressure, respectively; CG, SG, and LG - control, aortic stenosis, and lisinopril groups, respectively. *P<0.05 vs CG; # P<0.05 vs SG; + P<0.05 vs 6 weeks.

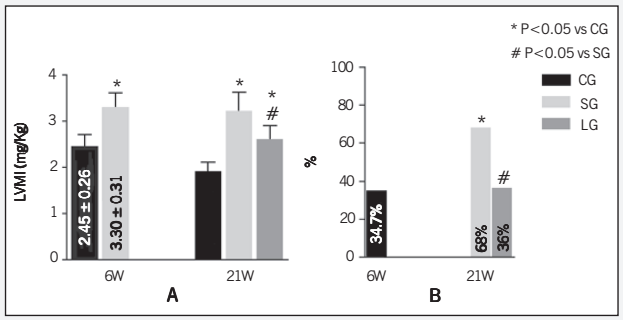

Fig. 4 - A: Left ventricular mass index (LVMI) observed in the control (CG), aortic stenosis (SG), and lisinopril (LG) groups, 6 (6W) and 21 (21W) weeks after simulated surgery or aortic banding. B: percentage of LVMI increase in SG and LG as compared with CG, observed 6 weeks and 21 weeks after the surgical procedures.

failure, and early death. These animal models are useful for long-term evaluations of the morphological, biochemical, and functional characteristics of left ventricular hypertrophy, as well as the assessment of the drug effect on mortality, left ventricular hypertrophy development, and heart failure.

When the aortic banding is performed in young rats, such as in our study, pressure overload is admittedly [21,24] mild initially and progressively increases as the animals grow, which, in a way, is similar to aortic stenosis in men. In this study, the assessment period of 21 weeks after AoS was chosen because it was associated with a high mortality rate, encompassing the transition to cardiac decompensation with alterations in gene reprogramming [14,21].

The results obtained in the echocardiographic (tab. I), hemodynamic (tab. II), and morphological (tab. III) evaluations performed 6 and 21 weeks after aortic banding or simulated surgery showed that the degree of pressure overload in animals with aortic stenosis was severe and remained stable for the 15 weeks of observation. In that period, treatment with lisinopril did not change the left ventricular systolic pressure values, therefore characterizing the persistence and equivalence of pressure overload in SG and LG.

In sustained pressure overloads, myocardial growth and function are modulated by the characteristics of the load and neurohumoral systems, of which, the RAAS stands out. The ascending aortic banding model is adequate for separating the effects related to the myocardial tissue RAAS inhibition from those associated with the reduction in the hemodynamic load. The results observed showed that chronic ACE inhibition with lisinopril reduces mortality, delays the transition to heart failure, favorably alters myocardial remodeling, and attenuates the degree of impairment of the left ventricular systolic and diastolic functions even in the presence of pressure overload.

Several factors may have contributed to the reduction in mortality in rats treated with lisinopril. The greatest frequency of deaths in the SG rats may be associated with the development of an earlier and more severe left ventricular dysfunction than that observed in the LG group. The greater impairment in left ventricular systolic and diastolic function and greater incidence of heart failure in SG observed at the end of the experiment reinforce that aspect. Another possibility for reducing mortality in the group treated is that ACE inhibition prevented the early deaths due to the arrhythmias triggered by myocardial ischemia. Several studies [25-27] have reported that, in that model, the coronary reserve is decreased, angiotensin II worsens and ACEIs protect against arrhythmias of reperfusion after transient myocardial ischemia in rats [26,28], and the myocardial tissue RAAS activity is exacerbated [12,21,29].

Before treatment, the SG animals had a 34.7% greater LVMI than that of the CG animals. At the end of the experiment, AoS caused 68% and 36% increases in the LVMI in SG and LG, respectively, as compared with that in CG. This represents a difference of 52.9% in the degree of LVHR. Regarding the dimensions of myocytes, 64% and 31% increases in the SA values were observed, representing a 51% difference between SG and LG.

Considering that, in the assessments performed 6 and 21 weeks after aortic banding, LVMI values increased in nontreated animals and were equivalent in lisinopril-treated animals, one may assume that the treatment with lisinopril prevented the aggravation in the degree of LVH (table III). One may also assume that the smaller SA observed in LG occurred predominantly due to a reduction in the parallel addition of myofibrils characteristic of heart hypertrophy triggered by pressure overload [22].

The LVHP measurements evidenced that the treatment with lisinopril reduced the accumulation of myocardial collagen. These results are in accordance with the reports in the literature indicating the participation of angiotensin II among the major mechanisms involved in the accumulation of myocardial collagen [30,31].

This study showed that the administration of lisinopril to rats with AoS prevents the increase in ventricular hypertrophy extension and reduces the accumulation of myocardial collagen even in the presence of PPO. These results support the proposition that isolated pressure overload is not the only determining factor of cardiac remodeling. In the present experimental conditions, one may infer that myocardial tissue RAAS also participates, accounting for, at least partially, the development of pathological remodeling that culminates in progressive left ventricular dysfunction. The smaller degree of hypertrophy, left ventricular dysfunction, and accumulation of myocardial collagen evidenced at the end of the experiment in the animals treated with lisinopril support those aspects.

Currently, it seems clear that heart enlargement may be modulated by changes in load and neurohumoral activation [1-9,10,11,21,29]. Some studies in animals undergoing banding of the proximal aorta or pulmonary artery simultaneously treated with agents that modulate RAAS activity had controversial results[24,32-36]. Of the 6 studies analyzed, 3 showed no effect of RAAS blockage on the extension of left or right ventricular hypertrophy[34-36], and 3 showed that RAAS blockage reduced or prevented ventricular hypertrophy[24,32,33]. The importance of the hemodynamic overload should not be minimized, because a recent study [37] showed that the removal of pressure overload promoted by banding of the thoracic aorta in guinea pigs resulted in complete reversion of left ventricular hypertrophy and function. Our results suggest that the RAAS blockage reduces the extension of left ventricular hypertrophy even in the presence of persistent pressure overload, PPO. Other neurohumoral

Table III - Morphological variables obtained 6 and 21 weeks after simulated surgery or aortic stenosis

Variables	Groups	6 weeks	21 weeks
BW	CG	302 ± 29	496 ± 55
(g)	SG	294 ± 27	481 ± 43
	LG	-	411 ± 20*
LVMI	CG	2.45 ± 0.26	1.91 ± 0.21
(mg/g)	SG	3.30 ± 0.31*	3.21 ± 0.41*
	LG	-	2.60 ± 0.3*#+
AS	CG	-	328 ± 17
(μm^2)	SG	-	537 ± 36*
	LG	-	429 ± 28*#
LVHP	CG	-	3.6 ± 0.6
(mg%)	SG	-	7.9 ± 1.1*
	LG	-	5.2 ± 0.7*#
H_2O Lu	CG	79.9 ± 1.0	80.4 ± 1.1
(%)	SG	80.2 ± 0.8	81.3 ± 0.9
	LG	-	81.2 ± 0.7
H_2O Li	CG	68.1 ± 0.8	67.8 ± 0.9
(%)	SG	67.6 ± 0.6	67.2 ± 0.8
	LG	-	68.3 ± 0.9

Values expressed as mean ± standard deviation; CG, SG, and LG: control, aortic stenosis, and lisinopril groups, respectively; BW: body weight; LVMI: left ventricular mass index; AS: sectional area of myocytes; LVHP: left ventricular hydroxyproline content; H_2O Lu and H_2O Li: water content in the lungs and liver, respectively. * P<0.05 *vs* CG; #P<0.05 *vs* SG; +P<0.05 *vs* 6 weeks.

mechanisms may have participated in the cardiac remodeling observed in this study. Angiotensin II increases the release of sympathetic neurotransmitters, and the smaller extension of left ventricular hypertrophy in animals treated with lisinopril could be partially related to the reduction in the trophic effect due to the sympathetic stimulation of the myocyte growth [1,8-10].

At the end of the experiment, in the presence of severe and equivalent pressure overload in SG and LG animals, the incidence of heart failure was 2.8 times smaller in treated rats. This showed that chronic ACE inhibition with lisinopril resulted in a significant reduction in the incidence of heart failure, and, therefore, attenuated the impairment of heart function. The results of the indicators of systolic (+dP/dt, ΔD%) and diastolic (LVEDP, E/A ratio, and –dP/dt) function in rats without heart failure showed that treatment with lisinopril was beneficial, promoting preservation of systolic function and attenuating impairment in left ventricular diastolic function.

Although myocardial remodeling may be considered an adaptive mechanism, because it preserves ventricular function, the process may become maladaptive when the triggering stimulus is pathological and continuous, such as in aortic stenosis and arterial hypertension. In such conditions, continuous myocardial remodeling usually evolves, for a variable length of time, with progressive ventricular dysfunction, which culminates in heart failure and early death, if the process is not effectively stopped as early as possible.

Several characteristics that occur during the process of cardiac remodeling triggered by pressure overload have been reported [16,29,30,38-43] as participating in functional cardiac impairment, such as the following: phenotypic changes due to reexpression of genes that codify the synthesis of myocardial proteins with fetal characteristics; hypertrophy of myocytes; accumulation of myocardial fibrosis; blood vessel deficit; reduction in coronary reserve; mitochondrial deficit; defective calcium cycle; synthesis of myosin with lower ATPase activity; and death of myocytes due to necrosis or apoptosis, or both.

This study showed that the beneficial effects of lisinopril on left ventricular remodeling and function did not depend on the reduction in load conditions or heart rate, which were equivalent in treated and nontreated rats. Therefore, if the beneficial effects of ACE inhibition were not due to an improvement in load conditions, and the treatment with lisinopril prevented deterioration of cardiac function, even in the presence of a lower degree of cardiac hypertrophy at the end of follow-up, alternative mechanisms through which the ACE inhibition preserves contractility need to be considered. Experimental studies [44-48] have shown that the treatment with ACEI improves the contractile response to extracellular calcium in rats with aortic banding [24,47] or acute myocardial infarction [46]. There is evidence that ACEIs reduce apoptosis in left ventricular hypertrophy triggered by pressure overload [48]. Therefore, another possible use of ACEI in aortic stenosis could be to reduce the loss of functioning myocytes due to angiotensin II-mediated apoptosis or necrosis, or both.

The emerging role of extracellular matrix in the process of myocardial remodeling can be evidenced in different animal models of left ventricular hypertrophy triggered by pressure overload [23,30,31,41-43]. In a simplified way, it has been proposed that excessive interstitial fibrosis may strangle the heart, initially during diastole, and, later, during systole. The greater impairment in left ventricular systolic and diastolic function and accumulation of myocardial collagen in SG, and the preservation of systolic function, attenuation of diastolic function impairment, and reduction in left ventricular hydroxyproline content in LG rats are in accordance with the propositions that the myocardial collagen matrix also participates in ventricular dysfunction.

Several studies and a literature review indicate that the treatment with ACEI may favorably change myocardial contractile capacity and reduce the accumulation of myocardial collagen [6,8,21,24,29,30,34,43-45]. This study could not identify the mechanisms responsible for the preservation of systolic function and for the attenuation of diastolic function impairment in rats treated with lisinopril. The lower diastolic function impairment observed in rats treated with lisinopril may be due to the lower myocardial distensibility impairment resulting from the less marked left ventricular hypertrophy or smaller accumulation of myocardial collagen, or both [23,30,41-43,48]. Alternatively, the attenuation of left ventricular diastolic function observed in LG could be due to the blockade or reduction in angiotensin II (AII) locally produced by the myocardial tissue RAAS [11,16,49,50]. Some studies [49,50] have reported that, in the presence of left ventricular hypertrophy, the activation of intracardiac AII delays and slows relaxation [49], and that the intravenous administration of ACEI in patients with severe AoS [50] improves myocardial distensibility and relaxation. Therefore, based on the results observed, one may infer that the RAAS accounts for, at least partially, the appearance of pathological cardiac hypertrophy, with consequent left ventricular systolic and diastolic dysfunction, cardiac decompensation, and early death through mechanisms distinct from those related to blood pressure elevation and the degree of left ventricular hypertrophy.

Our findings provide experimental support to the idea that the use of ACEI may have favorable effects in patients with aortic stenosis, for whom no clinical treatment that unquestionably modifies the natural history of the disease is yet available. However, it is worth noting that, although in the experimental model of this study left ventricular pressure overload is sustained, as it is in human AoS, the aortic banding is placed distally to the coronary ostia, and, therefore, coronary circulation is exposed to the same high left ventricular blood pressure levels. Therefore, although experimental studies [8,21,24,39,41,43] have suggested that the ACEI may have beneficial effects on patients with aortic stenosis, we should recognize the potential dangers of their use until clinical studies clearly demonstrate the benefits of that therapy in that group of patients, mainly in those with severe aortic stenosis or left ventricular dysfunction, or both.

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