Original Article

Blockade of Renin-Angiotensin System Attenuates Cardiac Remodeling in Rats Undergoing Aortic Stenosis

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Objective

To assess the role of the AT1 receptor blocker and the angiotensin-converting enzyme inhibitor in cardiac remodeling induced by aortic stenosis in rats.

Methods

Wistar rats were divided into the following 4 groups: 1) C - control (n=13); 2) AoS - aortic stenosis (n=11); 3) LIS - AoS treated with lisinopril, 20 mg/kg/day (n=11); and 4) LOS - AoS treated with losartan, 40 mg/kg/day (n=9). The treatments were initiated 3 days before surgery. After 6 weeks, the animals underwent echocardiographic study, and quantification of the hydroxyproline (HOP) concentration and the left ventricular (LV) myocyte cross-sectional area (CSA).

Results

Aortic stenosis induced an increase in left ventricular wall thickness. The LIS and LOS groups showed no difference as compared with the control group. The AoS and LIS rats had greater left atrial diameters than the control rats did, while no difference was observed in the LOS animals. The AoS animals had greater values of shortening percentage than control animals did. This fact was modified with neither LIS nor LOS. The cross-sectional area of the animals in the AoS group was greater than that in the control group. However, treatment with LOS and LIS attenuated the AoS-induced increase in area. Aortic stenosis caused an increase in HOP concentration, while the LOS group showed no difference as compared with the control group.

Conclusion

Blockade of the renin-angiotensin system with AT1 blocker and ACEI may attenuate the development of heart hypertrophy, but only the blockade of AT1 receptors attenuates left ventricular interstitial fibrosis.

Key words

hypertrophy, ventricular function, echocardiogram, fibrosis

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The process of cardiac remodeling is characterized by alterations in heart geometry, volume, mass, and constitution in response to a certain aggression. Myocardial hypertrophy is an important component of cardiac remodeling, and it usually occurs in response to chronic hemodynamic overload. This adaptive mechanism allows the heart to maintain its basic functions in the presence of an increase in load conditions. In the long run, however, hypertrophy represents an independent risk factor for cardiac morbidity and mortality [1-3]. Another important component of remodeling is the connective tissue. The myocytes represent only 30% of the total number of myocardial cells, the other cells being smooth muscle cells of the vessels, endothelial cells, and fibroblasts. A complex and organized collagen network exists surrounding and linking all those structures. The major functions of that network are as follows: to regulate apoptosis; to resist to pathological deformations; to maintain the alignment of the structures; and to regulate the transmission of force during cardiac fiber shortening. Therefore, collagen tissue is an important modulator of the cardiac architecture and function, and, depending on the stimulus, collagen may build up, characterizing myocardial fibrosis. This phenomenon may interfere with maintenance of the normal cardiac geometry or impair ventricular function, or both [4,5].

The stimuli for the development of cardiac remodeling include mechanical and biochemical factors, which have endocrine, paracrine, and autocrine actions. Such factors, acting on receptors, such as those of integrins, and ionic channels present in the sarcolemmal membrane activate signalers that cause an alteration in the gene expression and an increase in protein synthesis [6].

Among the stimuli involved in the remodeling process, angiotensin II (AII) stands out. Formed, both locally and systemically, from angiotensin I through the action of angiotensin-converting enzyme (ACE) and acting on AT1 receptors, AII stimulates cell growth and promotes collagen build up [5]. In the heart, however, the AII may be formed predominantly through the action of the chymase and not the ACE [7,8].

Considering the pathophysiological role of the renin-angiotensin system in the process of cardiac remodeling, several studies have already reported that ACE inhibitors (ACEI) attenuate the morphological and functional alterations triggered by several stimuli [9-12]. In aortic stenosis, in which persistent and severe elevation in left intraventricular pressure occurs with no elevation in systemic blood pressure, activation only of the cardiac renin-angiotensin system occurs. Thus, in that model, the benefit of the ACEI is less clear. In

addition, the role of the AT1 blockers in AoS is less clear. Because AII blockers block the AII action independently of ACE or chymase, they could be more effective than ACEI.

This study aimed at analyzing the effects of the renin-angiotensin system blockade with AT1 blockers or ACEI on the ventricular remodeling process (hypertrophy, and ventricular fibrosis and function) induced by aortic stenosis in rats.

Methods

The experimental protocol of this study was conducted according to the Ethical Principles in Animal Experimentation adopted by the Brazilian College of Animal Experimentation [13].

Male Wistar rats weighing between 90 and 100 g were used in this study. After anesthesia with ketamine hydrochloride (50 mg/kg, intramuscular) and xylazine hydrochloride (10 mg/kg, intramuscular), the rats underwent median thoracotomy. The ascending aorta was dissected, and a 0.6-mm-inner-diameter silver clip was placed at approximately 3 mm from the aortic root. The thoracic wall was closed, and the sternum, the muscle layers, and the skin were reconstructed with mononylon 4-0 suture thread. During the surgery, the animals were manually ventilated with positive pressure and 100% oxygen. The control animals underwent the same surgery, but no clip was placed.

The rats were divided into the following 4 experimental groups: 1) C - control (n=13); 2) AoS - aortic stenosis (n=11); 3) LIS - AoS treated with lisinopril, 20 mg/kg/day (n=11); and 4) LOS - AoS treated with losartan, 40 mg/kg/day (n=9). The drugs were added to the drinking water, initiated 3 days before surgery, and maintained for 6 weeks.

After 6 weeks, the animals were anesthetized with ketamine hydrochloride (50 mg/kg, intramuscular) and xylazine hydrochloride (1 mg/kg, intramuscular) for echocardiographic study [14]. After epilation of the anterior region of the chest, the animals were positioned in the left lateral decubitus for undergoing echocardiography with a Hewlett-Packard echocardiograph (Sonos 2000 model) equipped with a 7.5-MHz electronic transducer. The flows were assessed with the same transducer operating at 5.0 MHz. For measuring the cardiac structures, M-mode images were used with the ultrasound beam oriented by the 2-dimensional image, with the transducer in the short-axis parasternal view. The image of the left ventricular cavity was obtained by positioning the M-mode cursor right below the mitral valve plane between the papillary muscles. The images of the aorta and left atrium were also obtained in the short-axis parasternal view with the M-mode cursor positioned level with the aortic valve. The 1-dimensional image (velocity: 100 mm/s) was recorded by using a Sony Co printer, UP-890MD model. Later, the cardiac structures were manually measured with the aid of a pachymeter, according to the recommendations of the American Society of Echocardiography [15]. The cardiac structures were measured in at least 5 consecutive cardiac cycles. The left ventricular diastolic diameter (LVDD) and the left ventricular diastolic posterior wall thickness (LVDPWT) were measured at the moment corresponding to the maximum diameter of the cavity. The left ventricular systolic diameter (LVSD) was measured at the moment of the maximum systolic excursion of the posterior wall of the cavity. The left ventricular systolic function was assessed by calculating the percentage of systolic shortening (LVDD-LVSD function)/LVDD x 100. The transmitral diastolic flow (E and A waves) was obtained with the transducer at the apical 4-chamber view. The flow measurements were performed directly on the echocardiographic monitor.

One to 3 days after the echocardiographic study, the animals were weighed, anesthetized with sodium pentobarbital (50 mg/kg, intraperitoneal), and underwent median thoracotomy for heart removal. The right (RV) and left (LV) ventricles were separated and weighed separately. From the central part of the LV, a 2- to 3-mm-thick ring was sectioned encompassing all the extension of the wall. The material was immersed in neutral and buffered 10% formalin for 48 hours at 4^{0}C. After that period, the tissue was washed, dehydrated, and embedded in paraffin. The 5- to 7-mm-thick histological sections were stained with hematoxylin and eosin, and analyzed under optical microscopy. The morphometric analysis was performed by using a video camera coupled to a Leica microscope connected to a computer equipped with a program for image analysis (Image-Pro 3.0, Media Cybernetics, Silver Spring, Maryland, USA). In cross-sectional sections of the left ventricular subendocardial and subepicardial regions, the cross-sectional areas of at least 50 myocytes were measured, in which the nucleus was clearly identified in the center of the cell [16].

The hydroxyproline measurements were performed according to the Switzer technique [17]. After dehydration in alcohol, samples of the LV tissue were dried in a vacuum oven for 24 hours at a constant temperature of 60^{0}C. The previously dehydrated samples were hydrolyzed in a 6N solution of chloride acid (10 mg of dry tissue/mL of 6N HCl) for 16 hours at 110^0 C. The 50 mL aliquots of hydrolysate were dried in a vacuum oven for 24 hours and diluted in 1.6 mL of deionized water in a 150 ´ 16 mm test tube with a screw-type lid. This solution was alkalinized with 1 mL of borate buffer (pH=8.7), and then 0.3 mL of chloramine T were added. After a 20-minute rest at room temperature, 1.0 mL of 3.6 M sodium thiosulfate was added, and the test tube was vigorously agitated. Then, approximately 1.5 g of KCl were added to saturate the solution. The tubes were closed and warmed in boiling water for 20 minutes. After cooling of the sample, 2.0 mL of toluene were added, and the tubes were agitated for 5 minutes. After a short rest period, 1.0 mL of the supernatant toluene was transferred to a test tube, where 0.4 mL of the Ehrlich reagent were added (27.4 mL of sulfuric acid in 200 mL of ethanol; this was added to the solution of 120 g of p-dimethylaminobenzaldehyde in 200 mL of ethanol). After 30 minutes, optical density was read with a spectrophotometer at 565 nm, against a blank. The same sequences of reactions were performed in samples containing water and 20 mg/mL of the hydroxyproline solution, blank, and standard sample, respectively.

The data had a normal distribution and were expressed as mean ± standard deviation. The method used was analysis of variance (one-way ANOVA) complemented with the Tukey multiple comparisons test. The significance level adopted was 5%.

Results

The results of the morphometric study are shown in table I. The animals that underwent blockade of the renin-angiotensin system had lower body weight (BW) than those with no treatment. The AoS resulted in an increase in the LV/BW ratio (C=2.08±

0.11mg/g; AoS=3.48±0.32 mg/g; P<0.05). This increase was not influenced by treatment. The cross-sectional area of the animals in the AoS group was greater than that in control animals (C=262±32 μm²; AoS=361±43 μm²; P<0.05). However, the treatment with LOS and LIS attenuated the increase in the area induced by blood pressure overload (LIS=334±39 μm²; LOS =326±55 μm²; P>0.05 vs C). Regarding collagen quantification, AoS resulted in an increase in the HOP concentration (C=2.13±0.16 μg/mg; AoS=2.74±0.61 μg/mg; P<0.05). On the other hand, the group treated with LOS showed no statistically significant difference as compared with that in the control group (C=2.13±0.16 μg/mg; LOS=2.46±0.53 μg/mg; P>0.05).

The morphological results of the echocardiographic study are shown in table II. The ventricular diameter did not differ between the C and AoS groups. The systolic diameters were smaller in the LIS and LOS groups as compared with those in control animals. The AoS induced an increase in left ventricular wall thickness (C =1.40±0.12 mm; AoS=1.96±0.15 mm; P<0.05). The LIS and LOS group animals showed no difference in that variable as compared with that in control rats (C=1.40±0.12 mm; LIS= 1.56±0.14 mm; LOS=1.54±0.13 mm; P>0.05). Considering the left atrium, the animals in the AoS and LIS groups had greater diameters than control animals did (C=4.39±0.47 mm; AoS= 6.41±1.21 mm; LIS=6.34±1.11 mm; P<0.05 vs C). The animals undergoing treatment with LOS showed no difference compared with those without overload (C=4.39±0.47 mm; LOS =5.65±0.63 mm; P>0.05).

The results of the functional study are shown in table III. In regard to the shortening percentage, the animals with AoS had greater values as compared with control animals (C=57.6± 3.7%; AoS=67.0±6.9%; P<0.05). This was not modified by the treatment with LIS or LOS (LIS=73.6±6.8%; LOS=73.6±7.5%). No difference was observed between the values of the E/A ratio in the 4 groups studied.

Discussion

This study aimed at assessing the influence of the blockade of the renin-angiotensin system with the AII AT1 receptor blocker and ACEI on cardiac remodeling by analyzing the development of left ventricular hypertrophy, myocardial interstitial fibrosis, and ventricular function in rats undergoing supravalvular AoS. In this model, the administration of ACEI or AT1 blockers does not significantly alter the values of left intraventricular pressure [18]. Thus, the hemodynamic effects and the direct tissular effects caused by ACEI or AII AT1 receptor blockers may be dissociated from the cardiac remodeling variables. Because in this model activation of the cardiac renin-angiotensin system occurs, the possibility that the blockade with ACEI or AT1 blockers could attenuate cardiac remodeling was considered.

Our study showed that AoS caused left ventricular hypertrophy. The administration of LIS or LOS attenuated that process, because the 2 treatments resulted in a myocytic cross-sectional area similar to that of the control group. The echocardiographic assessment also characterized ventricular hypertrophy, based on the increase in left ventricular wall thickness with AoS [19]. Both treatments induced a smaller wall thickness as compared with that in the AoS group. Thus, our results suggest that the blockade of the renin-angiotensin system with ACEI or AT1 blocker attenuated the development of ventricular hypertrophy induced by AoS. In the literature, evidence that the blockade of the renin-angiotensin system attenuates the hypertrophic process in the AoS model is not consistent. Some authors [20,21] have reported a decrease in ventricular mass, while others have not [22,23]. It is worth emphasizing that the reasons for such discrepancies have not been clarified yet.

Regarding the content of interstitial collagen, in accordance with other studies [4,5], ours evidenced that cardiac pressure overload resulted in myocardial fibrosis, because an increase in HOP concentration was observed in the AoS group. Treatment with LIS did not influence the collagen build-up, because no difference was observed in that variable as compared with that in the AoS group without treatment. On the other hand, we may infer that the administration of LOS attenuated the development of fibrosis, because no difference was found in HOP concentration between the LOS animals and control animals. The AoS model comprises an activation of the cardiac tissue renin-angiotensin system, and an increase in messenger RNA for ACE has been documented [24] in the myocardium of rats undergoing aortic stenosis. However, in the heart, contrary to that which occurs in the circulation, the major pathway of transformation of AI into AII seems to be the chymase pathway [7,8]. Considering that AII is an important modulator of collagen synthesis [25], we may infer that, in that model, the blockade of AII AT1 receptors is more effective than the ACE blockade in preventing fibrosis.

The left ventricular functional analysis performed by use of echocardiography revealed that the AoS groups had similar systolic

Table I – Morphometric variables observed in the different groups

Variable	Group			
	Control	AoS	LIS	LOS
Weight (g)	373 ± 45	352 ± 38	265 ± 36*#	267 ± 36*#
LV (g)	0.78 ± 0.09	1.22 ± 0.13*	0.80 ± 0.14#	0.95 ± 0.08*#
RV (g)	0.23 ± 0.03	0.25 ± 0.04	0.19 ± 0.03#	0.22 ± 0.02
LV/BW (mg/g)	2.08 ± 0.11	3.48 ± 0.32*	3.02 ± 0.49*	3.63 ± 0.47*†
RV/BW (mg/g)	0.62 ± 0.06	0.70 ± 0.12	0.72 ± 0.09	0.80 ± 0.07*
CSA (μm)	262 ± 32	361 ± 43*	334 ± 39	326 ± 55
HOP (μg/mg)	2.13 ± 0.16	2.74 ± 0.61*	2.92 ± 0.43*	2.46 ± 0.53

AoS - aortic stenosis; LIS - aortic stenosis and lisinopril; LOS - aortic stenosis and losartan; LV - left ventricular weight; RV - right ventricular weight; LV/BW - left ventricular weight adjusted for body weight in the rat; RV/BW - right ventricular weight adjusted for body weight in the rat; CSA - myocytic cross-sectional area; HOP - myocardial concentration of hydroxyproline. * P < 0.05 vs Control; # P < 0.05 vs AoS; †P < 0.05 vs LIS; (ANOVA).

Table II - Echocardiographic morphological variables studied in the different groups

Variable	Group			
	Control	AoS	LIS	LOS
LVDD (mm)	7.34 ± 0.28	7.57 ± 0.66	7.21 ± 0.53	7.05 ± 0.51
LVSD (mm)	3.09 ± 0.44	2.41 ± 0.56	1.91 ± 0.54*	1.86 ± 0.52*
LVDPWT (mm)	1.40 ± 0.12	1.96 ± 0.15*	1.56 ± 0.14	1.54 ± 0.13
LVDPWT/LVDD	0.19 ± 0.01	0.25 ± 0.03*	0.22 ± 0.02	0.26 ± 0.03*
LA (mm)	4.39 ± 0.47	6.41 ± 1.21*	6.34 ± 1.11*	5.65 ± 0.63
AO (mm)	3.69 ± 0.36	3.84 ± 0.39	3.24 ± 0.23*	3.49 ± 0.23#

AoS - aortic stenosis; LIS - aortic stenosis and lisinopril; LOS - aortic stenosis and losartan; LVDD - left ventricular diastolic diameter; LVSD - left ventricular systolic diameter; LVDPWT - left ventricular diastolic posterior wall thickness; LVDPWT/LVDD - ratio between diastolic posterior wall thickness and left ventricular diastolic diameter; LA - left atrial diameter; AO - aortic diameter. * P < 0.05 vs Control; # P < 0.05 vs AoS; (ANOVA).

Table III - Echocardiographic functional variables studied in the different groups

Variable	Group			
	Control	AoS	LIS	LOS
HR (bpm)	290 ± 20	282 ± 30	319 ± 35*#	286 ± 24†
% Shor	57.6 ± 3.7	67.0 ± 6.9*	73.6 ± 6.8*	73.6 ± 7.5*
E/A	1.6 ± 0.2	1.6 ± 0.5	1.4 ± 0.2	1.3 ± 0.2

AoS - aortic stenosis; LIS - aortic stenosis and lisinopril; LOS - aortic stenosis and losartan; HR - heart rate; % Shor - shortening percentage; E/A - ratio between the E and A waves assessed from transmitral flow. * P < 0.05 vs Control; # P < 0.05 vs AoS; †P < 0.05 vs LIS; (ANOVA).

functions, which were better than that in the control group, because the left ventricular shortening percentage was greater in animals with cardiac remodeling. In accordance with our results, other authors [26,27] have reported that, in cardiac pressure overload, the heart has initially normal or hyperdynamic function, and, over time, it evolves with progressive ventricular dysfunction.

In regard to the left ventricular diastolic function, analysis of the size of the left atrium showed an increase in atrial dimensions in the AoS and LIS groups as compared with that in control animals. Because the AoS and LIS groups had a better systolic function than the control group did, atrial enlargement may be due to diastolic dysfunction, although the E/A ratio did not differ between the groups. On the other hand, the LOS group showed no statistically significant difference in atrial size as compared with that in the control group. The concept that interstitial fibrosis is an important determinant of the passive stiffness of the ventricular chamber has been well established [28]. Thus, our results allow the speculation that the treatment with LOS improved diastolic function, at least partially, due to attenuation of interstitial fibrosis.

Considering that, up to now, no clinical therapy exists to prevent or to attenuate the progression of the repercussions of aortic stenosis, our study suggests that the blockade of the renin-angiotensin system may be considered a therapeutic option in that situation. The data available on the comparison between AT1 blockers and ACEI in that model are scarce. In a study, Liu et al [21] reported that both captopril and losartan equally attenuated the development of hypertrophy, the decrease in SERCA-2, and the increase in beta-myosin, markers of the remodeling process, after 8 weeks of treatment. Although further studies are required for a definitive conclusion, our results suggest that AT1 receptor blockers may provide greater benefit compared with that of ACEI in that model.

Our study had 2 limitations. In some variables analyzed, the values of the LIS and LOS groups did not differ from those of the AoS group. However, differently from the AoS group, the values of the groups under treatment did not differ from those of the control group. Therefore, we interpreted that the treatment used resulted in intermediate values between those in control animals and in the AoS group. Another consideration refers to the dosages of lisinopril and losartan used. We used dosages that were equal or equivalent to those used in other studies, and the blockade of the renin-angiotensin system was performed with the 2 strategies. However, we did not perform tests to assess the pharmacological equivalence of the medications used in our experiment.

In conclusion, the data in the present study indicate that, in the AoS model in rats, the blockade of the renin-angiotensin system with AII AT1 receptor blockers and with ACEI may attenuate the development of cardiac hypertrophy. However, only the blockade of AT1 receptors attenuates left ventricular interstitial fibrosis.

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