Effect of bilateral sympathectomy in a rat model of dilated cardiomyopathy induced by doxorubicin

Raphael dos Santos Coutinho e Silva, BSc, Fernando Luiz Zanoni, DVM, PhD, Rafael Simas, PhD, Mateus Henrique Fernandes Martins da Silva, MD, Roberto Armstrong Junior, BSc, Cristiano de Jesus Correia, PhD, Ana Cristina Breithaupt Faloppa, PhD, and Luiz Felipe Pinho Moreira, MD, PhD

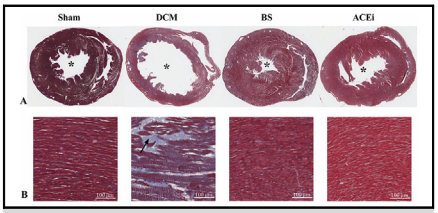
ABSTRACT

Objective: The study objective was to evaluate the effect of bilateral sympathectomy on ventricular remodeling and function in a rat model of dilated cardiomyopathy induced by doxorubicin.

Methods: Dilated cardiomyopathy was induced in male Wistar rats by weekly intraperitoneal injection of doxorubicin (2 mg/kg) for 9 weeks. Animals were divided into 4 groups: dilated cardiomyopathy; bilateral sympathectomy, submitted on day 15 of the protocol to bilateral sympathectomy; angiotensin-converting enzyme inhibitor, treated with enalapril through day 15 until the end of the experimental protocol; and sham, nonsubmitted through doxorubicin protocol, with weekly intraperitoneal injections of saline solution (0.9%). The left ventricular function was assessed, and the heart was collected for posterior analyses.

Results: The dilated cardiomyopathy group presented a significant decrease in the myocardial efficiency when compared with the sham group (33.4% vs 71.2%). Only the bilateral sympathectomy group was able to preserve it (57.5%; $P = .0001$). A significant dilatation in the left ventricular chamber was observed in the dilated cardiomyopathy group (15.9 μm^2) compared with the sham group (10.2 μm^2; $P = .0053$). Sympathectomy and enalapril prevented ventricular remodeling (9.5 and 9.6 μm^2, respectively; $P = .0034$). There was a significant increase in interstitial myocardial fibrosis in the dilated cardiomyopathy group (14.8%) when compared with the sham group (2.4%; $P = .0001$). This process was significantly reduced with sympathectomy and enalapril (8.7 and 3.9%, respectively; $P = .0001$).

Conclusions: Bilateral sympathectomy was effective in preventing remodeling and left ventricular dysfunction in a rat model of dilated cardiomyopathy induced by doxorubicin. (J Thorac Cardiovasc Surg 2020;160:e135-44)



BS attenuates ventricular remodeling and myocardial fibrosis.

Central Message

BS prevents LV remodeling and preserves cardiac function in a doxorubicin model of DCM.

Perspective

Our premise was that sympathetic blockade could attenuate LV dysfunction and remodeling in a DCM model. In rats, BS effectively preserved cardiac function and prevented LV remodeling, which was associated with diminished myocardial extracellular fibrosis and apoptosis.

See Commentaries on pages e145 and e147.

Dilated cardiomyopathy (DCM), characterized by ventricular dilatation associated with a variable degree of systolic dysfunction,[1] is an important cause of cardiac transplantation. The standard medical treatment involves angiotensin-converting enzyme inhibitors (ACEIs) and beta-blockers. Although they improve cardiac function and survival, they do not prevent disease progression.[2]

The use of doxorubicin as a chemotherapeutic agent frequently induces myocardial toxicity, making it an optimal and widely used experimental model for DCM. This anthracycline is associated with oxidative stress and mitochondrial damage, which together promote cardiomyocyte apoptosis,[3,4] leading to cardiac remodeling and subsequent loss of function.

Although sympathetic activation initially may preserve ventricular function, its long-term activity aggravates left ventricular (LV) remodeling, worsening cardiac function.[5,6]

From the Laboratório Cirúrgico de Pesquisa Cardiovascular (LIM-11), Instituto do Coração (Incor), Hospital das Clínicas HCFMUSP, Faculdade de Medicina, Universidade de São Paulo, São Paulo, Brazil.

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Address for reprints: Luiz Felipe Pinho Moreira, MD, PhD, Laboratório Cirúrgico de Pesquisa Cardiovascular (LIM-11), Instituto do Coração (Incor), Hospital das Clínicas HCFMUSP, Faculdade de Medicina, Universidade de São Paulo, São Paulo, BR, Av. Dr Eneas Carvalho de Aguiar, 44, Sao Paulo, SP 05403-900, Brazil (E-mail: luiz.moreira@incor.usp.br).

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Abbreviations and Acronyms

ACEI = angiotensin-converting enzyme inhibitor
BS = bilateral sympathectomy
DBT = dobutamine
DCM = dilated cardiomyopathy
HR = heart rate
ICAM = intercellular adhesion cellular molecule
LS = left sympathectomy
LV = left ventricular
LVEF = left ventricular ejection fraction
LVSW = left ventricular stroke work
MMP = matrix metalloproteinase
PRSW = preload recruitable stroke work
TBST = tris-buffered saline Tween-20
VEGF = vascular endothelial growth factor

In this regard, surgical thoracic sympathetic blockade has been investigated as an alternative treatment for heart failure.[7-9] In line with this, we have previously shown the importance of bilateral sympathectomy (BS) in the prevention of LV remodeling in a myocardial infarction model.[5] We aimed to evaluate the effects of thoracic BS on LV remodeling and function in a rat model of DCM induced by doxorubicin.

MATERIALS AND METHODS

The experiments were performed under the Brazilian College of Animals Experimentation ethical principles and approval from the Animal Subject Committee of the University of São Paulo Medical School (CEUA-FMUSP #079/16). This study was financed in part by the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior–Brasil-Finance Code 001.

Dilated Cardiomyopathy Induction and Experimental Groups

Thirty-nine male Wistar rats, weighing 350 ± 50 g, were randomly divided into groups: DCM only (n = 12), BS with DCM (n = 10), ACEI with DCM (n = 10), and a negative control group (sham) (n = 7). DCM induction was performed in the experimental groups through intraperitoneal injection of 18 mg/kg (cumulative) of doxorubicin (Laboratórios IMA S.A.I.C., Bueno Aires, Argentina), divided into 9 weekly doses of 2 mg/kg. The sham group received injections with the same volume containing only saline solution (0.9%).

Sympathectomy

Fifteen days after DCM induction, BS was performed through bilateral chemical sclerosis of the stellate ganglion with 100 μL of absolute ethanol in the BS group, using a modified percutaneous lateral technique.[10] BS was clinically confirmed through bilateral and nonreversible palpebral ptosis.

Pharmacologic Treatment

From day 15 of DCM induction to the end of the experimental protocol, the ACEI group received daily athwart water ingestion ad libitum enalapril maleate (Sigma-Aldrich, St Louis, Mo) at a 100 mg/L dilution.

Left Ventricular Function Evaluation

Ten weeks after the start of DCM induction, animals were anesthetized in a closed chamber with isoflurane in oxygen (fraction of inspired oxygen, 100%), orotracheal intubated, and mechanically ventilated in a rodent ventilator (Harvard Apparatus, Holliston, Mass). Anesthesia was maintained through the experiments with 2% isoflurane, a tidal volume of 10 mL/kg, and 70 breaths per minute. The right carotid artery was dissected, and a 2F microtip pressure-conductance catheter (SPR-838-MPVS-Ultra; Millar Instruments, Houston, Tex) connected to a data acquisition system (PowerLab, AD Instruments, Colorado Springs, Colo) was inserted into the LV for the continuum registry of the pressure volume loops. Data were obtained at steady-state conditions and after dobutamine (DBT) infusion for the following LV parameters: stroke work, stroke volume, end-diastolic volume, end-systolic volume, end-systolic pressure, ejection fraction, heart rate (HR), maximal slope of systolic pressure increment (dP/dt max), and time constant of pressure decay (tau).

To assess the LV response to a preload reduction, the inferior vena cava was transiently compressed. The following parameters were acquired after preload maneuver: preload recruitable stroke work (PRSW), end-diastolic volume relationship, slope of end-systolic - pressure volume relationship, slope of end-diastolic - pressure volume relationship. For the DBT protocol, we continuously infused 5 μg/kg/min of DBT for 10 minutes and acquired data for 5 minutes; after this, we doubled the dosage to 10 μg/kg/min for 10 minutes and acquired data for 5 minutes. The myocardial mechanical efficiency was calculated from the relationship between the pressure volume area and the left ventricular stroke work (LVSW) under steady-state and pharmacologic stress conditions. The volume calibration and parallel conductance volume calibration were performed as described by Zanoni and colleagues.[5]

Histologic Analyses

After LV function analyses, the rats were euthanized by infusion of hyperkalemic solution (19% KCl), which arrested the hearts in diastole. After organ harvest, the hearts were immersed in buffered formalin for 24 hours. Paraffin sections at the level of the papillary muscles were separated for histologic staining or immunohistochemistry reactions. For morphometric and histologic analyses, the slices were stained with Masson's trichrome staining. The following morphometric parameters were acquired: LV, right ventricular and interventricular septum thickness, and LV chamber size. Myocardial extracellular fibrosis was evaluated on the LV free wall under 20 × augmentation.

Immunohistochemistry

Previously paraffined heart sections (n = 6 for each group) were deparaffinized, hydrated, and incubated with citrate buffer (pH 6.0) for antigen retrieval. Nonspecific sites and endogenous peroxidase were blocked with tris-buffered saline Tween-20 (TBST) containing 2% bovine serum albumin and 2% hydrogen peroxide. Sections were incubated in TBST supplemented with 2% bovine serum albumin and rat antibodies to α-actin (1:500, Abcam, Burlingame, Calif), intercellular adhesion cellular molecule-1 (ICAM-1) (1:50, Santa Cruz, Dallas, Tex), vascular adhesion cellular molecule-1 (1:400, Abcam), rabbit antibodies to caspase-3 (1:100, Abcam), Bcl-2 (1:200, Abcam), or matrix metalloproteinase-9 (MMP-9, 1:200, Abcam). Sections were washed with TBST, incubated with anti-mouse or anti-rabbit secondary antibodies (1:200) conjugated to horseradish peroxidase (Millipore, Burlington, Mass), and counterstained with hematoxylin. Images were captured by a digital camera (DS-Ri1, Nikon, Tokyo, Japan) coupled to a microscope, and analyses were performed using the NIS-Elements-BR software (Nikon).

Enzyme-Linked Immunosorbent Assays

Previously frozen LV samples (n = 6 for each group) were weighed and homogenized with phosphate-buffered saline (3 mL/g tissue) in a TissueLyser. The LV homogenate supernatants were collected after centrifugation and stored at −80°C. The vascular endothelial growth factor (VEGF) concentration (R&D Systems Inc, Minneapolis, Minn) was determined according to the manufacturer's protocol.

mRNA Assays

After euthanasia, the LV myocardium free wall samples were conditioned in RNA later (n = 6 for each group), immediately frozen in liquid nitrogen, and stored at −80°C. A mirVana miRNA Isolation Kit (Ambion, Austin, Tex) was used for RNA extraction, according to the manufacturer's specifications. The cDNA was synthesized using the HighCapacity cDNA Reverse Transcription Kit (Applied Biosystems, Foster City, Calif). Real-time polymerase chain reaction was performed using TaqMan Universal PCR MasterMix and TaqMan Gene Expression Assay with the StepOnePlus Real-Time PCR System (Applied Biosystems) against MMP-9 (Rn00579162_m1) and β-actin (Rn00667869_m1). The result values were normalized in relation to the sham group data.

Statistical Analysis

The data were analyzed by Prism 6.0 (GraphPad Software Inc, San Diego, Calif), and all results were expressed as mean ± standard error of the mean or median and interquartile range. The minimal number of 7 animals per group was defined on the basis of an expected difference of more than 1 standard deviation in morphometric and hemodynamic variables. The differences between groups were assessed by 1-way or 2-way analysis of variance with repeated measures over the condition factor for morphometric or hemodynamic variables. These tests were followed by pairwise comparisons in relation to DCM group with Bonferroni corrections with *P* values adjusted to account for multiple comparisons. In the remaining data, the differences were assessed by Kruskal–Wallis test, followed by Dunn's comparison with *P* values adjusted to account for multiple comparisons.

RESULTS

There were no deaths in the sham group (n = 7). Ascites were observed in all animals submitted to the doxorubicin infusion, and 7 of these rats had died by the end of the experimental protocol. The DCM group had a higher mortality rate (42%), and both treated groups had a mortality rate of 10%, although no statistical differences were observed.

Analysis of Left Ventricular Function

Steady-state hemodynamics. The analyzed hemodynamics parameters are presented in Table 1. Under steady-state conditions, the DCM rats showed a significant decrease in left ventricular ejection fraction (LVEF), tau, and HR in relation to the sham group. There was also an increase in LV end-diastolic volume and LV end-systolic volume compared with the sham group. BS was able to prevent the decay in LVEF and increase LV systolic volume compared with the DCM group. There were no significant differences between the ACEI and DCM groups. As for the other parameters analyzed (LVSW, LV end-systolic pressure, and dP/dT max), no differences among the groups were observed.

Hemodynamics during dobutamine stimulation. Even after DBT infusion (Table 1), the DCM group presented with a reduced LVEF in relation to the sham group. An increase in LV end-diastolic volume and LV end-systolic volume, and a reduction in HR were observed in the DCM group compared with the sham group. Both BS and ACEI prevented LVEF, LVSW, and LV systolic volume decay when compared with the DCM group. The other measurements (LV end-systolic pressure, dP/dT max, and tau) were not significantly different among the groups.

Preload maneuver. The PRSW was reduced in the DCM group compared with the sham group. Only the BS group was able to improve this parameter in relation to the DCM group. There were no significant differences in the other measurements obtained after the preload maneuver among the groups (end-diastolic volume relationship, end-systolic - pressure volume relationship, and end-diastolic - pressure volume relationship) (Figure 1).

Myocardial mechanical efficiency. In regard to the myocardial efficiency analysis, the DCM group had a reduced contractile activity in all conditions compared with the sham group. The BS group was able to preserve myocardial efficiency in both steady-state and under DBT infusion. Unlike the BS group, the ACEI group recovered only after stimulation with DBT (Table 1).

Histologic Analysis

The histologic and morphometric data are presented in Table 2. In regard to the morphometric analysis, the DCM group showed an enlarged LV chamber compared with the sham group, and both treatments were able to reduce this dilation. As for the thickness parameters, the DCM group presented with a thinner LV and interventricular septum wall compared with the negative control group. Sympathectomy was able to inhibit the loss of LV cardiac muscle (Figure 2).

With regard to the myocardial fibrosis analysis, the DCM group presented with a significant increase in connective tissue when compared with the sham group (Table 2), whereas both treatment groups showed significant reductions when compared with the DCM group (Figure 2).

Alpha-Actin and Apoptosis Analyses

Figure 3 shows a nonsignificant increase in α-actin expression in the DCM group compared with the sham group. ACEI treatment was able to decrease this expression in myocardial tissue. Also, the DCM group had an increase in the expression of the pro-apoptotic protein, caspase 3, in comparison with the sham group; the BS group was effective in reducing this protein expression. Furthermore, both BS and ACEI groups showed a significant increase in the anti-apoptotic protein, Bcl-2, when compared with the DCM group (Figure 3).

TABLE 1. Left ventricular hemodynamic parameters

	Sham (n = 7)	DCM (n = 7)	BS (n = 9)	ACEI (n = 9)	*P* ANOVA
Stroke work (mm Hg*μL)					
Steady-state	6836 ± 908	3047 ± 611	8986 ± 781	6961 ± 1052	
Dbt 5.0	7118 ± 1410	5911 ± 1891	13,720 ± 2143*	12,030 ± 1691*	.0119
Dbt 10.0	7379 ± 1542	6430 ± 1974	14,870 ± 2464*	13,070 ± 1764*	
Stroke volume (μL)					
Steady-state	77.7 ± 7.9	56.7 ± 8.3	106.7 ± 6.9*	86.6 ± 10.8	
Dbt 5.0	91.3 ± 11.1	81.7 ± 10.5	126.6 ± 11.2*	129.2 ± 15.1*	.0156
Dbt 10.0	92 ± 12.4	92.2 ± 12.7	137 ± 15.3*	145.6 ± 16.2*	
End-diastolic volume (μL)					
Steady-state	125.3 ± 21.9*	241.6 ± 36.1	201.9 ± 17.2	210.6 ± 21.1	
Dbt 5.0	128.9 ± 24.3*	234.2 ± 31.6	178.1 ± 10.9	187.9 ± 19.8	.0239
Dbt 10.0	125.7 ± 24.1*	225.6 ± 30.5	175.6 ± 12.8	174.4 ± 19.7	
End-systolic volume (μL)					
Steady-state	68.6 ± 15.5*	170.0 ± 48.0	119.7 ± 13.7	156.3 ± 17.6	
Dbt 5.0	57.7 ± 15.9*	142.3 ± 48.2	70.2 ± 3.6	78.8 ± 11.2	.0655
Dbt 10.0	52.0 ± 15.6	122.7 ± 48.5	55.1 ± 5.8	47.3 ± 5.2	
End-systolic pressure (mm Hg)					
Steady-state	121.4 ± 5.6	95.67 ± 16.7	115.3 ± 12.6	111.8 ± 5.2	
Dbt 5.0	116.4 ± 5.8	103.5 ± 19.2	135.8 ± 13.3	110.6 ± 5.0	.315
Dbt 10.0	113.9 ± 5.0	101.2 ± 18.2	132.7 ± 13.0	105 ± 5.6	
Ejection fraction (%)					
Steady-state	61.7 ± 5.2*	29.7 ± 3.1	54.7 ± 3.5*	40.8 ± 4.3	
Dbt 5.0	69 ± 5.3*	44.5 ± 8.1	71.6 ± 2.4*	69 ± 3.2*	.0001
Dbt 10.0	69.9 ± 5.9	54.3 ± 11.5	77.9 ± 4.4*	84.3 ± 2.2*	
Heart rate (beats/min)					
Steady-state	381.1 ± 8.3*	271.0 ± 22.4	284.2 ± 11.7	288.3 ± 12.3	
Dbt 5.0	391.1 ± 9.5*	326.7 ± 28.7	362.2 ± 10.7	352.8 ± 16.0	.0121
Dbt 10.0	389.7 ± 9.0*	333.0 ± 24.4	373.9 ± 10.0	367.8 ± 14.8	
dP/dt max (mm Hg/s)					
Steady-state	6867 ± 229	4705 ± 1111	5624 ± 628	5923 ± 464	
Dbt 5.0	7501 ± 245	7170 ± 1679	8788 ± 934	8265 ± 461	.5953
Dbt 10.0	7510 ± 149	7122 ± 1597	9097 ± 1015	8420 ± 419	
Tau (ms)					
Steady-state	11.3 ± 0.4*	21.9 ± 6.3	16.4 ± 1.6	14.9 ± 2.1	
Dbt 5.0	10.5 ± 1.1	16.6 ± 4.7	12.2 ± 2.0	10.4 ± 0.9	.1599
Dbt 10.0	10.7 ± 1.1	17.5 ± 4.5	13.8 ± 2.8	10.8 ± 1.0	
Efficiency (%)					
Steady-state	71.2 ± 2.6*	33.4 ± 4.4	57.2 ± 3.1*	47.7 ± 5.6	
Dbt 5.0	83.1 ± 2.6*	48.5 ± 9.9	75.5 ± 3.4*	76.4 ± 3.3*	.0001
Dbt 10.0	86.8 ± 4.8*	57.5 ± 12.2	83.7 ± 2.4*	86.7 ± 2.0*	

Data presented as mean ± standard error of the mean. *DCM*, Dilated cardiomyopathy; *BS*, bilateral sympathectomy; *ACEI*, angiotensin-converting enzyme inhibitor; *ANOVA*, analysis of variance; *Dbt 5.0*, data acquired with DBT 5 μg/kg/min, *Dbt 10.0*, data acquired with DBT 10 μg/kg/min. *Bonferroni's multiple comparisons test $P < .05$ versus DCM group.

Protein Expression of Vascular Endothelial Growth Factor Concentration, Intercellular Adhesion Cellular Molecule, and Vascular Adhesion Cellular Molecule

The level of VEGF in the heart homogenate showed an increase in the DCM group in relation to the sham group. The BS group was able to normalize the levels of VEGF. There was no significant difference in ICAM-1 protein expression in heart tissue between the DCM and sham groups. However, both treatments amplified the ICAM-1 tissue expression in relation to the DCM group. As for the vascular adhesion cellular molecule-1 expression, no significant differences were observed between the groups (Figure 4).

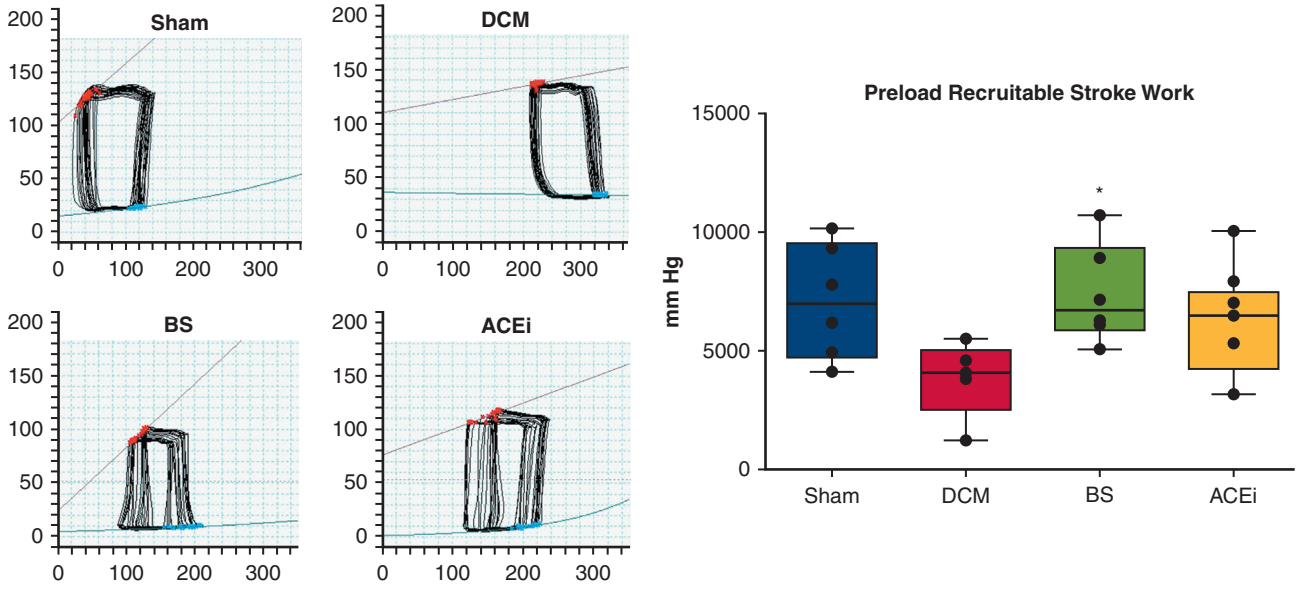

FIGURE 1. Data for PRSW. Representative pressure volume loop response to preload maneuver of each group. Data are not shown for dP/dt - end-diastolic volume relation, slope of end-systolic P-V relation, and slope of end-diastolic P-V relation. The *X-axis* shows the different groups. The *upper* and *lower* borders of the boxes represent the upper and lower quartiles. The *middle horizontal line* is the median value. Each measurement is shown as a *black dot*, and the *dots* outside of the box and *whiskers* represent outliers. *$P < .05$ versus DCM group. *Blue color*: sham group; *red color*: DCM group; *green color*: BS group; *yellow color*: ACEI group. *DCM*, Dilated cardiomyopathy; *BS*, bilateral sympathectomy; *ACEI*, angiotensin-converting enzyme inhibitor.

Matrix Metalloproteinase-9 Protein and Gene Expression

MMP-9 protein expression showed no differences between groups. Regarding extracellular matrix gene expression, the DCM group showed an upregulation of MMP-9 relative to the sham group. BS did not modify MMP-9 gene expression, and ACEI significantly downregulated MMP-9 when compared with the DCM group (Table 3).

DISCUSSION

The effects of BS and ACEI standard treatment on LV remodeling and function were evaluated using a DCM model, induced by doxorubicin infusion in rats. Untreated animals presented with LV functional decay across multiple parameters, associated with loss of myocardium tissue and a narrowed LV wall. Overall, myocardium mechanical efficiency was diminished, even after pharmacologic stimuli. Otherwise, BS preserved the LV function in both steady-state conditions, after pharmacologic stimuli with DBT, and through preload volume variations. LV remodeling was prevented by lower myocardial fibrosis. The expression of apoptotic markers was diminished, associated with attenuated LV tissue loss, characteristic of this DCM model (Figure 5). The ACEI standard treatment was also effective in preventing ventricular remodeling and reducing myocardial fibrosis. However, ACEI did not preserve LV wall thickness and LV function in steady-state conditions. Furthermore, these animals did not demonstrate an adequate response to preload maneuver, and only after DBT infusion was a functional improvement in the LV observed.

The beneficial effect of sympathetic blockade on cardiac diseases has been studied extensively, particularly the left

TABLE 2. Left ventricular histologic and morphometric analyses

	Sham (n = 7)	DCM (n = 7)	BS (n = 9)	ACEI (n = 9)	*P* ANOVA
LV chamber (μm^2)	10.21 ± 1.47*	15.97 ± 1.60	9.54 ± 1.18*	9.57 ± 0.62*	.0059
LV wall thickness (mm)	3.50 ± 0.17*	2.47 ± 0.17	2.94 ± 0.10*	2.48 ± 0.07	.0001
RV wall thickness (mm)	1.19 ± 0.07	0.91 ± 0.06	1.14 ± 0.13	1.18 ± 0.05	.0519
Septal thickness (mm)	2.51 ± 0.08*	1.99 ± 0.14	2.17 ± 0.17	1.88 ± 0.14	.0332
Fibrosis (%)	2.38 ± 0.78*	14.78 ± 0.91	8.73 ± 0.90*	3.91 ± 1.20*	.0001

Data are presented as mean ± standard error of the mean. *DCM*, Dilated cardiomyopathy; *BS*, bilateral sympathectomy; *ACEI*, angiotensin-converting enzyme inhibitor; *ANOVA*, analysis of variance; *LV*, left ventricle; *RV*, right ventricle. *Bonferroni's multiple comparisons test $P < .05$ versus DCM group.

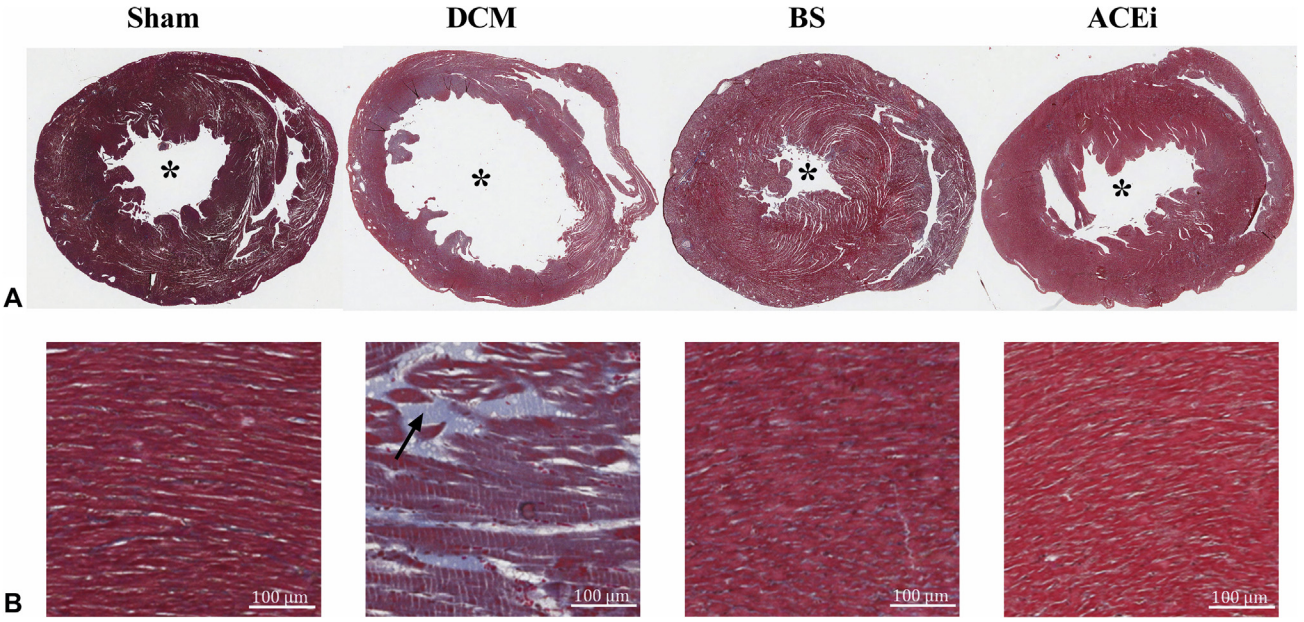

FIGURE 2. Representative photomicrographs of the LV myocardium stained with Masson's trichrome in gross section (A) or with 20 × augmentation, (B) showing the intensity of fibrotic tissue (*arrow*) in the 4 groups. *DCM*, Dilated cardiomyopathy; *BS*, bilateral sympathectomy; *ACEI*, angiotensin-converting enzyme inhibitor. *Represents the lumen of the left ventricle.

sympathectomy (LS) influence in patients with life-threatening arrhythmias, such as long QT syndrome.[11] In this regard, LS has been shown to be effective in reducing the incidence of implantable cardioverter-defibrillator use and improving the patients' quality of life.[12] Furthermore, LS was tested in patients with DCM with reduced ventricular function.[7,9] Despite the short follow-up time and the limited number of patients, LS could improve LVEF and ameliorate exercise performance.[7]

Nevertheless, other authors have not consistently shown positive effects of LS; this can be explained by nervous hypertrophy and the following structural changes in the superior cervical ganglion on the contralateral to the one on region that the resectomy was performed.[13] A nervous plasticity in the contralateral ganglion has been described after LS in patients with ventricular arrhythmia, and BS has been shown to be more beneficial than LS in terms of arrhythmia control in these patients.[14] Recently, BS was performed in a patient with nonischemic DCM and refractory ventricular tachycardia, New York Heart Association functional class IV, with 15% LVEF. After 1 year of follow-up, the patient had no implantable cardioverter-defibrillator shock, the patient's LVEF increased to 25%, and the patient was removed from the transplant list.[15]

In the same line, the effect of LS was compared with BS in infarcted rats; BS effectively controlled sympathetic

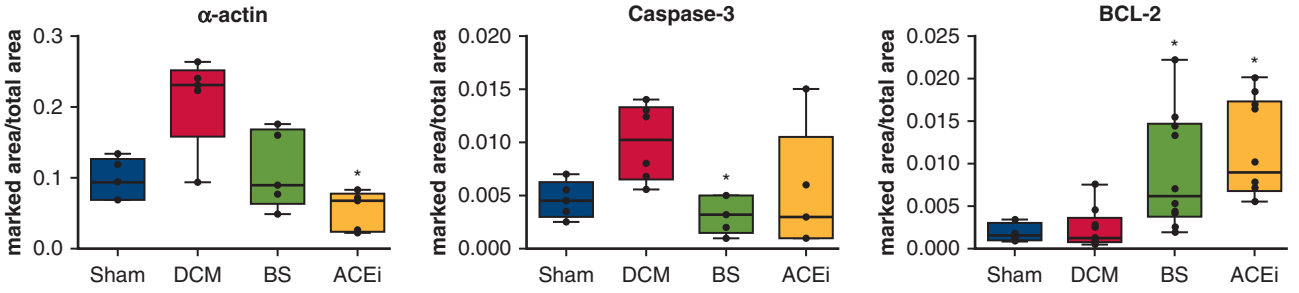

FIGURE 3. Alpha-actin, caspase-3, and BCL-2 protein expression in LV myocardium. The *X-axis* shows the different groups. The *upper* and *lower* borders of the boxes represent the upper and lower quartiles. The *middle horizontal line* is the median value. Each measurement is shown as a *black dot*, and the *dots* outside of the box and *whiskers* represent outliers. *$P < .05$ versus DCM group. *Blue color*: sham group; *red color*: DCM group; *green color*: BS group; *yellow color*: ACEI group. *DCM*, Dilated cardiomyopathy; *BS*, bilateral sympathectomy; *ACEI*, angiotensin-converting enzyme inhibitor.

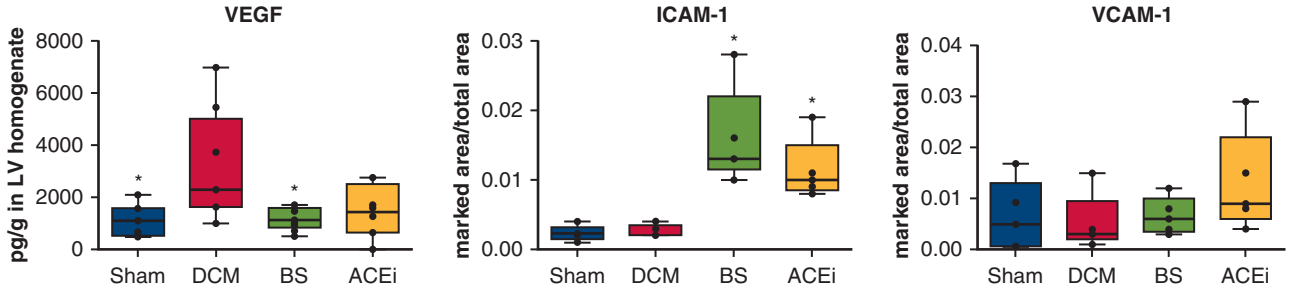

FIGURE 4. VEGF, ICAM-1, and vascular adhesion cellular molecule-1 (*VCAM-1*) protein expression in LV myocardium. The *X-axis* shows the different groups. The *upper* and *lower* borders of the boxes represent the upper and lower quartiles. The *middle horizontal line* is the median value. Each measurement is shown as a *black dot*, and the *dots* outside of the box and *whiskers* represent outliers. *$P < .05$ versus DCM group. *Blue color*: Sham group; *red color*: DCM group; *green color*: BS group; *yellow color*: ACEI group. *LV*, Left ventricle; *DCM*, dilated cardiomyopathy; *BS*, bilateral sympathectomy; *ACEI*, angiotensin-converting enzyme inhibitor; *VEGF*, vascular endothelial growth factor; *ICAM*, intercellular adhesion cellular molecule.

hyperactivity and the associated ventricular remodeling and dysfunction, whereas LS was unsuccessful in controlling these parameters.[5] These findings reinforce that the complete interruption of the sympathetic overdrive may disrupt different pathways associated with myocardial remodeling and can be of major importance in preserving cardiac function in the most diverse pathologies, such as myocardial infarction and DCM.

In the current study, the LV wall thickness maintenance, associated with diminished fibrosis, could be related to a better functional response in the BS group. Even under steady-state conditions, BS preserved LVEF and stroke volume. Animals in the BS group showed a positive response to the preload decrease by compression of the vena cava, assessed through PRSW. In contrast, steady-state LV performance and the positive response to the volume decrease were not observed in animals treated with ACEI, a finding that could be related to LV tissue loss.

Both BS and ACEI were effective in reducing myocardial fibrosis, an important mechanism related to DCM remodeling. Fibrosis develops from cardiomyocyte apoptosis and subsequent substitution of these cells for conjunctive tissue. Doxorubicin-induced DCM amplifies MMP-9 expression, a metalloproteinase involved in the degradation of the cardiac extracellular matrix, which can be associated with tissue fibrosis.[16] BS did not change protein or gene expression of MMP-9. In contrast, ACEI significantly reduced gene expression of this metalloproteinase. Augmented levels of MMPs are associated with increased fibrosis in myocardial tissue when their activity is elevated. In our study, the activity of MMPs was not measured, which precludes us from asserting that the elevated MMP-9 expression in the BS group is active, because the fibrosis was in fact diminished.

The type of lesion observed in doxorubicin-induced DCM elevates the proapoptotic protein expression of caspase-3, as well as reducing antiapoptotic protein expression of Bcl-2.[17,18] The protective role of BS was associated with diminished tissue apoptosis, in which caspase-3 tissue expression was reduced and that of Bcl-2 was augmented. The reduction of cellular death and consequent attenuation of the remodeling process through sympathetic inhibition is highly relevant, especially because BS was effective in the current study even on an experimental model that acts mainly through direct cardiomyocyte injury.

This reduction in apoptosis seems to be related to a decrease in LV protein levels of α-actin. High levels of this protein can be associated with an increase in cardiomyocyte apoptosis and consequent release of α-actin from the tissue in the untreated group. Once cellular death is attenuated in a model with direct fiber injury, we postulate eventual BS success on ventricular remodeling in patients with DCM triggered by other causes. Such influence has shown to be effective in a myocardial infarction model in rats, in which BS has decreased caspase-3 expression and

TABLE 3. Matrix metalloproteinase-9 protein and gene expression in left ventricle

MMP-9	Sham (n = 7)	DCM (n = 7)	BS (n = 9)	ACEI (n = 9)	*P* value
Protein expression (marked area/total area)	0.031 ± 0.009	0.042 ± 0.015	0.055 ± 0.007	0.077 ± 0.030	.4593
Gene expression (relative to sham)	-	22.80 ± 8.12	17.87 ± 6.54	0.79 ± 0.42*	.0012

Data are presented as median ± interquartile variation. *MMP-9*, Matrix metalloproteinase-9; *DCM*, dilated cardiomyopathy; *BS*, bilateral sympathectomy; *ACEI*, angiotensin-converting enzyme inhibitor. *Dunn's multiple comparisons test $P < .05$ versus DCM group.

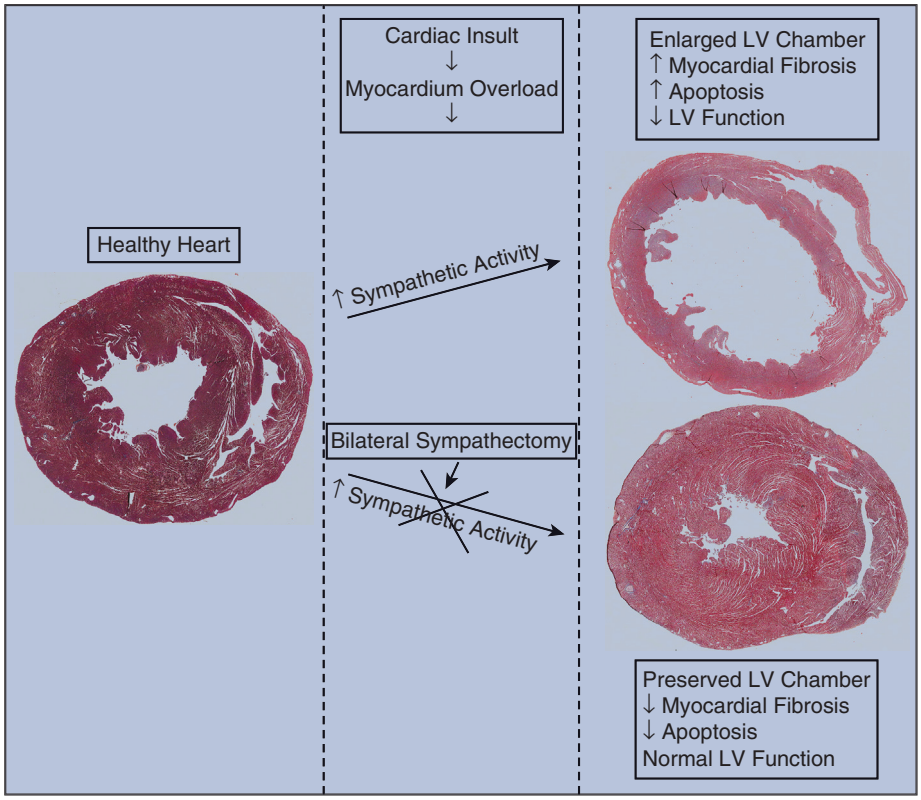

FIGURE 5. After a cardiac insult and myocardium overload, the sympathetic nervous system activity increases as a compensatory mechanism. However, this mechanism leads to further decompensation with ventricular remodeling observed by enlarged left ventricle, myocardial extracellular fibrosis, increased apoptosis and subsequent loss of function. With sympathetic blockade, LV remodeling was prevented and cardiac function was preserved. *LV,* Left ventricle.

augmented Bcl-2. Therefore, regarding LV remodeling, BS effectively preserved LV structure.[5]

The protective role of BS was also related to the increased ICAM-1 protein expression. An increase in ICAM-1 expression usually is associated with higher tissue inflammation, because ICAM-1 is a molecule that facilitates leukocyte rolling and adhesion to the blood vessel.[19] However, in skeletal muscle cell culture, ICAM-1 expression increased myogenesis events in which myotube formation, nuclei addition, alignment, fusion, protein synthesis, and hypertrophy occur without a negative influence on proliferation and differentiation.[20] The expression of ICAM-1 by myofibroblasts and satellite cells after muscle overload was related to a hypertrophic response on skeletal muscle, facilitating myeloid cell ligation and cell–cell communication. Therefore, we can speculate that in our model, the same mechanism could offer cardiac muscle protection and prevent ventricular remodeling.[21]

VEGF expression is another important mechanism associated with ventricular remodeling, known for its main role in angiogenesis, stabilization of newly formed vessels, vasodilatation, and stem cell recruitment.[22] In this study, VEGF was overexpressed in the control group, accounted by doxorubicin oxidative stress, which was responsible for activating VEGF pathway.[23] BS normalized VEGF protein expression, which was related to the reduction in apoptosis and fibrosis in myocardial tissue, and the consequent attenuation of LV remodeling. The same phenomenon was observed in infarcted rats submitted to BS; VEGF levels were decreased after treatment with BS, which was related to the attenuation of the LV remodeling process.[5]

The hypothesis that BS acts on remodeling and control of fibrosis by decreasing the cellular death in cardiac tissue is supported by the present results. The current literature corroborates the finding that BS is more effective than isolated LS in controlling the sympathetic hyperactivity and the occurrence of ventricular arrhythmias.[11,12] Reduced fibrosis is a major therapeutic mechanism because fibrotic tissue can disrupt heart electrophysiology and act as a potential trigger for life-threatening arrhythmias. Notably, sudden death is the second most common outcome in

patients with DCM, highlighting another potential beneficial effect of BS.[24,25]

However, stating the efficacy of pharmacologic treatment, specifically through the use of beta-blockers, is important.[26] More recently, clinical trials have inclusively shown a protective prophylactic effect of carvedilol beta-blockers in patients with cancer treated with anthracyclines.[27,28] Nonetheless, the continued use of beta-blockers was associated with a series of collateral effects. High-dosage exposure of nonselective beta-blockers was correlated with a higher incidence of mild and severe asthma exacerbation in asthmatic patients with cardiovascular diseases.[29] Beta-blocker toxicity usually results in cardiac shock due to myocardial depression, bradycardia, and reduced contractility.[30,31] Abrupt discontinuation of beta-blockers after long-term treatment can cause angina and increase the risk of sudden death.[32] According to the National Poison Data System, beta-blockers were responsible for 3.37% of deaths by toxicity in the United States in 2016. Furthermore, calcium channel blockers and beta-blockers are responsible for 65% of deaths resulting from cardiovascular medications.[33]

Study Limitations

The doxorubicin rat model induces DCM mainly through direct lesion of the cardiomyocyte, leading to cardiomyocyte apoptosis and further replacement for fibrotic tissue. The following ventricular remodeling process diminishes LV function. Although this model induces a similar DCM to the one observed in humans, it does not fully simulate the many forms of human DCM. Another limitation is that the doxorubicin model has a high mortality rate that precludes a longer follow-up and a major delay for the sympathectomy procedure. The current intervention time occurred after the beginning of the myocardial compromise process. However, the myocardial remodeling process is continuous in DCM, which means that greater beneficial effects can be expected for the performance of BS. This characteristic also justifies an expected benefit with the intervention at any time point.

Finally, a combined group of ACEI and BS was not included because an absence of detected interaction between both treatments would be expected because of the significant isolated effects of them in a small animal population. However, because of the observed difference between the mechanisms in both groups, some independent benefit should be expected in the clinical setting.

CONCLUSIONS

BS in experimental models showed positive results for preventing ventricular remodeling and preserving cardiac function. This encouraging response may be associated with the previously described positive results of thoracic sympathectomy in prevention of refractory ventricular arrhythmias and sudden death in patients with heart failure with various etiologies. However, only 2 clinical trials based on the use of sympathectomy are currently active. One study has aimed to investigate the use of LS on patients with DCM,[34] and another evaluated the use of BS in patients with ventricular tachyarrhythmia.[35] Therefore, the use of BS in patients with DCM should be further investigated in clinical trials to complement the current medical therapy.

Conflict of Interest Statement

Authors have nothing to disclose with regard to commercial support.

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