RAPHAEL DOS SANTOS COUTINHO E SILVA

Perspectivas da simpatectomia torácica bilateral como tratamento da insuficiência cardíaca

Perspectives of bilateral thoracic sympathectomy for treatment of heart failure

Tese apresentada à Faculdade de Medicina da Universidade de São Paulo para obtenção do título de Doutor em Ciências

Programa de Cirurgia Torácica e Cardiovascular

Orientador: Prof. Dr. Luiz Felipe Pinho Moreira

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São Paulo

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“A mind needs books as a sword needs a whetstone if it is to keep its edge.”

G.R.R.Martin

Acknowledgments

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Resumo

Coutinho e Silva RS. Perspectivas da simpatectomia torácica bilateral como tratamento da insuficiência cardíaca [tese]. São Paulo: Faculdade de Medicina, Universidade de São Paulo; 2021.

A insuficiência cardíaca é o estágio final de diversas cardiopatias. Apesar do constante avanço no tratamento clínico da insuficiência cardíaca, esta afecção continua a ter alta mortalidade e morbidade. Novas opções de tratamento são importantes, uma vez que o número de transplantes cardíacos é escasso em relação ao número de pacientes no estágio final da doença. Portanto, avaliamos o uso da simpatectomia torácica bilateral como opção de tratamento em modelos experimentais de insuficiência cardíaca. Para tal avaliação, utilizamos os modelos de cardiomiopatia dilatada induzida por doxorrubicina e de hipertensão arterial pulmonar com disfunção do ventrículo direito induzida por monocrotalina. O modelo de cardiomiopatia dilatada ocasionou um remodelamento ventricular significativo, com aumento das câmaras cardíacas, perda de espessura do miocárdio e fibrose difusa. Estas alterações repercutiram na função ventricular esquerda, com queda da fração de ejeção, diminuição da reserva miocárdica, aumento do volume diastólico final e redução da eficiência cardíaca. Por outro lado, a simpatectomia bilateral se mostrou eficaz na prevenção do remodelamento ventricular, com diminuição importante da fibrose e da dilatação ventricular. Este fato levou a manutenção da função ventricular esquerda, com volume sistólico pré recrutável preservado e eficiência cardíaca conservada. Alterações semelhantes foram observadas no modelo de hipertensão arterial pulmonar. O grupo não tratado evoluiu com hiperplasia da parede das artérias pulmonares e aumento da expressão da actina de músculo liso. Observou-se também aumento da resistência vascular pulmonar, com consequente aumento da pós carga cardíaca, hipertrofia das paredes do ventrículo direito e queda dos indicadores de sua eficiência contrátil, como a dP/dT máxima e o volume sistólico pré recrutável. A disfunção do ventrículo direito foi ainda associada a hipertrofia dos cardiomiócitos e ao aumento significativo do estresse mitocondrial. A simpatectomia bilateral reduziu o remodelamento das artérias pulmonares, mitigando o remodelamento e a disfunção ventricular direita, sendo também associada a diminuição do estresse oxidativo nos

cardiomiócitos e a manutenção da atividade mitocondrial. Com base nestes resultados, podemos concluir que a simpatectomia torácica bilateral atua na proteção do miocárdio tanto em cardiopatias de lesão direta ao músculo cardíaco, como a cardiomiopatia dilatada por toxicidade, quanto em cardiopatias reativas, como a insuficiência cardíaca secundaria a hipertensão pulmonar. Entretanto, estudos clínicos controlados serão necessários para avaliar o seu potencial como opção de tratamento para a insuficiência cardíaca.

Descritores: Simpatectomia; Insuficiência cardíaca; Cardiomiopatia dilatada; Hipertensão arterial pulmonar; Remodelação ventricular; Estresse oxidativo.

Abstract

Coutinho e Silva RS. Perspectives of bilateral thoracic sympathectomy for treatment of heart failure [thesis]. São Paulo: “Faculdade de Medicina, Universidade de São Paulo”; 2021.

Heart failure is the final stage of several cardiopathies. Despite constant advances in the clinical treatment approaches of heart failure, it still has a high mortality and morbidity. New treatment options are important, as the number of heart transplants is limited in relation to the number of patients at the final stage of the disease. Therefore, we assessed the use of bilateral thoracic sympathectomy for treatment in experimental models of heart failure. For this assessment, we used a dilated cardiomyopathy model induced by doxorubicin and pulmonary arterial hypertension with a right ventricular dysfunction model induced by monocrotaline. The dilated cardiomyopathy model led to significant ventricular remodeling, with augmented heart chambers, loss of myocardium thickness, and diffuse fibrosis. These alterations reverberated on left ventricular function, with a decrease in ejection fraction, loss of myocardium reserve, increase in end-diastolic volume, and reduction in heart efficiency. On the other hand, bilateral thoracic sympathectomy was effective in preventing ventricular remodeling, with an important reduction in fibrosis and ventricular dilation. This led to maintenance of left ventricular function, with preserved preload recruitment stroke work and heart efficiency. Similar alterations were observed on the pulmonary arterial hypertension model. The untreated group showed progressive pulmonary arterial wall hyperplasia and amplified smooth muscle actin expression. There was also an increase in pulmonary vascular resistance, with consequent augmentation of cardiac afterload, hypertrophy of the right ventricular wall, and decreased contractile efficacy indicators, such as dP/dT max and preload recruitable stroke work. Right ventricular dysfunction was associated with cardiomyocyte hypertrophy and a significant increase in mitochondrial stress. Bilateral thoracic sympathectomy was able to reduce pulmonary artery remodeling and mitigate right ventricular remodeling and dysfunction. Furthermore, it was associated with decreased oxidative stress in cardiomyocytes and the maintenance of mitochondrial activity. Based on these results, we can conclude that bilateral thoracic sympathectomy protects the myocardium in both cardiopathies with direct lesions on the cardiac muscle, such as toxic

dilated cardiomyopathy, and reactive cardiopathies, such as heart failure secondary to pulmonary hypertension. However, controlled clinical trials are warranted to fully assess its potential as a treatment option for heart failure.

Descriptors: Sympathectomy; Heart failure; Cardiomyopathy, dilated; Pulmonary arterial hypertension; Ventricular remodeling; Oxidative stress.

Chapter 1

1. General introduction

1.1 Background

Heart failure (HF) is a progressive clinical syndrome with a high global morbidity and mortality despite increasing advances in therapeutics. According to the World Health Organization, 17.9 million people died from cardiovascular diseases in 2019[1]. HF affects 2% of the adult population. The survival in the first 5 years of diagnosis is less than 50%[2].

HF can result from several different conditions. The main etiologies are ischemic heart disease; cardiomyopathy, such as, dilated, Chagas disease, diabetic, and drug-induced; and right HF secondary to lung diseases, such as pulmonary arterial hypertension, chronic obstructive pulmonary disease, and mitral valve disease[3].

HF is classified according to ejection fraction (EF) levels. Patients with left ventricular EF (LVEF) <40% are defined as having HF with reduced EF (HFrEF). Patients with LVEF >50% are classified as having HF with preserved EF. More recently, patients with LVEF between 40% and 49% are classified as having HF with mid-range EF[4].

1.2 Pathophysiology

Regardless of the primary condition, a reduction in cardiac output activates compensatory mechanisms. The sympathetic nervous system (SNS) releases catecholamines, mainly norepinephrine, and increases heart rate and myocardial contractility. The constant release of norepinephrine activates the renin-angiotensin-aldosterone system (RAAS), the final product of which is angiotensin-II, a systemic vasoconstrictor[5-11].

These mechanisms preserve cardiac function in the early stages of the disease. However, the constant activation of the SNS and RAAS, with increased chronotropy, inotropy, and systemic vasoconstriction eventually leads to maladaptive ventricular remodeling. The cardiac function continues to decrease, sustaining the activation of the compensatory systems and perpetuating the vicious cycle[12-15].

1.3 Sympathectomy

The neurohormonal system is the main therapeutic target for HF treatment. The thoracic trunk of the SNS has been targeted for the treatment of life-threatening arrhythmias[16]. Since then, the surgical procedure of sympathectomy has greatly advanced and is

currently performed via thoracoscopy. Many clinical trials have been performed and proven the effectiveness of sympathectomy in decreasing the number of malignant arrhythmias and mitigating the number of implantable cardioverter-defibrillator (ICD) shocks[17-19].

A few studies have been performed on the use of sympathectomy to treat patients with HF. The effect of left thoracic sympathectomy (LS) was assessed in 10 patients with HFrEF. In a short follow-up period of 6 months, the patients had improved LVEF and quality of life, demonstrating the feasibility of the procedure[20,21]. Another study performed epidural bilateral thoracic blockade in HFrEF patients to assess the safety of the procedure[22]. Most recently, bilateral thoracic sympathectomy (BS) was performed in a non-ischemic dilated cardiomyopathy patient with NYHA Class IV and 15% LVEF. In the 1-year follow-up period, the patient had improved LVEF to 25%, had no ICD shocks, and the patient's name was removed of the transplant list [23].

In the experimental setting, the BS effect was compared to LS in a rat model of myocardial infarction. As LS showed no benefit on cardiac function or decreased myocardial fibrosis and remodeling, BS was highly effective. BS was able to maintain LVEF levels, diminish myocardial fibrosis, mitigate ventricular remodeling, and prevent cardiac function decay[24]. The superiority of BS over LS was also observed in another study; BS was more beneficial than LS in patients with ventricular arrhythmias, in terms of preventing arrhythmias and decreasing the number of ICD shocks[18].

2. Scope of the thesis

Experimental and clinical trials evaluating the effect of BS on HF are scarce and do not fully express its potential as a treatment or explain its mechanisms. However, few publications have reported positive results that encourage further studies. Thus, we aimed to evaluate the potential of BS as a treatment in experimental models of HF.

In Chapter 2, we opt for one of the main causes of HF to study BS as a treatment. Therefore, we used a rat model of dilated cardiomyopathy induced by doxorubicin to assess the effects of BS on ventricular remodeling and function. In Chapter 3, we chose pulmonary hypertension as the cause of ventricular remodeling to determine the effect of BS on a different mechanism of HF. Hence, in a pulmonary arterial hypertension rat

model induced by monocrotaline, we performed BS to evaluate its effects on pulmonary arterial remodeling and secondary right ventricular remodeling and function.

Finally, in Chapter 4, we published a review article on neuromodulation therapies for the treatment of HF, with a focus on thoracic sympathectomy studies, to fully describe the future perspectives of BS.

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Chapter 2

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

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

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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 μm2) compared with the sham group (10.2 μm2; P = .0053). Sympathectomy and enalapril prevented ventricular remodeling (9.5 and 9.6 μm2, 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.

Keywords: apoptosis; dilated cardiomyopathy; doxorubicin; left ventricular function; left ventricular remodeling; sympathectomy.

Chapter 3

Thoracic bilateral sympathectomy attenuates oxidative stress and prevents ventricular remodeling in experimental pulmonary hypertension

Raphael dos Santos Coutinho e Silva, Lucas Moritz Wiggenhauser, Rafael Simas, Fernando Luiz Zanoni, Geisla Medeiros, Fernanda Beatriz da Silva, Daniel Cury Ogata, Ana Cristina Breithaupt Faloppa, Guido Krenning, Luiz Felipe Pinho Moreira.

In submission

Abstract

Aim

Pulmonary arterial hypertension (PAH) is a cardiopulmonary disease that affects the pulmonary vasculature, leading to increased afterload and eventually right ventricular (RV) remodeling and failure. Bilateral sympathectomy (BS) has shown promising results in dampening cardiac remodeling and dysfunction in several heart failure models. In the present study, we investigated whether BS reduces pulmonary arterial remodeling and mitigates RV remodeling and failure.

Methods and results

PAH was induced in male Wistar rats by intraperitoneal injection of monocrotaline. Rats were divided into 3 groups, involving untreated PAH, BS-treated PAH, and non-manipulated control rats. Three weeks after PAH induction, the rats were anesthetized, and RV function was assessed via the pressure-volume loop catheter approach. Upon completion of the experiment, the lungs and heart were harvested for further analyses. BS was found to prevent pulmonary artery remodeling, with a clear reduction in α-smooth muscle actin and endothelin-1 expression. RV end-systolic pressure was reduced in the BS group, and preload recruitable stroke work was preserved. BS therefore mitigated RV remodeling and cardiomyocyte hypertrophy and diminished oxidative stress.

Conclusions

We showed that thoracic BS may be an important treatment option for PAH patients. Blockade of the sympathetic pathway can prevent pulmonary remodeling and protect the RV from oxidative stress, myocardial remodeling, and function decay.

Keywords: sympathetic blockade[1]; pulmonary hypertension[2]; ventricular remodeling[3]; pulmonary artery remodeling[4]; oxidative stress[5].

1. Introduction

Pulmonary arterial hypertension (PAH) is a cardiopulmonary disease that affects both the pulmonary vasculature and the right ventricle (RV). Irrespective of the primary etiology, PAH is characterized by vascular remodeling of the pulmonary artery resulting in increased pulmonary vascular resistance (PVR), which leads to high RV afterload and the development of RV failure—the leading cause of death in PAH patients[1,2]. While current therapeutic approaches are symptomatic and based on vasodilative and anti-proliferative drugs, none of the standard treatments are curative[3].

A vicious cycle between states of oxidative stress and inflammation aggravates PAH development and activates the sympathetic nervous system (SNS)[4]. Norepinephrine released by the SNS promotes pulmonary arterial smooth muscle cell (PASMC) remodeling via the ERK-1/2 pathway[5]. Autonomic nervous system imbalance is associated with disease progression, which leads to RV failure and increased mortality[6]. Expectedly, sympathetic blockade has been shown to be effective in dampening ventricular dysfunction in several heart failure models.

Bilateral sympathectomy (BS) has been observed to prevent cardiac remodeling and preserve left ventricular function in experimental models of myocardial infarction[7] and dilated cardiomyopathy[8]. In PAH models, partial sympathetic blockade inhibited ERK-1/2-mediated vascular remodeling in PASMCs[9] and attenuated PAH progression via the nitric oxide (NO) pathway[10]. Nevertheless, the underlying mechanisms of sympathetic blockade still remain elusive.

As SNS seems to play a central role in PAH development and RV failure, we aimed to investigate whether BS ameliorates pulmonary artery remodeling and secondary RV failure and to evaluate its mechanism of action in a monocrotaline-induced PAH rat model.

2. Methods

All experiments were performed in accordance to the Ethical Principles for Animal Research proposed by the Brazilian College of Animal Experimentation. Approval was obtained from the Animal Subject Committee of the University of São Paulo Medical School (CEUA-FMUSP #033/17).

2.1 Animals and procedures

Male Wistar rats, weighing an average of 350 ± 30 g, were randomly divided into 3 groups the untreated PAH group (PAH, n=15), the BS-treated PAH group (BS, n=13), and the control group (n=13). PAH was induced in both the PAH and BS groups through a single intraperitoneal injection of monocrotaline (60 mg/kg; Sigma Aldrich, San Luís, Missouri, USA), while the control group was not manipulated.

Before PAH induction in the BS group, bilateral chemical sclerosis of the stellate ganglion was performed as previously described[8]. BS was confirmed by observation of bilateral and non-reversible palpebral ptosis.

2.2 Right ventricular function evaluation

Three weeks after PAH induction, the rats were anesthetized with 2% isoflurane (FiO_2 100%), followed by orotracheal intubation. Seven rats per group were placed in a mechanical rodent ventilator (Harvard Apparatus, Holliston, Massachusetts, USA) maintained at a tidal volume of 10 mL/kg, and a respiratory rate of 70 breaths per minute. A small window was created below the sternum, and a 2 F microtip pressure-conductance catheter (SPR-838; Millar Instruments, Houston, Texas, USA) was inserted through a small incision directly into the RV through the apex. After stabilization, signals were recorded using a pressure-volume conductance system (MPVS-Ultra, Millar Instruments, Houston, Texas, USA) connected to a data acquisition system (PowerLab, AD Instruments, Colorado Springs, Colorado, USA). Mean arterial pressure (MAP), RV stroke work (RVSW), RV stroke volume (RVSV), RV end-diastolic volume (RVEDV), RV end-systolic pressure (RVESP), ratio between MAP and RVESP (MAP-RVESP), RV ejection fraction (RVEF), heart rate (HR), maximal slope of the RV systolic pressure increment (dP/dT max), and time constant of RV pressure decay (tau) were measured under steady-state conditions. For the preload maneuver, the inferior vena cava was

compressed, and data were collected to assess preload recruitable stroke work (PRSW), dP/dT-end-diastolic volume relation (dP/dT-EDV), and slope of end-systolic and end-diastolic pressure-volume (P-V) relations (ESPVR and EDPVR). Volume calibration and parallel conductance volume calibration were performed as previously described[8].

2.3 Exhaled O_2 and CO_2 evaluation

Respiratory flow was evaluated using a small animal device (MLT1L Respiratory Flow Head, AD Instruments, USA) coupled to a respiratory gas analyzer (ML206 Gas Analyzer, AD Instruments). Percentages of exhaled O_2 and CO_2 were determined during RV evaluation.

2.4 Histological analyses

At the end of the experimental protocol, all the rats were euthanized by exsanguination. The lungs were removed, and the right lungs were insufflated with optimal cutting temperature solution (Tissue Teck, USA), immersed in hexane, and frozen with liquid nitrogen for posterior enzyme-linked immunosorbent assays (ELISAs). The left lungs were immersed in buffered formalin for 24 hours. The hearts were arrested in diastole by infusion of a hyperkalemic solution (19% KCl) and then immersed in buffered formalin for 24 h. Both organs were dehydrated and embedded in paraffin.

For histological analyses, the paraffinized heart and lungs were cut into a series of 3 mm thick slices and were subjected to either histological staining or immunohistochemistry reactions. Slices were stained with hematoxylin and eosin for histological analyses and with Masson's trichrome for fibrosis analyses. The slices were scanned, and a grid with 45 intersections was placed over the lung sections. The presence of pulmonary atelectasis, inflammatory infiltrate, perivascular edema, and extracellular fibrosis in each intersection was evaluated. Pulmonary arterial wall thickness was determined by a blind evaluator according to the average external-to-internal circumference ratios of randomly selected arteries. The hearts were analyzed for right and left ventricular thickness, along with RV chamber size. Myocardial extracellular fibrosis was evaluated on the RV free wall as previously described for the lung sections.

2.5 Immunohistochemistry

2.5.1 Lung analyses

Lung sections were deparaffinized, hydrated, and incubated with citrate buffer (pH 6.0) over 20 min at 100ºC for antigen retrieval. Non-specific sites were blocked with 2% bovine serum albumin (BSA) in Tris-buffered saline Tween-20 (TBST), followed by incubation with 2% hydrogen peroxide to block endogenous peroxidase. Sections were incubated with rat antibodies against smooth muscle α-actin (α-SMA; 1:500; Abcam, USA) or endothelin-1 (ET-1; 1:50; Santa Cruz, USA) in TBST supplemented with 2% BSA. The sections were then washed with TBST, incubated for 2 h at 37ºC with anti-mouse or anti-rabbit secondary antibodies (1:200) conjugated to horseradish peroxidase (HRP; Millipore, MA, USA), and counterstained with hematoxylin. Finally, images were captured using a digital camera (DS-Ri1; Nikon, Tokyo, Japan) coupled to a Nikon microscope, and were analyzed using the NIS Elements-BR software (Nikon, Tokyo, Japan).

2.5.2 Heart analyses

Whole heart sections (4 µm thick) were deparaffinized and rehydrated according to standard procedures. Antigen retrieval was performed using 10 mM citrate buffer (pH 6.0) at 95°C for 10 min. Sections were incubated with fluorescein-conjugated wheat-germ agglutinin (WGA; 20 µg/ml, ThermoFisher, Waltham, MA, USA) supplemented with 0.01 M $CaCl_2$ at room temperature for 1 h to visualize cell membranes. DAPI (0.3 µM in PBS) counterstain was added to visualize cell nuclei, and sections were mounted in Citifluor solution (Electron Microscopy Sciences, Hatfield, PA, USA). Imaging was performed on the same day using an AxioObserver Z1 microscope (Zeiss, Jena, Germany) in fluorescence mode. Cardiomyocyte hypertrophy was analyzed in both the left and right ventricles using the Fiji software[11].

2.6 Lung homogenate and enzyme-linked immunosorbent assays

Lung samples were weighed and homogenized with phosphate-buffered saline (PBS; 3 mL/g tissue) in TissueLyser, followed by centrifugation at 1500 rpm for 15 min at 4°C. Aliquots of the lung homogenate supernatants were stored at -80°C. The concentrations of vascular endothelial growth factor (VEGF; R&D Systems Inc., Minneapolis, USA)

and catecholamines (adrenaline and noradrenaline; Elabscience, USA) were determined by ELISA according to the manufacturer's protocol. The results were expressed as pg/g tissue.

2.7 Total nitric oxide determination

Total NO was determined by measuring the formation of nitrite (NO_2^-) and nitrate (NO_3^-). Previously homogenized lung samples were incubated with nitrate reductase (0.15 U/mL), and NO_2^- concentrations were determined using the Griess reagent reaction. The optical density (at 540 nm) was recorded using a microplate reader (BioTek Instruments), and NO_2^- levels were obtained using a standard curve of $NaNO_2$ (5Y60 2M).

2.8 Radical scavenging activity and lipid peroxidation

Whole heart sections (total 100 µm thickness) were homogenized in double distilled H_2O (ddH_2O) using TissueRuptor II (Qiagen, Hilden, Germany) and then sonicated 3 times at 20 kHz for 1 min (Sonopuls 2000, Bandelin, Berlin, Germany) and centrifuged at 14000 g to pellet insoluble proteins. The supernatants were used to assess radical scavenging activity by ABTS-radical decolorization, as previously described[12], and to evaluate lipid peroxidation in terms of reactivity to thiobarbituric acid, as previously described[13]. Total protein content of the supernatants was determined using the DC Protein Assay kit (Bio-Rad, Hercules, CA, USA), and was used for data normalization.

2.9 mtDNA copy number

Whole heart sections (total 50 µm thickness) were homogenized in DNA isolation buffer (100 mM NaCl, 10 mM EDTA, 0.5% SDS in 20 mM Tris-HCl, pH 7.4) containing 50 U/ml RNase I and 100 U/ml proteinase K (both Fermentas, Waltham, MA, USA). After overnight incubation at 55°C, total DNA was precipitated using 2-propanol. Aliquots of 10 ng DNA were amplified on a ViiA7 Real-time PCR system (ThermoFisher, Waltham, MA, USA), using iTaq Universal SYBR Green Supermix (Bio-Rad, Hercules, CA) and primers specific for mitochondrial DNA (MT-ND1; sense 5'-CCTCCTAATAAGCGGCTCCT-3', antisense 5'-GGCGGGGATTAATAGTCAGA-3') or nuclear DNA (NDUFA1; sense 5'-ATGGCCCGAACCAAGCAGACC-3', antisense 5'-TTAAGCTCTCTCCCCCCGTATCCG-3'). Amplification was performed

for 40 cycles of 15 s at 95°C for denaturation, and of 1 min at 60 °C for annealing and elongation. MtDNA copy number was calculated as: $mtDNA=2\times2^{Cq(NDUFA1)-Cq(MT-ND1)}$.

2.10 Cardiomyoblast oxidative stress assays

Rat H9C2 cardiomyoblasts (ATCC CRL-1446) were suspended in DMEM medium containing 10% fetal bovine serum and 1% penicillin-streptomycin solution (Sigma-Aldrich, St. Louis, MO, USA), and were passaged when a culture confluency of 70% was reached. Prior to all experiments, the cardiomyoblasts were seeded at a density of 0.6 x 10^5 cells/cm^2, and were serum-starved for 24 h.

To assess mitochondrial oxidative stress, the cardiomyoblasts were stimulated with 2 × 10^{-5} M phenylephrine for 24 h and then incubated in culture medium containing the mitochondrial-selective superoxide indicator mitoSOX (5 µM, ThermoFisher, Waltham, MA, USA) for 30 min. Fluorescence was recorded on a CLARIOStar Plus plate reader (BMG Labtech) at an $^{Ex}/_{Em}$ of $^{510}/_{580}$ nm and a bandwidth of 10 nm. Fluorescence recordings of culture medium containing 5 µM mitoSOX served as a background fluorescence control. The relative fluorescence intensities of 6 6 individual cell lines from each group were used for downstream analyses.

To assess cardiomyoblast hypertrophy, H9C2 cardiomyoblasts were lentivirally transduced with a hypertrophy-reported construct as previously described[14]. Cardiomyoblasts were stimulated with phenylephrine at a dose range of 1 × 10^{-11} to 2 × 10^{-5} M in the presence of either a vehicle (0.01% DMSO) or the antioxidant Trolox (30 µM, Sigma-Aldrich, St. Louis, MO) for 24 h. Cardiomyoblasts were lysed in passive lysis buffer (Promega, Madison, WI), and fluorescence intensity of the lysate was recorded on a CLARIO Star Plus plate reader (BMG Labtech) at an $^{Ex}/_{Em}$ of $^{488}/_{507}$ nm and a bandwidth of 10 nm. All experiments were performed in duplicates per condition, and the results were averaged. Data obtained from four individual experiments were used for downstream analyses.

2.11 Statistical analysis

All data were analyzed using Prism 9.0 (GraphPad Software Inc, California) and are expressed as median and interquartile interval. The minimal number of 7 rats per group was defined based on the expected difference of more than one interquartile variation in

morphometric and hemodynamic variables. The differences between groups were assessed via the Kruskal-Wallis test, followed by the Benjamini-Hochberg method of false discovery rate, with P-values adjusted to account for multiple comparisons.

3. Results

No deaths occurred in the control group, while PAH rats reported a 14% mortality rate. BS effectively decreased PAH-associated mortality, with no deaths reported in the BS group. At the end of the experimental protocol, the rats were randomly assigned for either hemodynamic analyses (n = 7 per group) or molecular and biochemical analyses (n = 6 per group). Morphological data of the lung and heart were obtained in all the surviving animals. Both exhaled O_2 and CO_2 and blood gases (PO_2 and PCO_2) did not differ between the groups.

3.1 Bilateral sympathectomy decreased pulmonary artery remodeling, atelectasis and fibrosis

As expected, the pulmonary arterial and arteriolar walls were thicker in untreated PAH rats compared to control rats (Figure 1A; PAH: 24,127 ± 11,854 μm²; control: 8,787 ± 2,666 μm²). BS decreased arterial wall thickness by 52.3%, and the arterial wall thickness of BS-treated PAH rats did not differ from that of control rats.

Untreated PAH rats exhibited increased arterial muscularization compared to control rats, as evidenced by an increase in αSMA-positive areas (Figure 1B). BS inhibited the increase in muscularization when compared to the untreated PAH group and had a tendency to decrease muscularization when compared to the control group.

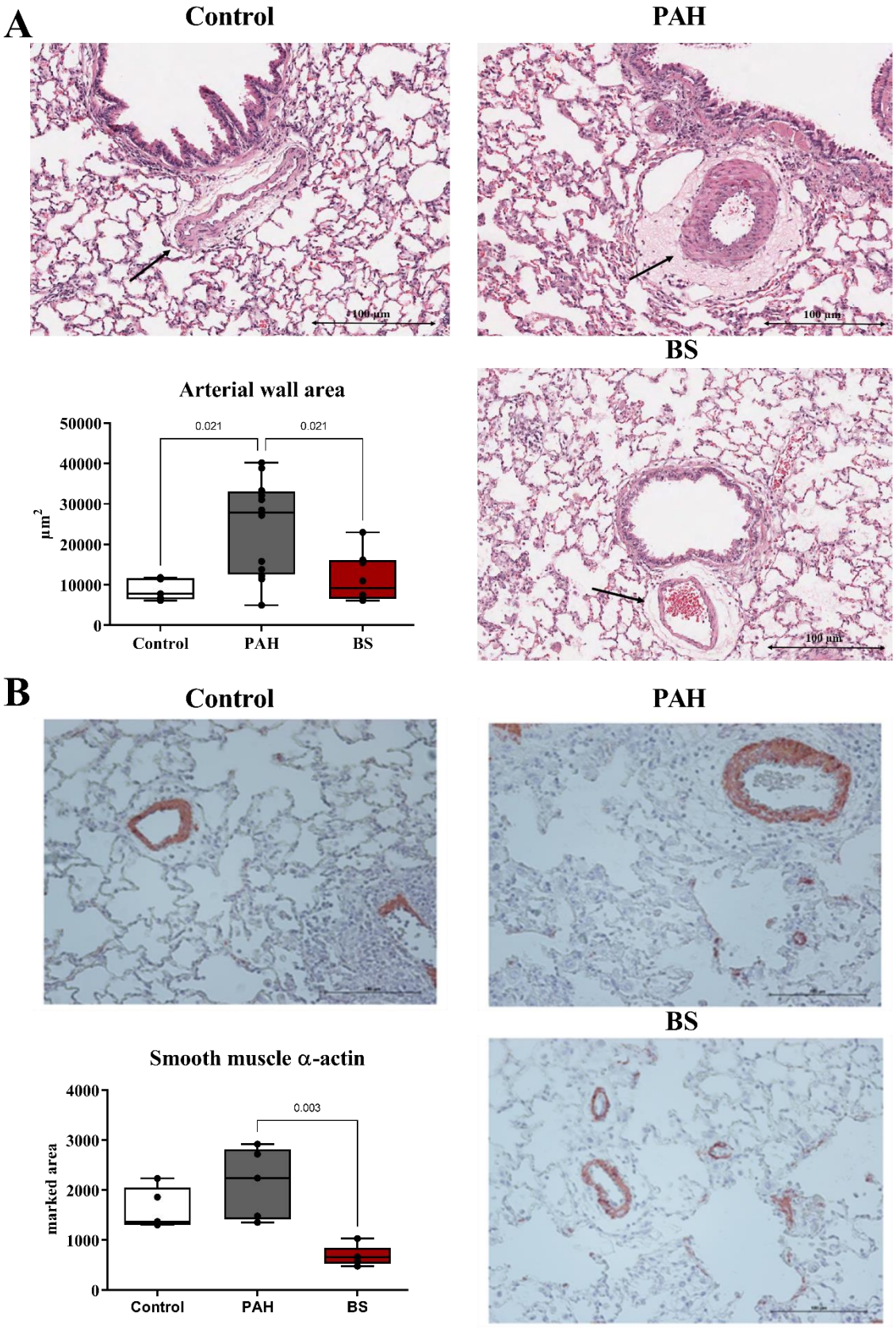
A
Control
PAH
100 µm
100 µm
BS
100 µm
Arterial wall area
50000
40000
30000
20000
10000
0
µm2
0.021
0.021
Control
PAH
BS
B
Control
PAH
BS
Smooth muscle α-actin
4000
3000
2000
1000
0
marked area
0.003
Control
PAH
BS

Figure 1. Arterial wall (n=13) and α-actin smooth muscle analysis (n=6). 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 represents the median value. Each measurement is shown as a black dot, and dots outside of the box and whiskers represent the outliers. White: Control group; grey: PAH group; red: BS group. PAH, pulmonary arterial hypertension; BS, bilateral sympathectomy. (A) Representative photomicrography under 20× augmentation of the arteries (arrow) stained with hematoxylin and eosin. Kruskal-Wallis P value = 0.013. (B) Representative photomicrography under 20× augmentation of the arteries (arrow) marked for smooth muscle α-actin, counterstained with hematoxylin. Kruskal-Wallis P value <0,001.

Besides pulmonary arterial remodeling, untreated PAH rats demonstrated a higher degree of pulmonary atelectasis when compared to control rats (Table 1). BS reduced, but did not mitigate, pulmonary atelectasis in PAH rats to levels similar to those of the control group. Atelectasis did not stem from the occurrence of pulmonary edema or inflammation, as these did not differ between the groups. However, pulmonary fibrogenesis was increased in untreated PAH rats, and was reduced in the BS group. Thus, BS reduced pulmonary arterial remodeling and atelectasis in PAH rats, presumably by mitigating fibrogenic responses.

Table 1. Lung histology

	Control, n=13	PAH, n=13	BS, n=13	*P* value
Pulmonary atelectasis, %	0.0 (0.0-3.9)*	17.8 (0.0-48.9)	0.0 (0.0-15.0)*	0.0001
Pulmonary edema, %	1.7 (0.6-3.4)	6.1 (2.1-13.3)	6.2 (0.0-7.1)	0.1189
Inflammatory infiltrate, %	2.8 (1.0-6.7)	4.5 (0.2-9.1)	7.0 (1.0-10.4)	0.4480
Pulmonary fibrosis, %	8.9 (5.6-15.6)*	21.1 (11.1-41.7)	12.2 (6.7-22.2)*	0.0140

Table 1. Lung histology. Data presented as median and interquartile interval. PAH, pulmonary arterial hypertension; BS, Bilateral sympathectomy. *Benjamini–Hochberg method of false discovery rate P < .05 versus PAH group.

3.2 Noradrenaline decrease attenuated vascular constriction

A decrease in noradrenaline expression (Figure 2A), but not in adrenaline expression (Figure 2B), was observed in the BS group. ET-1 expressions in lung blood vessels did not differ between the control and PAH groups, However, ET-1 expression in the BS

group was significantly lower than that of the PAH group (Figure 2C). VEGF expression in lung homogenates of the PAH group was lower than that of the control group. BS did not alter VEGF expression (Figure 2D). In terms of NO metabolites, no (significant) differences were shown in the nitrate (Figure 2E) and nitrite (Figure 2F) levels of PAH and BS lung homogenates.

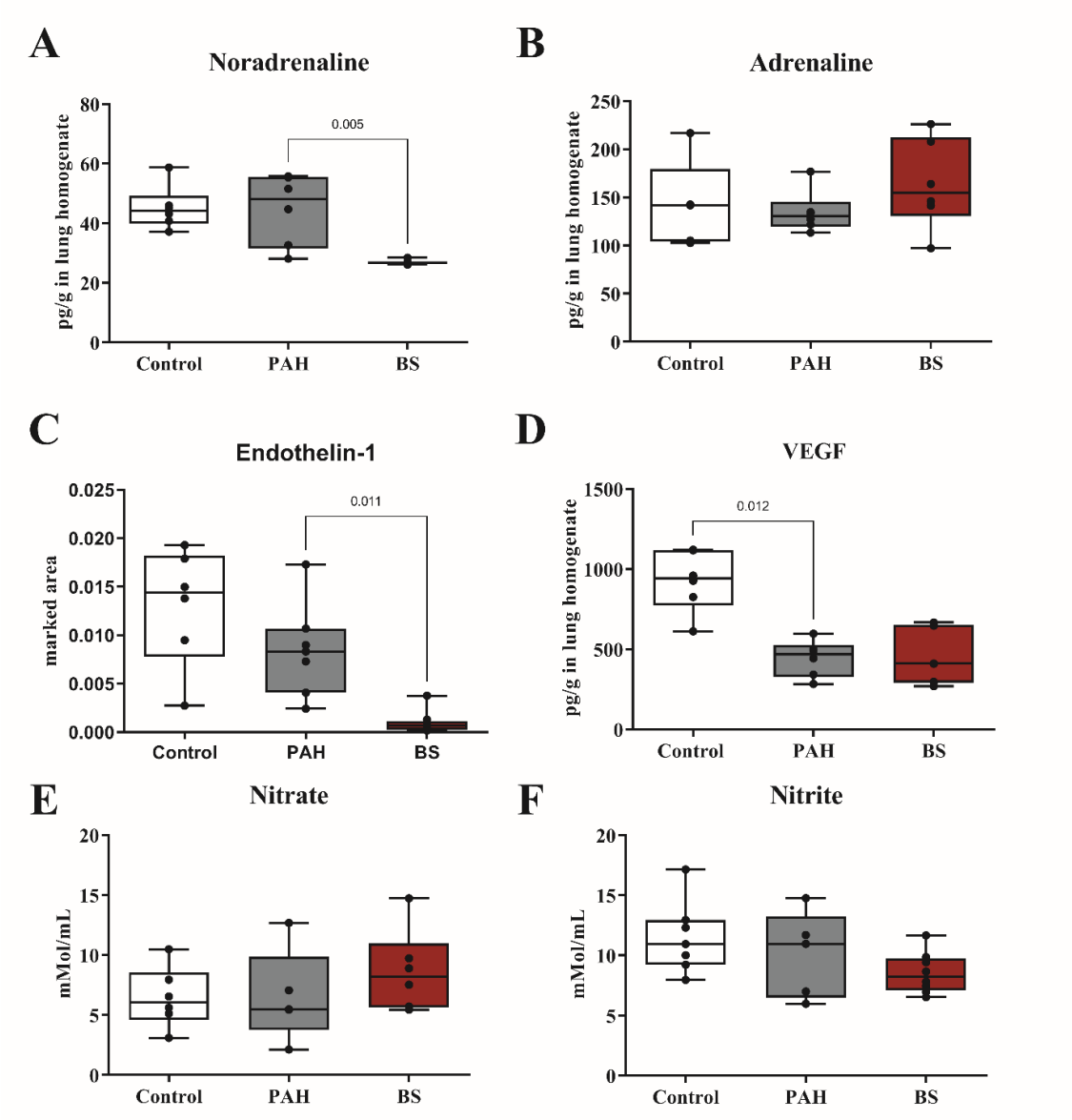

Figure 2. Noradrenaline (A), adrenaline (B), endothelin-1 (C) and VEGF (D) protein expression and nitric oxide metabolites (E and F) in lung homogenates. N = 7 per group. The X-axis shows the different groups. The upper and lower borders of the boxes represent the upper and lower quartiles. The middle horizontal line represents the median value. Each measurement is shown as a black dot, and dots outside of the box

and whiskers represent the outliers. White: Control group; grey: PAH group; red: BS group. PAH, pulmonary arterial hypertension; BS, bilateral sympathectomy.

3.3 Bilateral sympathectomy mitigated RV remodeling and dysfunction

Untreated PAH rats showed greater RV remodeling compared to control rats (Figure 3). In PAH rats, RVESP and PVR were significantly higher than those of control rats, while HR was not (Table 2), suggesting the development of RV failure. Indeed, the indicators for contraction efficacy, including PRSW and dP/dTmax, were lower in PAH rats than in control rats, while those for relaxation efficacy did not differ between the 2 groups. BS restored PRSW and normalized both RVESP/MAP and PVR, which may be associated with the prevention of RV failure (Figure 4A). No differences in the remaining parameters were observed among the groups.

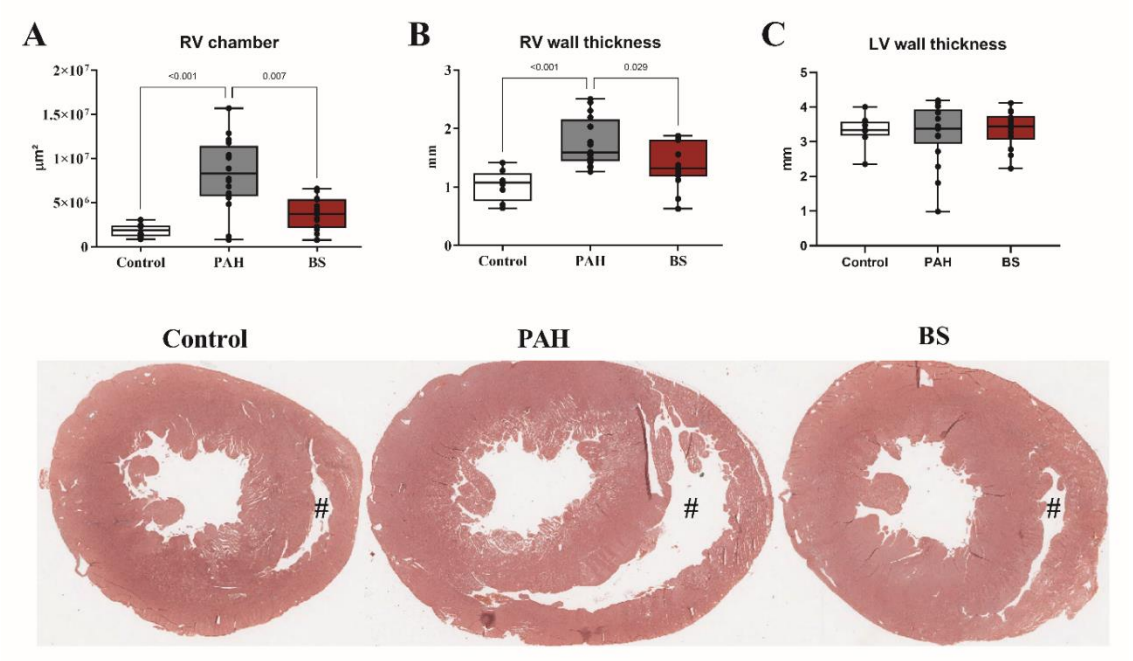

Figure 3. Cardiac morphometric analyses and representative photomicrographs photomicrographs of gross heart sections stained with hematoxylin and eosin. Right ventricular chamber (A), right ventricular (B) and left ventricular (C) wall thickness. N = 13 per group The X-axis shows the different groups. The upper and lower borders of the boxes represent the upper and lower quartiles. The middle horizontal line represents the median value. Each measurement is shown as a black dot, and dots outside of the box and whiskers represent the outliers. White: Control group; grey: PAH group; red: BS group. PAH, pulmonary arterial hypertension; BS, bilateral sympathectomy. # Represents the lumen of the right ventricle.

Table 2. Heart function

	Control, n=7	**PAH, n=7**	**BS, n=7**	***P* value**
Global hemodynamics				
Heart rate, bpm	328 (263 - 353)	319 (289 - 370)	334 (283 - 372)	0.533
Mean arterial pressure, mmHg	68 (63 - 85)	82 (71 - 95)	93 (78 - 105)	0.085
Cardiac output, ml/min	24.6 (13.3 - 36)	26.2 (8.9 - 45.4)	27.1 (8.4 - 44)	0.945
RV function				
End-diastolic pressure, mmHg	2.2 * (1.2 – 6.2)	8.9 (1.7 – 13.9)	1.6 * (0.3 – 12.8)	0.0007
End-systolic pressure, mmHg	25 * (18 - 29)	52 (36 - 82)	42 (28 - 53)	0.0005
End-diastolic volume, μL	120.9 (85.7 – 171.3)	121 (83.2 – 178.6)	134.4 (95.9 – 176.2)	0.492
End-systolic volume, μL	54.9 (30.9 -71.7)	57.3 (20.3 – 81.8)	66 (10.1 – 85.5)	0.693
Ejection fraction, %	65 (36 - 87)	70 (33 - 86)	56 (31 - 71)	0.339
Stroke volume, μL	79 (40.5 - 106)	80.1 (27.3 – 122.7)	72.8 (26.5 – 126.2)	0.978
Stroke work, mmHg*mL	2.06 (0.58 - 3.54)	2.2 (0.39 - 10.33)	3.09 (0.39 - 4.85)	0.917
dP/dT min, mmHg/s	-860 * (-1349 - -600)	-2332 (-6298 - -1204)	-2229 (-2557 - -910)	0.001
dP/dT max, mmHg/s	1072 * (862 - 1861)	2443 (1465 - 7299)	2166 (1294 - 2850)	0.002
tau , ms	14.5 (8.9 – 28.7)	11.6 (3.1 – 34.5)	10.8 (7.7 – 33.2)	0.850

Table 2. Global and right ventricular hemodynamics parameters. Data presented as median and interquartile interval. RV, right ventricular; PAH, pulmonary arterial hypertension; BS, Bilateral sympathectomy. *Benjamini–Hochberg method of false discovery rate P < .05 versus PAH group.

PAH rats demonstrated maladaptive remodeling with enlarged RV chambers and thicker RV wall than the control group (Figure 3). BS was shown to prevent RV chamber dilation in relation to the PAH group. Furthermore, the BS group exhibited thinner RV walls compared to the PAH group, but no differences were observed in terms of left ventricular (LV) wall thickness. There was no difference in the percentage of RV myocardial

connective tissue between the three groups [Control: 0.7% (0.2-1.6); PAH: 1.1% (0.3-3.7); BS: 0.7% (0.1-1.4); P = 0.318].

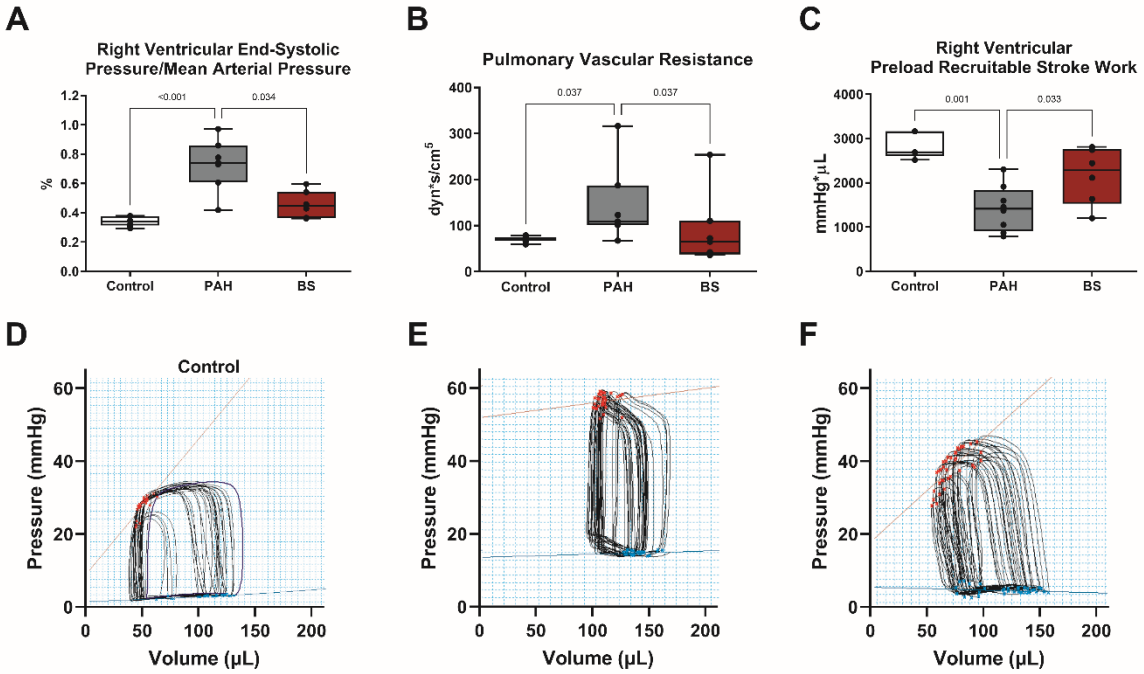

Figure 4. Data for right ventricular end-systolic pressure/mean arterial pressure relation (A), pulmonary vascular resistance (B) and right ventricular preload recruitable stroke work (C). The representative PV loop response to preload maneuver of each group (D, E and F). N = 7 per group. The X-axis shows the different groups. The upper and lower borders of the boxes represent the upper and lower quartiles. The middle horizontal line represents the median value. Each measurement is shown as a black dot, and dots outside of the box and whiskers represent the outliers. White: Control group; grey: PAH group; red: BS group. PAH, pulmonary arterial hypertension; BS, bilateral sympathectomy.

3.4. RV failure associated with cardiomyocyte hypertrophy and mitochondrial stress, which were precluded by BS

The RV failure in PAH rats coincided with RV cardiomyocyte hypertrophy, which was precluded by BS (Figure 5A). LV cardiomyocytes were unaffected, and their sizes did not differ between the groups. Cardiomyocyte hypertrophy associates with mitochondrial stress. Indeed, myocyte mtDNA copy number was lower in PAH rats than in control rats, and the loss of mtDNA was precluded by BS (Figure 5B). Concurrently, oxidative stress, as indicated by radical scavenging activity (Figure 5C) and lipid peroxidation products

(Figure 5D), was apparent in the RV of untreated PAH rats, and was also mitigated by BS.

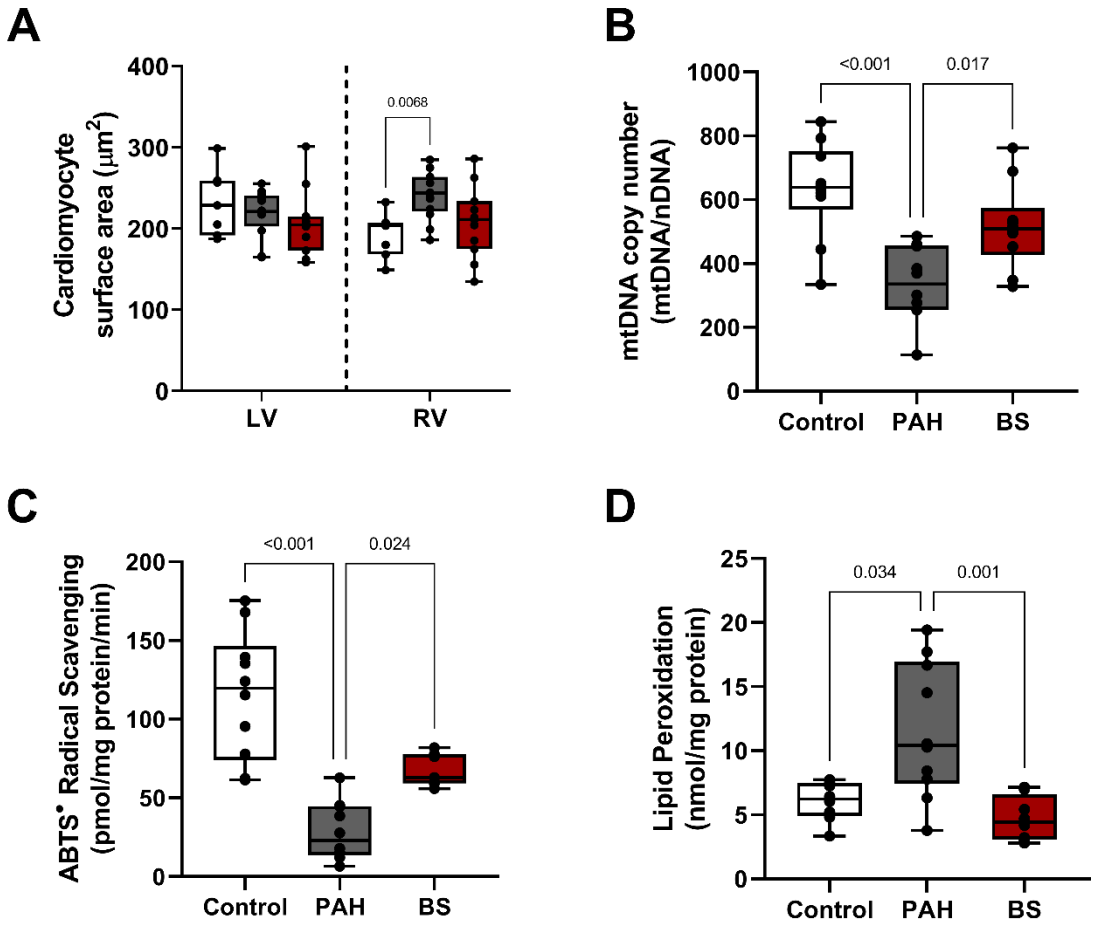

Figure 5. Right ventricular cellular analyses. Cardiomyocyte cellular hypertrophy (A) measured in the right and left ventricle. Mitochondrial copy number (B). Radical scavenging activity (C). Lipid peroxidation (D). N = 13 per group. The upper and lower borders of the boxes represent the upper and lower quartiles. The middle horizontal line represents the median value. Each measurement is shown as a black dot, and dots outside of the box and whiskers represent the outliers. White: Control group; grey: PAH group; red: BS group. PAH, pulmonary arterial hypertension; BS, bilateral sympathectomy.

3.5 Sympathetic stimuli associated with increased oxidative stress in cardiomyoblasts

Adrenergic stimulation by phenylephrine is known to induce cellular hypertrophy in cultured cardiomyoblasts. Stimulated cardiomyoblasts in our study showed increased mitochondrial oxidative stress, which was mitigated by the strong antioxidant Trolox

(Figure 6A). As expected, phenylephrine stimulation increased the activity of a hypertrophy-reported construct, which was reduced by co-treatment with Trolox (Figure 6B). These data suggest that adrenergic stimulation of cardiomyocytes directly evokes mitochondrial stress, which can culminate to cellular hypertrophy.

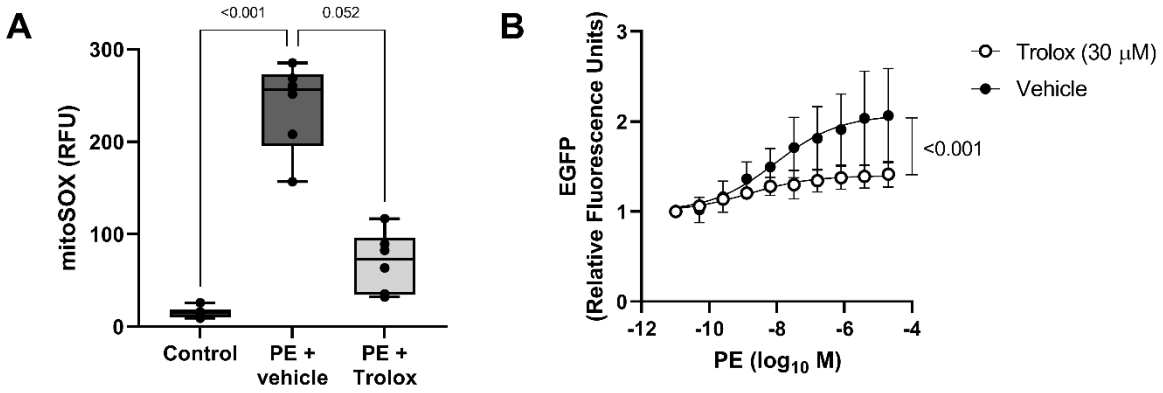

Figure 6. H9C2 cardiomyoblast assays. MitoSOX analysis (A) and hypertrophy reporter assay (B). N = 6 per group. The X-axis shows the different groups. The upper and lower borders of the boxes represent the upper and lower quartiles. The middle horizontal line represents the median value. Each measurement is shown as a black dot, and dots outside of the box and whiskers represent outliers. White: Control group; dark grey: PE + vehicle group; light grey: PE + Trolox group. PE, phenylephrine.

4. Discussion

Our study showed that BS can mitigate pulmonary vascular remodeling and RV failure in a rat model of PAH. Untreated PAH rats presented with pulmonary blood vessel wall hypertrophy, accompanied by increased PVR, lung fibrosis, and atelectasis. Such pathological changes were mitigated by BS. Pulmonary vascular remodeling and increased PVR result in the development of RV failure, which represents the primary cause of mortality in PAH patients. Untreated PAH rats developed RV failure secondary to PAH, and presented with increased PVR, RVESP, dP/dT max, and RVESP/MAP, as well as decreased PRSW. At the pathophysiological level, the RV of PAH rats showed maladaptive hypertrophy and dilatation, with cardiomyocyte hypertrophy, oxidative stress, and diminished mitochondrial copy number, which were mitigated following treatment with BS. Hence, we conclude that BS can elicit beneficial effects in experimental PAH and may offer a therapeutic benefit to PAH patients.

Sympathetic overactivity has been linked to pulmonary artery remodeling in PAH[9]. In pulmonary arteries, vascular contraction is primarily controlled by the α1-adrenoceptor, which has a high affinity for norepinephrine. Norepinephrine induces PASMCs[5], which results in increased muscularization of the pulmonary arteries. Blockade of the sympathetic tone attenuates ERK activation, and thus diminishes norepinephrine-induced pulmonary artery muscularization[9].

Sympathetic blockade has been investigated as an alternative to pharmacological therapy in the treatment of PAH. Pulmonary artery denervation (PADN) represents one of the alternative treatments based on modulation of the sympathetic system. PADN was performed on a monocrotaline-induced PAH dog model and was shown to improve hemodynamics and pulmonary artery remodeling[15]. Chen et al. evaluated the effects of PADN on patients with idiopathic PAH with 3 months of follow-up. PADN decreased mean pulmonary arterial pressure and improved the 6-minute walk test[16]. In a larger study[17] involving 66 patients who underwent PADN and discontinued other targeted treatment, 94% reported at least a 10% decrease in mean pulmonary arterial pressure within 1 year of follow-up. However, no control group was included in this study.

Thoracic sympathetic blockade has emerged as an alternative management of PAH[9,10]. In this regard, a left superior cervical ganglion blockade has been reported to prevent PASMC proliferation and increase NO availability in the lungs[10]. Similarly, transection of the right cervical sympathetic trunk suppressed pulmonary artery remodeling and prevented RV failure[9]. In a myocardial infarction model[7], BS has been proven more effective in protecting the myocardium from remodeling than unilateral sympathectomy. Based on this, the present study opted for bilateral, rather than unilateral, sympathetic blockade, and aimed to protect both the RV and the pulmonary arteries.

Endothelial dysfunction in the pulmonary arteries contributes to vascular remodeling and the increase in PVR in PAH[18]. In our model, ET-1 did not differ between the control and PAH groups. However, serum ET-1 levels have been described to be elevated in PAH patients[1]. In healthy subjects, noradrenaline and ET-1 synergism regulate vascular tone[19]. In our study, BS decreased noradrenaline levels in the lungs, consequently diminishing ET-1 expression in the pulmonary blood vessels. As a consequence, vasoconstriction was decreased, as observed by the lowered PVR and α-SMA expression, which associated with decreased vascular proliferation in this study and others[9,20]. ET-1 is a potent vasoconstrictor associated with PASMC vascular remodeling, and PVR increases with PAH progression. Notably, ET-1 has been a target for PAH treatment, particularly with endothelin receptor antagonists such as Bosentan[21].

Alongside endothelin, NO plays an important role in PAH development. To analyze NO levels, most studies quantify endothelial nitric oxide synthase protein expression in the lungs. However, lung endothelial nitric oxide synthase expression is mostly unchanged or increased in experimental PAH studies, but enzyme activity is uncoupled, which produces superoxide instead of NO[22]. Since nitrates and nitrites represent the major subproducts of NO, quantifying their levels in lung homogenates allows for the assessment of NO availability in the tissue. In our study, NO levels remained unchanged in the untreated PAH group, suggesting that BS is not involved in this pathway.

In terms of VEGF, most studies have confirmed that it is overexpressed in the lungs of both chronic hypoxic and monocrotaline-induced PAH models[23,24]. However, VEGF blockade associated with worsened pulmonary fibrosis in a rat model of PAH[25].

Interestingly, our results showed diminished VEGF lung expressions in the PAH group and the lack of effects of sympathetic blockade on these levels.

With regards to myocardial changes, BS has been shown to be superior to unilateral sympathectomy in a myocardial infarction model. BS not only preserved LV function, but also prevented LV remodeling and myocardial fibrosis. In contrast, unilateral left sympathectomy failed to prevent LV remodeling, and associated with increased fibrosis and decay of function[7]. BS was equally effective in a rat model of doxorubicin-induced heart failure. It was shown to decrease apoptotic markers and reduce myocardial fibrosis, and thereby associated with maintained LV ejection fraction and myocardial contractile efficiency, as well as increase PRSW[8]. Since RV remodeling and further heart failure represent the leading cause of death in PAH patients, RV protection is an important outcome to be analyzed.

Pathological remodeling increases energy demand through unfavorable cardiac geometry, with increased neurohormonal stimulation and impaired calcium handling, which can result in mitochondrial stress and metabolic imbalance[26]. Untreated PAH rats in our study demonstrated an increase in myocardial peroxidation damage associated with diminished endogenous radical scavenging activity. The increase in oxidative stress was accompanied by a decrease in myocardial mitochondrial copy number. Sympathetic blockade by BS therefore associated with preserved radical scavenging activity, reduced levels of lipid peroxidation, and consequently the maintenance of mitochondrial copy number.

As a result of preserved metabolic balance, RV remodeling was prevented. By preserving myocardial reserves, thoracic BS associated with the maintenance of both cardiomyocyte size and RV geometry. While RV hypertrophy can initially compensate for the augmented afterload (PVR) and maintain cardiac output, it is rarely fully compensatory and will eventually lead to RV failure. In line with this, our analysis of PRSW in untreated PAH rats demonstrated a lack of stroke work reserve in the RV.

The aforementioned results suggest a potential link between adrenoceptor activity, mitochondrial stress, and cardiomyocyte hypertrophy. To investigate this correlation, we stimulated H9C2 cardiomyoblasts with phenylephrine, an adrenergic α_1-agonist, and

found that adrenergic stimuli associated with an increase in mitochondrial superoxide, which was alleviated by the addition of an antioxidant. We further investigated the relationship between adrenergic stimulated oxidative stress and cardiomyoblast hypertrophy, and observed that phenylephrine-stimulated cardiomyoblasts were indeed hypertrophied. Notably, stimulated cardiomyoblasts treated with an antioxidant demonstrated reduced cellular hypertrophy, suggesting a strong link between the sympathetic pathway and myocardial oxidative stress, and its consequences to RV remodeling.

4.1 Study Limitation

The monocrotaline model is one of the most commonly used animal models for PAH. Monocrotaline induces PAH through direct endothelial lesion formation, which is comparable to drug- and toxin-related PAH seen in humans. However, the monocrotaline model does not fully mimic complex idiopathic PAH, which is, clinically, the most common form of PAH[27]. Another limitation involves the time points used in assessing the effects of BS on PAH. Therefore, it was not possible to distinguish the direct effects of BS on the lungs from those on the RV, particularly because PVR is closely linked to afterload and thus RV failure. Since we studied BS prior to monocrotaline induction, we can only theorize its effects on patients with developed PAH. Nevertheless, given that myocardial remodeling is a continuous process, BS interventions could provide beneficial effects at any time point. Additionally, sympathetic blockade was able to prevent PASCM muscularization, the primary pathological change in PAH, as previously reported by other authors[9,10]. Nonetheless, further investigations regarding BS are warranted to assess its potential benefits in pulmonary artery reverse remodeling.

4.2 Conclusions

Bilateral thoracic sympathetic blockade prevented pulmonary vascular remodeling and right ventricular failure in our experimental PAH model. While sympathetic activation evoked pulmonary arterial muscularization, elevated pulmonary vascular resistance, augmented myocardial oxidative stress, and consequently induced cardiac remodeling and failure, BS prevented all of these pathophysiological changes and maintained RV function. Our data suggest that BS may provide a therapeutic benefit to PAH patients.

5. Sources of funding

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6. Declaration of interest

None.

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Chapter 4

Perspectives of bilateral thoracic sympathectomy for treatment of heart failure

Raphael dos Santos Coutinho e Silva, Fernando Luiz Zanoni, Rafael Simas, Luiz Felipe Pinho Moreira.

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REVIEW ARTICLE

Perspectives of bilateral thoracic sympathectomy for treatment of heart failure

Raphael dos Santos Coutinho e Silva[I,*] Fernando Luiz Zanoni[I] Rafael Simas[II] Luiz Felipe Pinho Moreira[I]

[I]Laboratorio Cirurgico de Pesquisa Cardiovascular (LIM-11), Instituto do Coracao (Incor), Hospital das Clinicas HCFMUSP, Faculdade de Medicina, Universidade de Sao Paulo, Sao Paulo, SP, BR. [II]The Rolf Luft Research Center for Diabetes and Endocrinology, Karolinska Institutet, Stockholm, Sweden.

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*Corresponding author. E-mail: raphacoutinho@usp.br

Surgical neuromodulation therapies are still considered a last resort when standard therapies have failed for patients with progressive heart failure (HF). Although a number of experimental studies have provided robust evidence of its effectiveness, the lack of strong clinical evidence discourages practitioners. Thoracic unilateral sympathectomy has been extensively studied and has failed to show significant clinical improvement in HF patients. Most recently, bilateral sympathectomy effect was associated with a high degree of success in HF models, opening the perspective to be investigated in randomized controlled clinical trials. In addition, a series of clinical trials showed that bilateral sympathectomy was associated with a decreased risk of sudden death, which is an important outcome in patients with HF. These aspects indicates that bilateral sympathectomy could be an important alternative in the treatment of HF wherein pharmacological treatment barely reaches the target dose.

KEYWORDS: Heart Failure; Sympathectomy; Myocardial Infarction; Dilated Cardiomyopathy; Pulmonary Hypertension.

BACKGROUND

Heart failure (HF) affects approximately 2% of the adult population in developed countries. This number increases to 10% among people over the age of 70 years (1). Even in developed countries, 50% of patients with HF die within 5 years after diagnosis. HF progression is highly linked to an overactivated sympathetic nervous system (SNS) and renin angiotensin aldosterone system (RAAS), which makes them the main targets for medical treatment (2,3). Although current medical treatments have improved survival after diagnosis, mortality remains high (1).

HF patients have been treated with a combination of angiotensin-converting enzyme inhibitor (ACEI) or angiotensin-II receptor blocker (ARB) if ACEI is not tolerated, a b-blocker (BB), and a mineralocorticoid receptor antagonist (MRA) (4). Most recently, a new drug was approved for treatment of HF, a first-class angiotensin receptor neprilysin inhibitor (ARNI) that contains an ARB, sacubitril, and an inhibitor of neprilysin, valsartan (5,6).

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Even though clinical treatment guidelines support the combined therapy based on large randomized controlled trials, medication use and dosage in clinical practice are suboptimal. In the United States, the CHAMP-HF registry presented significant gaps in the use and dose of the current medical treatment for HF. Among patients eligible for the combined therapy, 26.6%, 33%, and 76.6% did not receive ACEI/ARB/ARNI, BB, and MRA, respectively. Additionally, less than 30% of the patients received the target doses of BB and ACEI/ARB/ARNI. Only 1% of the patients received optimal treatment (ACEI/ARB/ARNI+BB+MRA) and target dosage (7).

Due to the continuing high mortality in patients with HF and the continuous progression despite treatment, strategies to improve this setting continues to be required. Blockade through thoracic sympathectomy might be an alternative for halting disease progression using different pathways.

PATHOPHYSIOLOGY

The most recent classification of HF stratifies into 2 groups based on the contractile function of the left ventricle. Hence, HF is classified in HF with reduced ejection fraction (HFrEF), i.e., patients with left ventricular ejection fraction (LVEF) lower than 40%, and HF with preserved ejection fraction (HFpEF), i.e., patients with LVEF higher than 50%. A more recent classification, HF with midrange ejection fraction (HFmrEF) englobes those with LVEF between 41 to 49%. On this review, we will be focusing on HFrEF, once the development of this HF is directly linked to compensatory mechanisms that aim to improve cardiac function. HFrEF is

mainly caused by ischemic heart disease, dilated cardiomyopathies of different etiologies or right heart failure secondary to pulmonary arterial hypertension. As for the main compensatory mechanisms, there are the neurohormonal systems (SNS and RAAS), Frank Starling mechanism (stretch-induced increase of preload) and myocardial structural changes (hypertrophy and hyperplasia) (2).

Regardless of the cause of HF, a reduction in cardiac output leads to an increase in the contraction force by elongation of the cardiomyocyte sarcolemma (8). In addition, the SNS and RAAS are activated and have pivotal roles in HFrEF progression. The release of catecholamines by the SNS, mainly norepinephrine, enhances heart rate and myocardial contractility, which in the early clinical phase of the disease, preserves cardiac output (9-12). Additionally, circulating norepinephrine activates the RAAS, with subsequent release of angiotensin-II. Angiotensin-II enhances the effects of norepinephrine, with systemic vasoconstriction, as well as water and sodium retention, augmenting the venous return to the heart and increasing cardiac filling pressure (13,14).

When healthy, the heart is able to derive energy from fatty acids, glucose, ketone bodies, and amino acids. Under normal conditions, fatty acids are the main source of energy and are responsible for up to 90% of ATP production. These metabolic flexibilities support increased cardiac workload (15). Another important compensatory mechanism is myocardial remodeling through cardiomyocyte hypertrophy and an accelerated apoptosis/regeneration cycle (2,16).

However, constant activation of the aforementioned mechanisms leads to decompensation. Continued increases in chronotropy and inotropy reduce coronary perfusion, leading to myocardial ischemia and downregulation of myocardial beta-adrenergic receptors, especially beta-1 receptor, thus reducing inotropic response (17). Furthermore, systemic vasoconstriction elevates afterload and augments ventricular wall stress (18). Consequently, there is an increase in myocardial oxygen demand despite the limited supply due to myocardial ischemia (19).

As HFrEF progresses, the main source of energy shifts from fatty acids to glucose oxidation. The diminished oxygen supply reduces cardiomyocyte production of high-energy phosphate availability, which is responsible for transporting ATP to the myofibers. This metabolism changes with the accumulation of toxic intermediates, leads to mitochondrial dysfunction and increased oxidative stress (15). Damage by reactive oxygen species (ROS) leads to further impairment of cardiac energy homeostasis due to poor mitochondrial repair capacity (20). As energy depletes and excessive elongation of the fiber leads to the failure of muscle contractile unity, apoptosis and necrosis intensify. Cardiomyocytes are replaced by fibrotic tissue, and the ventricular remodeling process becomes pathological (21).

The development of myocardial fibrosis alters the mechanical properties of the heart during contraction by untying the cardiomyocyte contact and limiting the oxygen supply. The band separation of the cardiomyocytes leads to a non-uniform anisotropy in conduction speed, inducing micro-ischemic conditions and prolonging the duration of action potential. Increased SNS activity also has an arrhythmogenic effect on HFrEF. The constant activation of B1 receptors induces refractory tachycardia and malignant ventricular arrhythmias. In addition, fibrotic tissue can act as a potential trigger for reentry arrhythmia and is associated with sudden death, the second most prevalent outcome in patients with HFrEF (22,2).

Along with life-threatening arrhythmias, HFrEF progression can lead to the development of pulmonary hypertension (PH). An elevation in the left ventricular (LV) filling pressure causes an increase in pulmonary venous pressure (23,24). The resulting vascular remodeling increases pulmonary vascular resistance, leading to a high right ventricular (RV) afterload, further deteriorating the damaged heart. PH can also cause HFrEF as idiopathic PH leads to increased RV afterload due to pulmonary vascular remodeling (25,26). In addition, the SNS promotes pulmonary vascular remodeling and is further activated by it (27,28). Regardless of whether PH is a cause or consequence of HFrEF, the presence of PH is associated with a poor prognosis for mortality in patients with HFrEF (29).

PHARMACOLOGICAL THERAPY

With increasing stiffness of the heart, cardiac systolic function decreases even further. Eventually, the compensatory mechanisms are overwhelmed and unable to sustain cardiac function without further decompensation. The neurohormonal system is the main target for the treatment of HFrEF to dampen this vicious cycle (9-12).

The ACEI/ARB/ARNI and MRA target the RAAS, while the SNS is directly targeted by the BB (5-7). Antiarrhythmic drugs such as amiodarone and implantable cardioverter defibrillators (ICDs) are also important in preventing sudden death from life-threatening arrhythmias (30,31). However, as previously mentioned, less than 30% of patients on gold standard therapy receive the optimal dosage (7). Since pharmacological therapy has a low percentage of optimal dosage, surgical neuromodulation therapies could offer a greater benefit, particularly those that target the SNS.

NEUROMODULATION THERAPIES

Renal denervation (RD) has been studied using various experimental models and clinical trials. RD is based on dampening renal sympathetic activity, which is responsible for increasing sodium and fluid retention and activating the RAAS. In experimental models of myocardial infarction in rats, RD was associated with less fibrosis and cardiac remodeling, better cardiac function, decreased SNS activation, and improved hemodynamics (32,33). In the clinical setting, although the safety of the RD procedure is assured, its efficacy in patients with HFrEF is not as clear. The REACH trial showed that RD was associated with symptom and exercise capacity improvements; however, the small group size and lack of control groups preclude any robust conclusions (34).

Another important point to discuss is whether reinnervation occurs after RD. Using a sheep model, Booth et al. showed that 5 months after the RD procedure, almost complete functional and anatomical reinnervation occurred. Eleven months later, no differences were found in RD and non-RD animals, in relation to the renal distribution of afferent and efferent nerves and renal NE levels (35). There is still a lack of clinical trials with long-term follow-up and strong clinical endpoints to demonstrate whether RD might be an effective alternative for patients with HFrEF.

Vagal nerve stimulation (VNS) is another therapy option, which is based on the aforementioned disbalance between the SNS and the parasympathetic nervous system in HFrEF. Rather than attempting on or dampen SNS activation, VNS

tries to balance the autonomic nervous system by stimulating the vagal nerve with an implanted device. In experimental studies using rat models of HFrEF, VNS baroreflex activation decreased cardiac oxygen consumption, inflammation, SNS activity, and overall mortality (36-38). In a canine model of HFrEF, VNS was associated with improved LV function and decreased levels of several cardiac biomarkers (39,40).

The success of VNS in experimental models has not been replicated in clinical trials. In the randomized, sham-controlled, double-blind NECTAR-HF trial, VNS did not improve LV remodeling for 6 months, nor did it achieve any of the secondary efficacy endpoints (41). INOVATE-HF was another study that investigated VNS use in HFrEF. This was a multicenter, randomized trial with chronic HFrEF patients (NYHA III, left ventricular ejection fraction 040%) who were enrolled for 16 months. The trial concluded that VNS does not reduce mortality in patients with chronic HFrEF or diminish HF events (42).

Pulmonary artery denervation (PADN) is an alternative option for primary pulmonary arterial hypertension (PAH) in an attempt to prevent HFrEF development. The PADN mechanism is based on the removal of sympathetic nerves from the main pulmonary artery trunk, thereby inhibiting excessive activation of the SNS. An experimental model of PH showed improved hemodynamics and diminished pulmonary artery remodeling (43). In patients with idiopathic PH, PADN decreased the mean pulmonary arterial pressure and improved the 6-minute walk test in a 3-month follow-up (44). However, the small number of patients and the lack of larger studies preclude the assessment of PADN efficacy.

THORACIC SYMPATHECTOMY

Thoracic sympathectomy was first described as an alternative for arrhythmia control in patients with angina and ventricular arrhythmia (45). Thoracic sympathectomy surgery has improved and is now performed by thoracoscopy with high levels of success and minimal collateral effects. One of the main sympathectomy mechanisms is the increase in the fibrillation threshold, in addition to enhancing efferent vagal nerve activity. Besides to ICD therapy, left thoracic sympathectomy (LS) is one of the main treatment options for patients with long QT syndrome, sustained ventricular tachycardia, and other heart rhythm disorders (46).

Although LS has positive results in controlling arrhythmias, nervous plasticity of the stellate ganglion was observed after LS treatment. Unilateral sympathectomy causes hypertrophy of the contralateral ganglion (47,48). This can be seen when LS is compared with bilateral sympathectomy (BS). Furthermore, LS and BS were compared in a rat model of myocardial infarction, and BS was shown to be effective in protecting LV function and morphology, while LS failed to do so (49). In patients with ventricular arrhythmia, BS was more beneficial than LS in terms of arrhythmia control and decreased ICD shocks (47). In developing countries, this is an important result, considering the high cost of such mechanical devices. In comparison, BS surgery is a safe and low-cost procedure.

One of the main concerns about performing BS rather than unilateral sympathectomy was the maintenance of a minimal adrenergic tonus in a patient with HFrEF. A clinical trial verified the safety of BS in patients with ventricular tachyarrhythmia (47). Another study confirmed the safety of this procedure in patients with severe HFrEF. Also, it showed that epidural thoracic blockade was responsible for completely decreasing the sympathetic influence (50). In an experimental setting, a study evaluated the effects of BS on physiological scenarios. Interestingly, this study suggested the possibility of an extracardiac sympathetic compensation pathway that sustains the sympathetic tonus. The higher concentration of peripheral catecholamines and increased heart rate at rest in BS rats compared to non-operated rats supported this hypothesis (51).

The potential benefits of LS were assessed in 10 patients with dilated cardiomyopathy (DCM) and NYHA II or III with reduced LVEF. On a short follow-up of 6 months, LS improved the LVEF, exercise performance, and quality of life. These results showed that LS is feasible; however, a larger study is required to assess its long-term effects (52,53).

Recently, BS was performed in a patient with nonischemic DCM, NYHA class IV, and 15% LVEF. After 1 year, the patient had no ICD shock, improved LVEF to 25%, and was removed from the transplant list, showing the potential benefit of BS in a HFrEF patient (54). In another study, an epidural thoracic blockade was performed in 20 patients, followed for 30 days, with an increase in LVEF, reduction in LV dilatation, and improvement in NYHA class. Although it was a temporary blockade, it showed encouraging results (50). However, no other clinical trial determining BS potential benefits for patients with HFrEF has been performed or is currently active.

Although clinical trials are lacking, experimental studies in different DCM models are being published. Zanoni compared the effect of LS to BS as a treatment for myocardial infarction in rats. BS effectively controlled the overactive SNS, preventing LV remodeling and decay of function. In contrast, LS-treated rats showed a loss of wall thickness, increased fibrosis, and decreased heart function. The potential mechanism by which BS acts on ventricular remodeling is by decreasing myocardial apoptosis. BS-treated rats had diminished expression of apoptosis proteins, as LS and untreated rats had increased expression of these proteins. In addition, BS decreased the expression of matrix metalloproteinases, which are known to play a role in myocardial remodeling (49).

Once BS was shown to be superior to LS, BS was tested in a DCM model induced by doxorubicin (55). DCM rats were treated with either BS or conventional ACEI therapy. After dobutamine stimulation, both treatments preserved LV function; however, only BS was able to preserve LVEF and myocardial efficiency under steady-state conditions. Additionally, only BS animals were able to respond adequately to the preload maneuver, with increased preload recruitable stroke work. These different responses in LV function were correlated with histological analyses. On one hand, both treatments decreased LV dilatation; on the other hand, only BS was able to prevent the decrease in LV wall thickness. This prevention was associated with diminished expression of apoptosis proteins after BS treatment and could explain the difference in function from ACEI treatment (55).

In another unpublished experimental study using a PAH model, BS effects were evaluated in both lung microcirculation and in the RV (Unpublished data). BS was successful in preventing lung arterial wall hypertrophy, which is the primary pathological alteration observed in PAH. An interesting finding was the diminished expression of a-smooth muscle actin (a-SMA) by BS, since the augmented expression

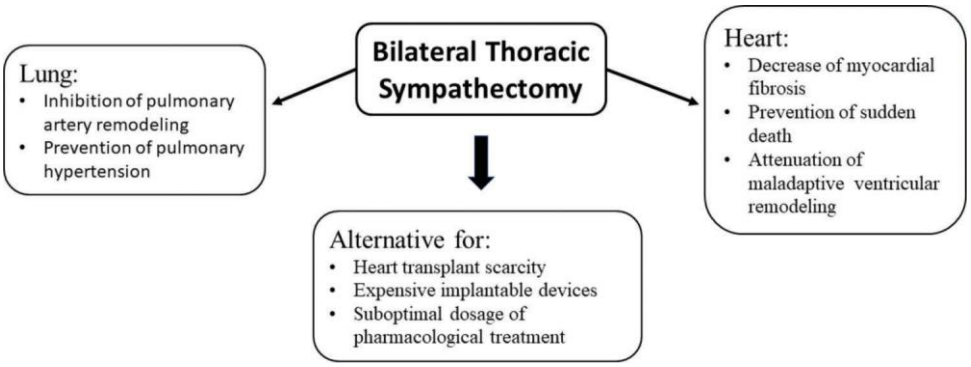

Figure 1 – Bilateral sympathectomy potential benefits for patients with heart failure.

of this particular protein is associated with endothelial dysfunction and vascular remodeling. BS protected the RV from dilation and hypertrophy by decreasing pulmonary vascular resistance. Consequently, RV function was preserved under steady-state conditions and responsive to preload changes. In untreated PAH, cardiomyocyte hypertrophy was associated with mitochondrial stress, decreased mitochondrial copy number, and increased oxidative stress. BS could decrease mitochondrial stress by enhancing radical scavenging activity and mitigating oxidative stress. These results could indicate a new mechanism by which BS halts the vicious cycle of SNS-induced lesions by protecting the heart from further damage and might contribute to reverse remodeling (Unpublished data).

In both the myocardial infarction and PAH models, the main cause of cardiac dysfunction was derived from an overactivated SNS in response to a lesion. In contrast, the doxorubicin model caused direct injury to cardiomyocytes. This direct injury leads to cardiomyocyte death and replacement of fibrotic tissue. It is important to highlight the success of BS in all three models, even in a direct lesion model. These results support the hypothesis that BS has a protective role in myocardial apoptosis through an unknown pathway. This mechanism might be key in preventing LV remodeling and halting the progression of HFrEF. The doxorubicin model showed that this mechanism might be different from that of ACEI and could be synergistic.

FUTURE PERSPECTIVES

Thoracic BS is a minimally invasive and promising procedure for patients with HFrEF (50,54). Experimental (49) and clinical (47,48) studies have shown that BS is superior to LS. Furthermore, BS seems to have a number of potential benefits for patients with HFrEF (Figure 1), such as attenuation of maladaptive ventricular remodeling or even induction of reverse remodeling; prevention of sudden death by decreasing myocardial fibrosis, which is the trigger for life-threatening arrhythmias; inhibition of pulmonary artery remodeling; and consequent prevention of primary or secondary pulmonary hypertension.

The current pharmacological treatment does not prevent the progression of HFrEF, as its dosage is mostly suboptimal in patients (7). In addition, implantable devices, such as LV mechanical assist devices and ICDs are expensive, especially in developing countries. Therefore, along with its potential benefits, BS could be an alternative for the scarcity of heart transplants, the only option for end-stage patients with HFrEF. The mechanisms by which BS acts has already been studied in a number of experimental studies, but with few clinical information, precluding definitive conclusions about its potential benefits. The absence of severe complications and side effects in patients submitted to BS for treatment of ventricular arrhythmias (47) or hyperhidrosis (56) also does not exclude possible risks in HF patients. Randomized controlled trial in patients with HFrEF must be performed to fully assess the effects of thoracic BS and provide real evidence of its perspective as an alternative to the current treatments.

AUTHOR CONTRIBUTIONS

All the authors were responsible for the manuscript drafting, editing and review.

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Chapter 5

Final considerations

The current treatment approaches for HF are constantly improving. Each year, many studies are been published to better understand disease progression. Despite this, the number of deaths within 5 years of diagnosis is 50% in developed countries. This high mortality rate urges new approaches for the management of HF.

In Chapter 1, a brief introduction is provided on the understanding of HF pathophysiology, its treatment, and the potential link with the SNS. It also delineates the scope of the thesis and describes the experimental models used to assess the effects of BS.

In Chapter 2, BS was performed in a doxorubicin-induced dilated cardiomyopathy model. BS prevented ventricular remodeling with a decrease in myocardial fibrosis. In parallel, LV function was preserved in steady-state conditions and after pharmacological stimuli with dobutamine and through preload volume variations. Overall, myocardial efficiency was maintained, and the preservation of cardiac function was associated with a decrease in apoptosis.

Following this study, in Chapter 3, we investigated the effect of BS in another experimental model, pulmonary arterial hypertension induced by monocrotaline, with secondary right ventricular failure. BS reduced pulmonary artery remodeling and hypertrophy. It also decreased pulmonary vascular resistance and consequent ventricular remodeling. In cardiomyocytes, BS was effective in reducing oxidative stress and protecting mitochondrial function. Hence, right ventricular function was preserved, and the effect of pulmonary arterial hypertension was mitigated by BS.

Finally, Chapter 4 is a review of the literature comprising the latest publications on neuromodulation therapies for the treatment of HF. In addition to a brief overview of the HF current pharmacological therapy, thoracic sympathectomy studies are discussed, and its potential is described.

Therefore, BS is a promising alternative for HF treatment. It is a minimally invasive procedure, with a low cost compared to implantable devices, such as mechanical assist devices and implantable cardioverter-defibrillators. It is also an important alternative to

the current setting of the global scarcity of heart transplants, whose demand exceeds the supply.

Experimental studies have shown potential in dampening the ventricular remodeling process characteristic of HF and is responsible for the progressive decay of function. The decrease in myocardial apoptosis, possibly through the mitigation of oxidative stress and mitochondrial protection, requires further attention. Regardless, BS was effective in different HF models in halting the loss of cardiac function.

However, clinical information is scarce and still warranted to fully assess its effects on patients with HF. However, the absence of side effects following BS for the treatment of hyperhidrosis and ventricular arrhythmias is encouraging. Currently, there are no active clinical trials investigating the potential of BS in patients with HF. Hopefully, recent experimental trials may encourage researchers to evaluate BS as an alternative for patients with HF.