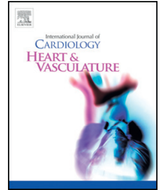
IJC Heart & Vasculature 21 (2018) 22–28

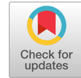
Contents lists available at ScienceDirect

IJC Heart & Vasculature

journal homepage: http://www.journals.elsevier.com/ijc-heart-and-vasculature



Atrial fibrillation promotion in a rat model of heart failure induced by left ventricle radiofrequency ablation



Luis dos Santos [a,1], Ednei L. Antonio [a,1], Andrey J. Serra [b,1], Amanda Yoshizaki [a,1], Larissa Seibt [a,1], Flavio A. Silva [a,1], Gisele K. Couto [c,1], Luciana V. Rossoni [c,1], Paulo Tucci [a,1], Angelo A. de Paola [a,1], Guilherme Fenelon [a,*,1]

[a] Department of Cardiology, Paulista School of Medicine, Federal University of São Paulo, Rua Pedro de Toledo 781 10° - Vila Clementino, 04039-032 São Paulo, SP, Brazil
[b] Biophotonics Program, Nove de Julho University (UNINOVE), Rua Vergueiro, 235/249 - Vergueiro, 015 04000 São Paulo, SP, Brazil
[c] Department of Physiology and Biophysics, Institute of Biomedical Science, University of São Paulo, Av. Professor Lineu Prestes - 1524- Cidade Universitária, 05508-000 São Paulo, SP, Brazil

ARTICLE INFO

Article history:
Received 28 June 2018
Accepted 11 September 2018
Available online xxxx

Keywords:
Radiofrequency ablation
Atrial fibrosis
Heart failure
Atrial fibrillation

ABSTRACT

Background: Atrial fibrillation (AF) frequently coexists with congestive heart failure (CHF). The increased susceptibility to AF in CHF has been attributed to a variety of structural and electrophysiological changes in the atria, particularly dilation and interstitial fibrosis. We evaluated atrial remodeling and AF vulnerability in a rat model of CHF induced by left ventricle (LV) radiofrequency (RF) ablation.

Methods: Wistar rats were divided into 3 groups: RF-induced CHF (Ab, n = 36), CHF animals treated with spironolactone (AbSpi, n = 20) and sham controls (Sham, n = 29). After 12 weeks, animals underwent echocardiographic and electrophysiological evaluation and were sacrificed for histological (atrial fibrosis) and Western blotting (TGF-β1, collagen I/III, connexin 43 and $Ca_V1.2$) analysis.

Results: Mild LV dysfunction and marked atrial enlargement were noted in both ablated groups. AF inducibility (episodes ≥2 s) increased in the Ab group compared to sham animals (31/36, 86%; vs. 15/29, 52%; $p = 0.005$), but did not differ from the AbSpi group (16/20, 80%; p = NS). Sustained AF (>30 s) was also more frequent in the Ab group compared to shams (56% vs. 28%; $p = 0.04$). Spironolactone reduced atrial fibrosis ($p < 0.01$) as well as TGF-β1 ($p < 0.01$) and collagen I/III ($p < 0.01$) expression but did not affect connexin 43 and $Ca_V1.2$ expression.

Conclusions: Rats with RF-induced CHF exhibit pronounced atrial structural remodeling and enhanced AF vulnerability. This model may be useful for studying AF substrate in CHF.

© 2018 The Authors. Published by Elsevier B.V. This is an open access article under the CC BY-NC-ND license (http://creativecommons.org/licenses/by-nc-nd/4.0/).

1. Introduction

Atrial fibrillation (AF) frequently coexists with congestive heart failure (CHF), as CHF promotes AF and as AF worsens CHF [1]. The increased susceptibility to AF in CHF has been attributed to a variety of structural and electrophysiological changes in the atria, particularly dilation and interstitial fibrosis [2]. Heart failure increases expression of transforming growth factor beta 1 (TGF-β1) which plays a significant role in the genesis of atrial fibrosis [3]. Furthermore, CHF is associated with decreased expression of connexins 40 and 43 (major atrial gap junctional proteins) [4,5]. These abnormalities promote slow local conduction favoring sustained reentry. In addition, heart failure-induced atrial remodeling is commonly associated with reduced expression of L-type Ca^{2+} channels which may change action potential configuration and atrial refractoriness [6]. It has been shown in animal models that mineralocorticoid receptor blockers such as spironolactone and eplerenone, which exhibit potent antifibrotic properties, attenuate CHF-induced atrial fibrosis and atrial tachyarrhythmias [1,7,8].

The rat model of myocardial infarction induced by ligation of the left coronary artery has long been validated to simulate human CHF and left atrial remodeling [9]. However, this model has drawbacks such as elevated significant immediate mortality and high variability of myocardial infarction size [10–11]. As an alternative to coronary occlusion, we have recently demonstrated a method of inducing myocardial infarction in rats by left ventricle radiofrequency (RF) ablation which is associated with homogenous infarct size and low mortality [12–14]. In addition, the histopathologic evolution, severity of left ventricular dysfunction and CHF outcome reproduced a myocardial infarction from coronary occlusion [12]. However, atrial structural alterations and AF susceptibility were not evaluated.

* Corresponding author.
E-mail address: guilhermefenelon@uol.com.br (G. Fenelon).
[1] This author takes responsibility for all aspects of the reliability and freedom from bias of the data presented and their discussed interpretation.

https://doi.org/10.1016/j.ijcha.2018.09.003
2352-9067/© 2018 The Authors. Published by Elsevier B.V. This is an open access article under the CC BY-NC-ND license (http://creativecommons.org/licenses/by-nc-nd/4.0/).

We hypothesized that our CHF model promotes left atrial dilation and interstitial fibrosis increasing vulnerability to AF. Therefore, we have assessed atrial structural remodeling and induction of AF in rats with CHF induced by RF ablation. Additionally, we evaluated the impact of spironolactone on atrial fibrosis and AF inducibility.

2. Methods

This study was approved by the Ethics in Research Committee of the Federal University of São Paulo (protocol number: 8052201213) and was conducted in accordance with "Laws related to animal models in Brazil" (http://www.ccs.ufpb.br/pesqccs/animal.htm).

2.1. Open-chest preparation and ablation protocol

Under sterile conditions, 85 male Wistar rats (300 to 320 g) were anesthetized, mechanically ventilated, and a thoracotomy in the 4th intercostal space was performed to expose the heart. One RF ablation (12 W for 12 s) per animal was performed on the free wall of the left ventricle using a customized catheter as previously described [12–14]. Then, the heart was rapidly placed back inside the thorax, and the chest was closed with simple suture. After recovery, animals were maintained for 12 weeks in conditioned cages in a light cycle of 12/12 h.

2.2. Subdivision of animals

Three groups were studied: (1) CHF induced by left-ventricle RF ablation (Ab group, n = 36); (2) CHF animals treated with spironolactone (100 mg/kg per gavage once daily) during the 12 weeks of follow-up (AbSpi group, n = 20); and (3) sham-operated animals (SHAM, n = 29) which underwent thoracotomy and exposure of the heart but not ablation.

At the end of the follow-up period (12 weeks), rats were reanesthetized for echocardiography and electrophysiological study. After that, animals were sacrificed, and the hearts excised for left atrial histological and Western blotting evaluation.

2.3. Echocardiography

Transthoracic echocardiography studies were performed using the HP SONOS 5500 echocardiograph (Philips Medical System, Andover, MA) with a 12 MHz transducer, 2 cm depth. In addition to left ventricular function analysis, left atrial diameter, area, and left atrial/aorta ratio were also evaluated as previously described [15].

2.4. Arrhythmia inducibility

Percutaneous electrophysiological studies were performed using an octapolar catheter (EPR catheter 1.6F, AD instruments) positioned in the right atrium via the right jugular vein [16]. Surface ECG and bipolar intracardiac electrograms (3 channels) were displayed on a monitor and stored by a computerized recording system (PowerLab®, Australia). Sinus node function and atrioventricular conduction intervals were not evaluated. Arrhythmia inducibility was tested with up to 5 burst pacing attempts at 20 and 40 milliseconds for a maximum of 30 s using current strengths from 1200 to 1500 mV (STG3008 stimulator, MultiChannels - Reutlingen, Germany). AF was defined as ≥2 s of irregular atrial electrogram (>800 bpm) with irregular ventricular response. AF episodes were categorized as follows: a) No AF induction (episodes <2 s); b) Non-sustained AF (≥2 s and ≤30 s); c) sustained AF (>30 second duration); d) sustained long-lasting AF (>15 minute–900 second duration). AF duration was measured from the end of the burst stimulus until the first P wave of sinus rhythm post-AF and was determined in each rat as the mean duration of all AF episodes.

2.5. Atrial refractoriness analysis

Due to poor catheter stability, we were not able to reliably assess right atrial refractory periods by extra-stimulus testing. Therefore, we used the AF cycle length recorded in the intracavitary electrodes as an estimate of atrial refractoriness [17–18]. The AF cycle length analysis consisted of manually measuring using electronic calipers (same blind observer) the atrial electrical activity intervals (averaging 10 to 20 cycles) during the 31st second of the AF episode of animals with sustained AF. We have chosen this time frame to allow accommodation of atrial refractoriness. The mean, median and shortest intervals of AF cycles were evaluated. AF displaying multicomponent fractionated electrograms or with motion artifacts were excluded.

2.6. Histology

Atrial fibrosis was quantified with Picrosirius red staining [19]. Quantitative analysis of collagen (measured in pixels) was performed after sequential images (10 fields per atrium) along the atrial fibers and blindly measured using Image Tool® software.

2.7. Western blotting

To determine protein expression of TGFβ1, collagen I/III, connexin 43 and L-type Ca^{2+} channels ($Ca_V1.2$) left atria were homogenized with cold RIPA lysis buffer (RIPA, Millipore, USA), containing phenylmethyl sulfonyl fluoride (PMSF, 1 mM), sodium orthovanadate (10 mM), protease inhibitor cocktail (2 μL/mL, Sigma, USA), sodium fluoride (100 mM) and sodium pyrophosphate (10 mM) [14]. The homogenates were subsequently centrifugated (1500*g* for 20 min at 4 °C) and the supernatants were isolated. Equal amounts of proteins (30 μg) of the samples, the molecular weight marker (Precision Plus Protein, Kaleidoscope, Bio-Rad, USA), and positive controls (30 μg of fibroblast homogenates as a positive control for collagen I/III, TGFβ1 and $Ca_V1.2$, and 30 μg of mouse encephalon homogenates as a positive control for connexin 43 were electrophoretically separated on gradient polyacrylamide gel (4–20%, Bio-Rad, USA) in an apparatus for minigel (Mini Protean III, Bio-Rad, USA) and transferred to polyvinylidene difluoride (PVDF) membranes (Amersham-GE Healthcare, UK) overnight at 4 °C using a Mini Trans-Blot Cell system (Bio-Rad, USA). After blocking, the membranes were incubated overnight at 4 °C with blocking solution containing the following antibodies: anti-collagen I/III (1:500; Calbiochem, California, USA, cat. n° 234169), anti-TGFβ1 (1:1000; Santa Cruz Biotechnology, Texas, USA, cat. n° sc-146), anti-connexin 43 (1:5000, Abcan, Massachusetts, USA, cat. n° ab11370) or anti-$Ca_V1.2$ (1:1000, Alomone Labs, Jerusalem, Israel, cat. n° ACC-003). After labeling with primary antibody, the membranes were incubated for 90 min with blocking solution containing appropriate peroxidase-conjugated secondary antibody (anti-rabbit; Jackson Immuno Research, USA): 1:10,000 for collagen I/III, 1:4000 for TGFβ1 and Connexin 43 or 1:2000 for $Ca_V1.2$. After washing, the fluorescence kit detection reagents (ECL, Amersham-GE Healthcare, UK) were added and the membrane chemiluminescence was detected using Amersham Imager 600 (Amersham-GE Healthcare, UK). Optical densitometric analysis of the bands was performed by the Image J program (Wayne Rasband, National Institutes of Health, USA). The loading control was validated by staining the membrane with Ponceau S.

2.8. Statistical analysis

Data were analyzed with the Prism 4.0 program (GraphPad Prism Software Inc., San Diego, CA, USA). The Fisher's exact test was used to compare AF inducibility. AF duration is expressed as median and interquartile range (25% to 75%) and was analyzed with a Kruskal-Wallis test followed by Dunn's test. Data normality was examined with Shapiro-Wilk test. One-way ANOVA was applied to compare continuous

Download English Version:

https://daneshyari.com/en/article/11029178

Download Persian Version:

https://daneshyari.com/article/11029178

Daneshyari.com