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Bucindolol improves right ventricle function in rats with pulmonary arterial hypertension through the reversal of autonomic imbalance

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ABSTRACT

Pulmonary arterial hypertension (PAH) is characterised by an elevation in afterload imposed on the right ventricle (RV), leading to hypertrophy and failure. The autonomic nervous system (ANS) plays a key role in the progression to heart failure, and the use of beta-blockers attenuates this process. The aim of this study was to verify the role of bucindolol, aβ1-, β2- and α1-blocker, on the ANS, and its association with RV function in rats with PAH. Male Wistar rats were divided into four groups: control, monocrotaline, control+bucindolol, and monocrotaline+bucindolol. PAH was induced by a single intraperitoneal injection of monocrotaline (60 mg/kg). After two weeks, animals were treated for seven days with bucindolol (2 mg/kg/day i.p.) or vehicle. At the end of the treatment, animals underwent echocardiographic assessment, catheterisation of the femoral artery and RV, and tissue collection for morphometric and histological evaluation. In the monocrotaline+bucindolol group, there was a decrease in mean pulmonary artery pressure (33%) and pulmonary congestion (21%), when compared to the monocrotaline. Bucindolol treatment also reduced RV pleomorphism, necrosis, fibrosis and infiltration of inflammatory cells. An improvement in RV systolic function was also observed in the monocrotaline+bucindolol group compared to the monocrotaline. In addition, bucindolol promoted a decrease in the cardiac sympathovagal balance (93%) by reducing sympathetic drive (70%) and increasing parasympathetic drive (142%). Bucindolol also reduced blood pressure variability (75%). Our results show that the beneficial effects from bucindolol treatment appeared to be a consequence of the reversal of monocrotaline-induced autonomic imbalance.

1. Introduction

Pulmonary arterial hypertension (PAH) is a disease characterised by vascular pulmonary remodelling (Aggarwal et al., 2013), with an estimated prevalence of 10–52 cases per million people (Jansa et al., 2014; Peacock et al., 2007). The vascular remodelling leads to a gradual reduction of the lumen of pulmonary arterioles, resulting in a progressive increase in pulmonary vascular resistance (PVR) and mean pulmonary artery pressure (mPAP), increasing the RV afterload. In response, the stress on the luminal wall is intensified, which is attenuated by hypertrophy (Bogaard et al., 2009). Structural and functional remodelling of the RV is an important determinant of PAH progression (Sandoval et al., 1994). These adaptations are initially favourable, however, progression of the disease leads to the worsening of cardiac function (Vonk-Noordegraaf et al., 2013).

Acute activation of the sympathetic nervous system (SNS) occurs as an adaptive response, in order to re-establish or maintain cardiac output (CO) and pressure levels. However, long-term sympathetic hyperactivity has deleterious effects and accelerates the disease progression (Triposkiadis et al., 2009), contributing to maladaptive RV

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remodelling (Bogaard et al., 2009). In addition to the increased sympathetic activity, a decrease in parasympathetic activity has also been observed in PAH (Rigatto et al., 2013; Rosas-Peralta et al., 2006). Recent studies have shown improved RV function in PAH by blocking adrenergic receptors (Bogaard et al., 2010; Okumura et al., 2015; Perros et al., 2015). However, there is no clinical evidence that beta-blockers must be used for the treatment of PAH, due of possible detrimental effects on hemodynamic and exercise capacity (Galiè et al., 2015). Therefore, more research is urgently needed (Bandyopadhyay et al., 2015; De Man and Handoko, 2015).

In order to better understand the pathophysiology of PAH and to determine the efficacy and safety of novel therapeutic strategies, several experimental models have been developed. Monocrotaline treatment is the most commonly used model to mimic PAH (Ryan et al., 2013). Monocrotaline is a vegetal alkaloid that promotes structural and functional changes in lung and pulmonary vasculature, through its active metabolite dehydromonocrotaline (Schultze and Roth, 1998; Wilson et al., 1992). The cardiovascular and pulmonary changes induced by monocrotaline are very similar to those observed in PAH patients, as well as other functional and biochemical outcomes (Brown et al., 1998; Ryan et al., 2013).

Bucindolol is a β1- and β2-blocker, that also functions as an α1-antagonist (Hershberger et al., 1990). In addition, bucindolol has sympatholytic properties, which is a unique characteristic compared to other beta-blockers. Bucindolol has not shown any evidence of intrinsic sympathomimetic activity in the human heart (Black-Maier et al., 2015). Bucindolol has been shown to reduce mPAP (Eichhorn, 1993) improve cardiac contractility, improve left ventricular systolic and diastolic function, prevent of atrial fibrillation and maintain oxygen consumption (Aleong et al., 2013; Eichhorn et al., 1990). Thus, the aim of the present study was to verify the effects of bucindolol on autonomic modulation and its association with RV function in an experimental model of monocrotaline-induced PAH.

2. Materials and methods

2.1. Ethical approval

All procedures performed in this study were in accordance with the guidelines of the Directive 2010/63/EU of the European Parliament on the protection of animals used for scientific purposes, and also conformed with the ethical standards determined by the Universidade Federal do Rio Grande do Sul research committee (protocol number 26244). All efforts were made to minimise suffering in the experimental animals.

2.2. Experimental design

Male Wistar rats (*Rattus norvegicus*) weighing 180 ± 5 g were obtained from the Center of Reproduction and Experimentation of Laboratory Animals of the Universidade Federal do Rio Grande do Sul, RS, Brazil. All animals (n=36) received water and food *ad libitum* and were housed at a temperature of 20–25 °C with 70% humidity, under a 12 h light/dark cycle. The sample size for each experimental group started with 8 animals per group. However, since a 20% mortality rate was reported, and some animals (those whose heart rate was above 300 bpm) were excluded from the echocardiographic analysis, the number of animals varied, and it is indicated in each outcome. Animals were divided into four groups: (1) animals that did not receive monocrotaline or treatment with bucindolol (CTR); (2) animals that did not receive monocrotaline but were treated with bucindolol (CTR +BCD); (3) animals that received monocrotaline but were not treated with bucindolol (MCT); and (4) animals that received monocrotaline and bucindolol treatments (MCT+BCD). The animals in the MCT and MCT+BCD groups received a single intraperitoneal injection of monocrotaline (60 mg/kg) (Sigma-Aldrich, Saint Louis, MO, USA), and animals in the CTR and CTR+BCD groups received saline (same volume) (Farahmand et al., 2004; Werchan et al., 1989). After two weeks, animals in the CTR+BCD and MCT+BCD groups were given an intraperitoneal injection of bucindolol (Santa Cruz Biotechnology, Santa Cruz, CA, USA) every day for 7 days (2 mg/kg per day), and the CTR and MCT groups were injected with vehicle (1% DMSO, 2% Tween 20 and 0.9% NaCl), adapted from Baker et al. (2011). At the end of treatment, animals were anesthetised with an intraperitoneal injection of ketamine (90 mg/kg) and xylazine (10 mg/kg) (Flecknell, 2015), after which echocardiography and catheterisation of femoral artery and RV was performed. Immediately after, while still anesthetised, animals were decapitated for tissues collection (heart, lung, and liver).

2.3. Echocardiographic assessment

Images were obtained in the two-dimensional mode, M-mode and pulsed wave Doppler mode (HD7 Ultrasound System; Philips, Andover, MA, USA) using a S12-4 transducer (Philips, Andover, MA, USA). The following parameters were measured: diastolic and systolic RV diameter (RVDd and RVSd), area of the RV in diastole and systole, stroke volume (SV), cardiac output (CO), tricuspid annular plane systolic excursion (TAPSE), RV fractional shortening (FS) and RV fractional area change (FAC) (Urboniene et al., 2010; Rudski et al., 2010).

2.4. Measurement of arterial blood pressure

After echocardiography, the right femoral artery of each animal was exposed and a polyethylene catheter (P-10) filled with saline was inserted into the femoral artery. In order to assess systolic arterial pressure (SAP), diastolic arterial pressure (DAP) and heart rate (HR), the arterial pressure was monitored using a pressure transducer (Miniature Pulse Transducer PR-155; Narco Biosystems, Houston, TX, USA) connected to a signal amplifier (HP 8805 C; Hewlett Packard, Andover, MA, USA). The results for analogic pressure (mmHg) were digitized (WinDaq Data Acquisition System; Dataq Instruments Inc., Akron, OH, USA) over a frequency range of 2000 Hz (Baraldi et al., 2013; Jespersen et al., 2012).

2.5. Autonomic evaluation

After detecting the pulse intervals obtained by catheterisation of the femoral artery, heart rate (HR) was calculated on a beat-to-beat basis within the time interval between two consecutive systolic peaks or pulse intervals. Sequences of 250 beats were randomly chosen, and if an inconsistent pattern was observed this sequence was discarded and a new random selection was performed. The analysis of HR and arterial blood pressure was performed with an autoregressive algorithm of the interval sequences (tachograms). Two spectral components were considered in this study, low frequency (LF) from 0.10 to 1.00 Hz and high frequency (HF) from 1.00 to 5.00 Hz. The spectral components were expressed as their absolute (abs) and normalised units (nu). The ratio between power spectrum of LF and HF bands is called LF/HF index and represents the sympathovagal balance (Malliani et al., 1991; Montano et al., 1994).

2.6. Measurement of intraventricular pressure

The jugular vein of each animal was exposed and a polyethylene catheter (PE-50) filled with saline was inserted into the RV. The right ventricular systolic pressure (RVSP), right ventricular end diastolic pressure (RVEDP), dP/dt max (maximum rate of rise of RV pressure), and dP/dt min (maximum isovolumetric rate of relaxation) were monitored using a pressure transducer (Narco Biosystems) connected to a signal amplifier (Hewlett Packard). The analogic pressure data (mmHg) was digitized (WinDaq Data Acquisition System) over a