Biomedicine & Pharmacotherapy 99 (2018) 704–714

Contents lists available at ScienceDirect

Biomedicine & Pharmacotherapy

journal homepage: www.elsevier.com/locate/biopha

Bucindolol attenuates the vascular remodeling of pulmonary arteries by modulating the expression of the endothelin-1 A receptor in rats with pulmonary arterial hypertension

Bruna Gazzi de Lima-Seolin[a], Matheus Mittmann Hennemann[a], Rafael Oliveira Fernandes[a], Rafael Colombo[b], Jéssica Hellen Poletto Bonetto[a], Rayane Brinck Teixeira[a], Neelam Khaper[c], Alessandra Eifler Guerra Godoy[d], Isnard Elman Litvin[d], Alex Sander da Rosa Araujo[a], Paulo Cavalheiro Schenkel[a], Adriane Belló-Klein[a,*]

[a] Laboratory of Cardiovascular Physiology and Reactive Oxygen Species, Institute of Basic Health Science, Universidade Federal do Rio Grande do Sul, Rio Grande do Sul, Brazil
[b] Laboratory of Pharmacology and Physiology, Universidade de Caxias do Sul, Rio Grande do Sul, Brazil
[c] Northern Ontario School of Medicine, Lakehead University, Ontario, Canada
[d] Research Institute for Multicenter Studies, Universidade de Caxias do Sul, Rio Grande do Sul, Brazil

ARTICLE INFO

Keywords:
Adrenergic receptor blocker
Bucindolol
Endothelin-1 receptor
Monocrotaline
Nitric oxide synthase

ABSTRACT

The aim of this study was to investigate the role of the ß-adrenergic blocker bucindolol on endothelial dysfunction and pulmonary vascular remodeling in rats with pulmonary arterial hypertension (PAH). Male Wistar rats were divided into four groups: control, monocrotaline (MCT), control + bucindolol and monocrotaline + bucindolol (MCT + BCD). PAH was induced by an injection of monocrotaline (60 mg/kg i.p.). After two weeks, the animals were treated for seven days with bucindolol (2 mg/kg/day i.p.) or vehicle. Echocardiography was performed upon treatment completion to analyze pulmonary vascular resistance (PVR) and right ventricle (RV) myocardial performance index. Lungs were collected for oxidative stress and western blot analysis, and the pulmonary artery was analyzed for histological and immunohistochemical parameters. The MCT + BCD group showed a decrease (32%) in the protein expression of endothelin-1 type A receptor (ETAR) and in the ratio of ETA/endothelin-1 type B receptor (ETBR) (62%) as compared to the MCT group. Bucindolol treatment did not alter oxidative stress, as determined by lipid peroxidation analysis and antioxidant enzyme activities and expression, endothelial nitric oxide synthase immunocontent and decreased nitrotyrosine levels. Moreover, bucindolol improved vascular remodeling of the pulmonary artery in the MCT + BCD group by decreasing (21%) PVR and increasing RV workload in relation to MCT.

1. Introduction

Pulmonary arterial hypertension (PAH) is characterized by an inflammatory response that causes an imbalance between vasoactive molecules, such as endothelin-1 (ET-1) and nitric oxide (NO) [1]. NO is a potent vasodilator [2] and inhibitor of smooth muscle cell proliferation [3] that is released in the vascular lumen, and its synthesis is catalyzed by the endothelial nitric oxide synthase (eNOS) enzyme [2]. On the other hand, ET-1 is a potent endogenous vasoconstrictor with proinflammatory, mitogenic, and profibrotic effects in smooth muscle cells. ET-1 effects are exerted by the stimulation of two types of receptors: endothelin-1 type A receptor (ETAR) and endothelin-1 type B receptor (ETBR) [4]. An imbalance between NO and ET-1 promotes a reduction in the vessel's lumen, resulting in an increase in pulmonary vascular resistance (PVR) and mean pulmonary artery pressure (mPAP). Recent studies implicate oxidative stress as a mediator of PAH and the associated endothelial dysfunction. In fact, an increase in reactive oxygen species (ROS) production also contributes to vascular dysfunction [5].

Various models have been developed to understand the pathophysiology of PAH [6]. The most widely used model involves the pyrrolizidine alkaloid monocrotaline, which is present in plants of the

* Corresponding author at: Rua Sarmento Leite, 500, Bairro Farroupilha – CEP: 90050-170, Porto Alegre / RS/Brazil.
E-mail addresses: bruna.gazzi@hotmail.com (B.G. de Lima-Seolin), matheusmhennemann@gmail.com (M.M. Hennemann), rafafernandes.fisio@gmail.com (R.O. Fernandes), rcolombo1@ucs.br (R. Colombo), jpbonetto@gmail.com (J.H.P. Bonetto), raybrinck@gmail.com (R.B. Teixeira), nkhaper@nosm.ca (N. Khaper), aeggodoy@gmail.com (A.E.G. Godoy), ielitvin@ucs.br (I.E. Litvin), alex.rosa@ufrgs.br (A. Sander da Rosa Araujo), paulo.schenkel@ufrgs.br (P.C. Schenkel), belklein@ufrgs.br (A. Belló-Klein).

https://doi.org/10.1016/j.biopha.2018.01.127
Received 22 November 2017; Received in revised form 26 January 2018; Accepted 28 January 2018

Crotalaria genus [7,8]. It is known that its active metabolite, dehydromonocrotaline, accumulates in erythrocytes, enhancing the transit of this metabolite from the liver to the lungs, where it is released in the pulmonary endothelium [9]. Thus, dehydromonocrotaline promotes structural and functional changes in lung and pulmonary vasculature, leading to a sustained elevation of mPAP with clinical features that resemble human idiopathic PAH [10]. Recent studies have shown that adrenergic receptor blockade decreases mPAP and improves right ventricle (RV) function in PAH [11,12,13]. However, the use of ß-blockers for the treatment of PAH is controversial, due to possible negative effects on hemodynamics and exercise capacity [14]. Thus, more research is warranted to study ß-blocker efficacy.

Bucindolol is a lipophilic β-blocker agent with a hepatic metabolism by cytochrome P450. The half-life of bucindolol is 8 ± 4.5 h [15], and it is a β1-β2-blocker with mild vasodilatory properties by an α1-antagonism [16]. Moreover, bucindolol presents sympatholytic effects and reduces systemic norepinephrine levels in patients with increased sympathetic activity [17]. In a recent study of bucindolol in a model of PAH induced by monocrotaline, our group has shown attenuation of mPAP and an improvement in RV systolic function [11]; however, its effects on lung tissue and pulmonary artery are unknown. Thus, the present study aimed to evaluate the effects of bucindolol on vasoactive mediators, lung oxidative stress, and pulmonary artery remodeling induced by monocrotaline.

2. Methods

2.1. Ethical approval

All animal procedures performed in this study were in accordance with the "Guide for the Care and Use of Laboratory Animals" published by the National Institutes of Health (NIH Publication, 8th Edition, 2011), and conformed to the Ethical Committee on the Use of Animals of the Universidade Federal do Rio Grande do Sul (approval number 30484).

2.2. Experimental design

Male Wistar rats weighing 180 ± 5 g were obtained from the Center of Reproduction and Experimentation of Laboratory Animals at the Universidade Federal of Rio Grande do Sul. All animals (n = 36) received water and food *ad libitum* (Nuvilab CR-1; Nuvital, São Paulo, SP, Brazil) and were housed at a temperature of 20–25 °C with 70% humidity, under a 12-hour light/dark cycle. Animals were divided into four groups: 1) CTR - animals that did not receive monocrotaline or treatment with bucindolol; 2) MCT - animals that received monocrotaline alone; 3) CTR + BCD - animals that were treated with bucindolol alone; and 4) MCT + BCD - animals that received monocrotaline and bucindolol treatments. 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 in the same conditions [18]. After two weeks, animals in the CTR + BCD and MCT + BCD groups were given intraperitoneal injections of bucindolol (Santa Cruz Biotechnology, Santa Cruz, CA, USA) daily for 7 days (2 mg/kg per day), and the CTR and MCT groups were injected with vehicle (saline) [19]. At the end of the treatment, animals were anesthetized with an intraperitoneal injection of ketamine (90 mg/kg) and xylazine (10 mg/kg) for RV function assessment using echocardiography. Subsequently, the animals were sacrificed for tissue collection (lungs, pulmonary artery, RV, and right tibia). The lungs were immediately frozen in liquid nitrogen and then stored at − 80 °C for enzymatic and Western blot analysis. The pulmonary artery was immersed in formalin buffer for histological and immunohistochemical assessment. The RV weight and length of the tibia were measured for the morphometric analysis.

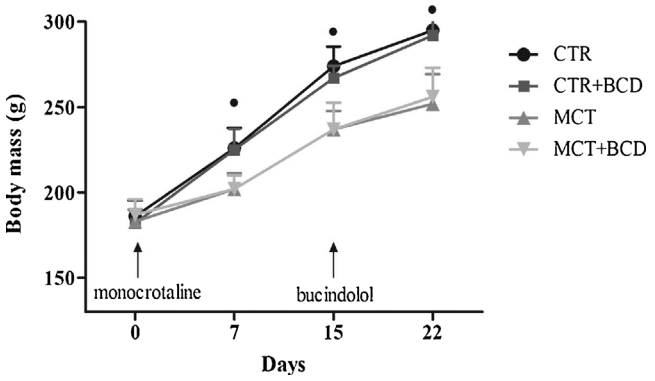

Fig. 1. Body weight gain in Wistar rats receiving monocrotaline (60 mg/kg – i.p.) or saline. Vehicle or bucindolol (2 mg/kg/day – i.p.) was started at day 15 for 7 days. Values are expressed as mean ± SD (n = 7 –10). Temporal analysis was assessed by repeated measures ANOVA. $^{\bullet}P < .05$ MCT and MCT + BCD vs. CTR and CTR + BCD. CTR: received saline and vehicle; MCT: received monocrotaline and vehicle; CTR + BCD: received saline and bucindolol; and MCT + BCD: received monocrotaline and bucindolol.

Table 1
Effects of daily treatment with bucindolol (2 mg/kg – i.p.) or vehicle for 7 days on morphometric and echocardiograph parameters in Wistar rats treated with saline or monocrotaline (60 mg/kg).

Parameters	CTR	MCT	CTR + BCD	MCT + BCD
RV (g)	0.19 ± 0.04	0.25 ± 0.06$^{\bullet}\triangledown$	0.18 ± 0.02	0.24 ± 0.03$^{\bullet *}$
RV/tibia length (mg/mm)	5.68 ± 1.10	7.49 ± 1.79$^{\bullet}\triangledown$	5.28 ± 0.69	7.27 ± 0.94$^{\bullet *}$
AT/ET (s/s)	0.34 ± 0.01	0.23 ± 0.02$^{\bullet}\triangledown$	0.32 ± 0.04	0.28 ± 0.02$^{\bullet}$†
MPI	0.19 ± 0.01	0.38 ± 0.12$\triangledown$	0.29 ± 0.12	0.26 ± 0.04†

Values are expressed as mean ± SD (n = 6–10). $^{\bullet}P < .05$ MCT and MCT + BCD vs. CTR and CTR + BCD; $\triangledown P < .05$ MCT vs. CTR; $^{*}P < .05$ MCT + BCD vs. CTR + BCD; †$P < .05$ MCT + BCD vs. MCT. RV: right ventricle; AT/ET: relationship between acceleration time through the pulmonary artery and the ejection time through the pulmonary artery; PVR: pulmonary vascular resistance; MPI: RV myocardial performance index. CTR, received saline and vehicle; MCT, received monocrotaline and vehicle; CTR + BCD, received saline and bucindolol; MCT + BCD, received monocrotaline and bucindolol.

2.3. Functional assessment

Echocardiographic images were obtained in the two-dimensional mode and pulsed wave Doppler mode (HD7 Ultrasound System; Philips, Andover, MA, USA) using a S12-4 transducer (Philips, Andover, MA, USA). The PVR was calculated by the relationship between acceleration time to ejection time through the pulmonary artery (AT/ET). The RV myocardial performance index (MPI) was determined using the following formula: MPI = (time of closing of the tricuspid valve - time of ejection through the pulmonary artery) / time of ejection through the pulmonary artery [20,21].

2.4. Morphometric assessment

The body weight of the animals was measured weekly to assess the effect of the PAH and the treatment on the body mass gain. The RV hypertrophy was determined by the ratio between RV mass/tibia length [22].

2.5. Histological analysis

Pulmonary arteries were fixed in 4% formalin for 24 h. Subsequently, the samples were immersed in paraffin overnight before tissue processing. The histological laminas were prepared as 3-μm thick sections, which were stained with hematoxylin and eosin [23], then observed with an optical microscope (DME; Leica Microsystems Inc.,