Pulmonary Pharmacology & Therapeutics 30 (2015) 57–65

Contents lists available at ScienceDirect

Pulmonary Pharmacology & Therapeutics

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

Effects of captopril on cardiovascular reflexes and respiratory mechanisms in rats submitted to monocrotaline-induced pulmonary arterial hypertension

Verônica Lourenço Wittmer [a, b], Élio Waichert Junior [b], Pablo Lúcio Gava [a, b], Fausto Edmundo Lima Pereira [c], Marco Cesar Cunegundes Guimarães [d], Suely Gomes de Figueiredo [b], Hélder Mauad [b, *]

[a] Department of Integrated Health Education, Center of Health Science, Federal University of Espírito Santo, Vitória, ES, Brazil
[b] Department of Physiological Sciences, Center of Health Science, Federal University of Espírito Santo, Vitória, ES, Brazil
[c] Pathology, Center of Health Science, Federal University of Espírito Santo, Vitória, ES, Brazil
[d] Department of Morphology, Center of Health Science, Federal University of Espírito Santo, Vitória, ES, Brazil

ARTICLE INFO

Article history:
Received 4 July 2014
Received in revised form
29 September 2014
Accepted 4 November 2014
Available online 20 November 2014

Keywords:
Pulmonary Arterial Hypertension
Monocrotaline
Captopril
Chemoreflex
Baroreflex

ABSTRACT

Background: Pulmonary Arterial Hypertension (PAH) is a disease associated with increased arteriolar resistance in the lungs. Due to hypoxemia, some physiological mechanisms can be posteriorly affected, including respiratory and cardiovascular reflexes, but this has not yet been fully investigated. This study aimed to evaluate how these mechanisms were affected by monocrotaline (MCT)-induced PAH and the possible therapeutic role of angiotensin converting enzyme inhibitor (ACEi), captopril, in reversing this remodeling process.

Methods and results: Groups of Wistar rats received MCT injections (60 mg kg^{-1}). Three weeks later, they received captopril (CPT, 100 mg kg^{-1}) in their drinking water (MCT + CPT) or water alone (MCT) for 2 weeks. As control, saline-treated animals received captopril in their drinking water (CPT) or water alone (CON), also for 2 weeks. Results showed that PAH was fully induced in the MCT group, evidenced by a high pulmonary index. Gasometrical and respiratory analyses showed hypoxemia and compensatory hyperventilation. CPT treatment brought these parameters to similar values to those observed in the CON group. We observed that autonomic dysfunction in the MCT group was suppressed by CPT. Finally, cardiovascular reflexes analysis showed increased chemoreflex responses in the MCT group, while baroreflex sensibility was decreased. Surprisingly, CPT normalized these reflex responses to values similar to the CON group.

Conclusions: The present study demonstrates that MCT-induced PAH induces compensatory respiratory responses, dysautonomia, and baroreflex dysfunction and increases chemoreflex responses. The data also indicate that CPT was effective in reversing these cardio-respiratory disorders, suggesting that ACEi could be a potential therapeutic target for PAH.

1. Introduction

Pulmonary Arterial Hypertension (PAH) is characterized by an elevated, sustained mean pulmonary arterial pressure greater than 25 mmHg at rest, measured by right-heart catheterization, with a normal pulmonary wedge pressure <15 mm Hg [1]. It is a chronic and progressive clinical picture leading to right-heart failure and death if untreated.

The etiology of PAH is not completely understood, and several possible underlying mechanisms have been suggested to explain the pathogenesis. The hallmark of this disorder is an increased pulmonary arterial resistance, which is usually induced by structural vascular remodeling resulting from intimal lesions, medial hypertrophy, and thickening of the pulmonary arteries [2].

Despite the significant advances in PAH therapies, most of the treatments are only symptomatic and cannot prevent disease

* Corresponding author. Departamento de Ciências Fisiológicas, Centro de Ciências da Saúde, Universidade Federal, do Espírito Santo, Av. Marechal Campos 1468, Vitória, ES, 29042-751, Brazil. Tel.: +55 27 3335 7336; fax: +55 27 3335 7342.
E-mail address: hmauad@terra.com.br (H. Mauad).

http://dx.doi.org/10.1016/j.pupt.2014.11.001
1094-5539/

evolution, which progresses rapidly and ultimately leads to right ventricular failure [3]. In this way, it is reasonable to suppose that anti-remodeling treatment may be a potential therapeutic option against PAH.

The involvement of the rennin-angiotensin system (RAS) in PAH has not been studied properly, and many questions remain to be answered. There are well known biological effects of angiotensin II (Ang II) in stimulating growth, differentiation and matrix synthesis in a number of mesenchymal cell types, and these effects could underlie the involvement of Ang II in multiple remodeling settings [4]. In addition, it is also known that the AT_1 receptor mediates constriction and hypertrophy in systemic vessels and produces inotropy, chronotropy and hypertrophy in the heart [5]. However, the role of Ang II and the AT_1 receptor in the pathogenesis of PAH is not known.

Therefore, the hypothesis of our study was that activation of the RAS could play a key role in the development of PAH, which led us to hypothesize that the use of angiotensin converting enzyme inhibitor (ACEi) drugs can limit disease progression and reverse vascular remodeling and its cardiovascular consequences.

In the present study, we used monocrotaline (MCT) as an experimental model to induce PAH in rats. MCT has been used in several studies [2,3]. Although the exact mechanism of MCT-induced PAH is not clear, it is known that MCT causes increasing resistance, medial hypertrophy and thickening of the pulmonary arteries, inducing significant right ventricular hypertrophy and dysfunction. The presence of these cardiopulmonary changes defines the MCT model for the study of PAH [6].

Considering the severity of the changes observed in the hearts and lungs in animals that were PAH-induced by MCT, other questions can arise that have not received due attention in the literature, such as the role of cardiovascular reflexes and their interplay with autonomic and respiratory systems. It is known that both baroreflexes and chemoreflexes play significant roles in blood pressure regulation by modulating autonomic pathways in the heart and arterial vessels [7]. Chemoreflexes, in turn, also modulate respiratory parameters, inducing tachypnea and hyperpnea, when chemically stimulated by potassium cyanide or hypoxia [8,9]. It is likely that chemoreceptors could be responsible for the survival of the PAH-induced animal, considering that hypoxia is usually observed in this model, but the role of chemoreflexes, as well as the baroreflex responses in PAH, remains unclear.

Thus, this study was performed a) to elucidate the therapeutic potential of RAS by evaluating the effects of ACEi captopril in reversing the cardio respiratory changes in MCT-induced PAH animals, and b) to evaluate the sensitivity of baro- and chemoreflexes in animals with MCT-induced PAH and the potential effects of captopril in modulating these cardiovascular reflex responses.

2. Methods

All experiments were conducted in accordance with the U.S. National Institutes of Health Guidelines for the Care and Use of Laboratory Animals, and study protocols were previously approved by our Institutional Ethics Committee for the Use of Animals (Protocol n° 008/2012).

2.1. Animal models

Nine-week-old male Wistar rats, weighing initially 180–220 g, were used in the experiments. They were maintained on a standard laboratory diet and tap water and exposed to a 12/12 h light–dark cycle at 23 °C. The animals were randomly divided into four groups: CON (control, saline-treated, $n = 18$), MCT (monocrotaline-treated, $n = 18$), CPT (captopril treated, $n = 18$) and MCT + CPT (monocrotaline and captopril-treated, $n = 18$). Rats from the MCT and MCT + CPT groups were induced to PAH through a single intraperitoneal (ip) injection of MCT (60 mg/kg, Sigma, St. Louis, MO, USA). Control rats received an equal volume of vehicle (saline, ~0.8 mL). Animals treated with captopril (100 mg/kg/day, Huahai, Zhejiang, China) (CPT and MCT + CPT groups) received this drug in their drinking water for 14 days. The dose of captopril was based on the work of Bolterman et al. [10].

2.2. Cardiovascular recordings

To permit evaluations of cardiovascular reflex and autonomic activities, one day before the experiments, with the animals under pentobarbital sodium anesthesia (40 mg/kg ip; Sigma Chemical, St. Louis, MO), a catheter (PE-10 connected to PE-50, Clay Adams, Parsippany, NJ) was inserted into the abdominal aorta through the femoral artery to measure the pulsatile arterial pressure (PAP). A second catheter was inserted into the femoral vein for drug injections. Cardiovascular recordings were performed with the animals under conscious, free-moving conditions. The PAP and mean arterial pressure (MAP) were measured using a pressure transducer (MLT0699) connected to a PowerLab® Data Acquisition System, Sydney, NSW, Australia. The MAP, systolic arterial pressure (SAP), diastolic arterial pressure (DAP) and heart rate (HR) were obtained from the PAP using Software Chart V4.2 (AD Instruments, USA). The sampling frequency was fixed at 1000 Hz. The animals were allowed 15-min before the recording began in order for them to adapt to the ambient environment.

2.3. Hemodynamic pressure measurements of the ventricles

Under pentobarbital sodium anesthesia (40 mg/kg ip), animals had a polyethylene catheter (PE-50) first inserted into the right jugular vein and navigated to the right ventricle (RV) to permit measurement of the right ventricular peak systolic pressure (RVPSP). To measure the left ventricular peak systolic pressure (LVPSP), a polyethylene catheter (PE-50) was inserted into the right carotid artery and positioned inside the left ventricle (LV). All hemodynamic variables were recorded for 15-min using a PowerLab® Data Acquisition System (Software Chart V4.2, AD Instruments, USA).

2.4. Determination of pulmonary index

The animals were euthanatized with an overdose of anesthesia. Subsequently, their hearts were dissected, the RV-free wall was separated from the LV and septum (LV + S), and both ventricles were weighed and normalized to body weight (BW). The ratios of RV/BW and LV + S/BW were used as the pulmonary index as previously described [11] to evaluate the development of PAH.

2.5. Respiratory measurements

Respiratory parameters were measured via whole-body plethysmography, as described by Malan [12]. This technique is based on monitoring small pressure changes within an isovolumetric animal chamber. A highly sensitive differential pressure transducer (Model 270, Hewlett Packard Co.) connected to a carrier amplifier (Model 8805B, Hewlett Packard Co.) and Biopac System Inc. (Model MP100) was used to record the respiratory parameters: tidal volume (V_T), respiratory rate (RR), ventilation per minute (V_{min}) and alveolar ventilation (V_A). To determine the V_A, 0.5 mL was considered to be the volume of dead space, according to a previous study [13]. During the recording period, each animal was kept in a closed chamber, and every 5-min, the chamber was open to ambient air