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Trapidil improves hemodynamic, echocardiographic and redox state parameters of right ventricle in monocrotaline-induced pulmonary arterial hypertension model

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ABSTRACT

Background: Pulmonary arterial hypertension is a disease characterized by increased pulmonary vascular resistance and redox imbalance, leading to failure of right ventricle. Trapidil has been described to improve the redox balance and cardiac conditions.
Hypothesis: Trapidil can improve the redox balance and contribute to functional improvements of the RV in PAH.
Methods and Results: Male, 5week-old Wistar rats were divided into four groups: Control, Control + Trapidil, Monocrotaline and Monocrotaline + Trapidil. PAH was induced by an intraperitoneal injection of monocrotaline 60 mg/kg at day 0. Treatment started at day 7 (5 or 8 mg/kg/day) until day 14, when animals were euthanized after echocardiography and catheterism. Right ventricular systolic pressure and pressure/time derivatives were increased in monocrotaline animals. The increased right ventricular diameters in monocrotaline groups were reduced with trapidil. Monocrotaline groups showed higher lipid peroxidation and glutathione peroxidase activity. Trapidil reduced NADPH oxidases activities and increased the reduced glutathiones/total glutathiones ratio. Protein expression of phospholamban in RV was diminished in monocrotaline groups, whereas expression of RyR and SERCA was enhanced in the groups treated with trapidil.
Conclusion: Our data suggest that trapidil induces an improvement in RV remodeling in PAH model, mitigating the progression of the disease.

1. Introduction

Pulmonary arterial hypertension (PAH) is a comorbidity which affects both lung and heart function [1]. In this disease, there is increased pulmonary vascular resistance and pressure, causing increased afterload to the right ventricle (RV), and consequently, augment in wall thickness and contractility deficiency. These changes culminate in poor adaptive response, characterized by dilation, right ventricular dysfunction and failure [2,3].

Evidences in the literature suggest the involvement of oxidative stress in cardiac hypertrophy and heart failure [4]. In its classical definition, oxidative stress is characterized by the imbalance between reactive oxygen species (ROS) production and the antioxidant defense system, factors that may be determinant for disease progression and severity [5,6]. In cardiomyocytes, ROS are derived from many sources, including mitochondria and pro-oxidant enzymes, such as nicotinamide adenine dinucleotide phosphate (NADPH) oxidases and uncoupled nitric oxide synthase [7]. The oxidative stress promotes damage to macromolecules and cell signalling impairment, resulting in cell dysfunction, necrosis and/or apoptosis [5].

The contractile function in cardiomyocytes may also be impaired by increased ROS levels established in the PAH. This occurs through interruption of the calcium cycle, alteration in myofilament proteins, as well as through deleterious effects on energy metabolism [4]. Additionally, the ROS can damage sarcolemma calcium channels, suppress sarcoplasmic reticulum activity and Sarcoplasmic Endoreticulum Calcium ATPase (SERCA), impairing contractility [8].

There is still no specific therapy to prevent or treat cardiac changes

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derived from PAH, so the search for new therapeutic options becomes necessary [2]. On the order hand, the drug trapidil (N, *N*-diethyl-5-methyl- [1,24] triazol [1,5] pyrimidine-7-amine) is a triazolpyrimidine widely used in clinic in cardiac patients due to its vasodilatory activity [10]. The mechanism of its vasodilatory action occurs not only by inhibition of phosphodiesterases, but also antagonizing platelet-derived growth factor (PDGF) [10]. Trapidil also has an antithrombotic action by inhibiting the synthesis of thromboxane A2, an important systemic vasoconstrictor and activator of platelet aggregation [10,11]. In addition to the already well-established vasodilator and antithrombotic effects, other studies highlight the anti-inflammatory and antioxidant potential of trapidil [9,12,13]. Therefore, the use of trapidil as a therapeutic alternative for the treatment of PAH can be highly promising and relevant. From this point of view, the objective of the present study was to evaluate the effects of trapidil on hypertrophy, ventricular function and RV contractility, as well as analyse parameters of oxidative stress in an experimental model of PAH.

2. Material and methods

The experimental procedures of our study were divided into two different moments, initially being carried out with the dose of trapidil fixed at 5 mg/kg/day (Experiment 1) and later in another study with the dose of trapidil fixed at 8 mg/kg/day (Experiment 2) [12,14,15,16].

2.1. Animals

Male Wistar rats weighing approximately 100 g from the Center for Reproduction and Laboratory Animal Experimentation of the Universidade Federal do Rio Grande do Sul (UFRGS) were housed in polypropylene boxes (33 × 17 × 40 cm), four animals per box, under standard animal conditions: 21 °C, light-dark cycle of 12 h and humidity of 70%. Water and commercial feed were offered ad libitum. Weight gain was measured daily. Our experimental protocol was carried out in accordance with the International Guidelines for Use and Care of Laboratory Animals and with Brazilian Laws for the Scientific Use of Animals. The experimental protocol began only after it had been approved by the Ethical Committee for Animal Experimentation at UFRGS (#28515).

2.2. Experimental groups

The animals were divided into 4 groups: **Control (CTR)** - Animals that received only the vehicle of trapidil (0.9% NaCl) and monocrotaline (0.9% NaCl); **Control + Trapidil (CTR + TRAP)** - Animals that received monocrotaline vehicle and treatment with trapidil; **Monocrotaline (MCT)** - Animals that received monocrotaline and only the vehicle of trapidil; **Monocrotaline + Trapidil (MCT + TRAP)** - Animals that received monocrotaline and treatment with trapidil.

2.3. Pulmonary arterial hypertension induction

For the induction of pulmonary arterial hypertension, animals from the MCT and the MCT + TRAP groups received a single intraperitoneal dose of 60 mg/kg of monocrotaline (Crotaline - C240 Sigma Aldrich®) [17]. Animals from the CTR and CTR + TRAP groups received a single intraperitoneal dose of saline (0.9% NaCl) in the same volume.

2.4. Trapidil administration

Animals from the CTR + TRAP and the MCT + TRAP groups received trapidil (T1820000 - Sigma-Aldrich®) via intraperitoneal 5 mg/kg diluted in 0.9% NaCl once daily from day 7 to day 14 (Experiment 1) or 4 mg/kg diluted in 0.9% NaCl twice daily (8 mg/kg/day) from day 7 to day 14 (Experiment 2). During this period, animals from the CTR and MCT groups received 0.9% NaCl in the same volume applied to the animals of the treated groups.

Therefore, two experiments were carried out at different times: Experiment 1 (trapidil dose of 5 mg/kg/day) and Experiment 2 (trapidil dose of 8 mg/kg/day).

2.5. Echocardiography

The animals were anesthetized (ketamine 90 mg/kg, xylazine 10 mg/kg, intraperitoneal), submitted to trichotomy of the thoracic region and placed in lateral decubitus position. The images were obtained using a Philips HD7 Ultrasound System (Andover, MA, USA) with a S12-4 transducer. The following parameters were analysed: tricuspid annular plane systolic excursion (TAPSE); right ventricular shortening fraction (RVSF), right ventricular myocardial performance index (MPI), RV stroke volume (RVSV), RV fractional area change (RVFAC), relation between pulmonary artery acceleration time and ejection time (AT/ET) and flow rate during fast and slow emptying of the right ventricle (ratios between E/A peaks). The RV systolic and diastolic diameters were also measured and evaluated.

2.6. Right ventricular pressure record

Animals were anesthetized (ketamine 90 mg/kg, xylazine 10 mg/kg, intraperitoneal) and submitted to trichotomy of the right jugular region. The jugular was dissected and isolated. Blood flow was blocked using a metal occluder. With obstructed flow, an incision was quickly made in the vessel's upper wall to allow the introduction of a PE-50 polyethylene catheter filled with 0.9% NaCl. The catheter was inserted into the right ventricle and its position was determined by the observation of the characteristic ventricular pressure waveform. After 5 min of stabilization, right ventricular systolic pressure and right ventricular end-diastolic pressure were recorded. The analog pressure signals were digitized (Windaq-Data Acquisition System, PC) with sampling rate of 2000 Hz, expressed in mmHg. The values of the contraction derivative (dP/dt_{max}) and the relaxation derivative (dP/dt_{min}) were obtained from the derivation of the right ventricular pressure wave and detection of maximum and minimum points for each cardiac cycle.

2.7. Morphometric analysis of right ventricle

After euthanasia, the hearts were rapidly withdrawn and placed in 1.15% KCl solution on ice. The atria were separated and discarded, while the ventricles were weighed. Right ventricle was separated from the left ventricle + septum for evaluation of right ventricular hypertrophy using the following ratios: right ventricular mass/body mass, right ventricular mass/tibia's length, right ventricular mass/left ventricular + septum mass.

2.8. Preparation of the homogenates and analysis of the RV redox State

The hearts were immediately stored in liquid nitrogen after withdrawal and subsequently stored at −80 °C until analysis. The homogenization of the RV was performed for 40 s in the presence of 1.15% KCl (5 ml/g tissue) and 100 mM phenyl methyl sulfonyl fluoride. The homogenates were centrifuged for 20 min at 10.000 × *g* at 4 °C and supernatant was collected and stored at −80 °C for further analyses.

2.9. Protein quantification

Protein concentration in the homogenates was estimated by Lowry's method using bovine serum albumin solution as standard [18].

2.10. Determination of lipid peroxidation

Lipid peroxidation was evaluated by the chemiluminescence (QL)