Respiratory Physiology & Neurobiology 186 (2013) 61–64

Contents lists available at SciVerse ScienceDirect

Respiratory Physiology & Neurobiology

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

Improvement of exercise capacity in monocrotaline-induced pulmonary hypertension by the phosphodiesterase-5 inhibitor Vardenafil

Stephan Schroll[a,*], Daniela Sebah[a], Michael Wagner[a], Viktoria Popara[a], Michael Pfeifer[a,b], Friedrich Blumberg[a,c]

[a] Department of Internal Medicine II, Pneumology, University Hospital Regensburg, Germany
[b] Department of Pneumology, Donaustauf Hospital, Donaustauf, Germany
[c] Department of Internal Medicine II, Prosper-Hospital, Mühlenstraße 27, 45659 Recklinghausen, Germany

ARTICLE INFO

Article history:
Accepted 6 November 2012

Keywords:
Pulmonary hypertension
Vardenafil
Exercise capacity
Monocrotaline

ABSTRACT

Vardenafil, a phosphodiesterase-5 inhibitor, is approved for the therapy of erectile dysfunction. However, in contrast to Sildenafil and Tadalafil, little is known about its effects on pulmonary hypertension.

Four weeks after monocrotaline-administration rats exhibited a significant increase in right ventricular pressure (RVSP, 94 mmHg vs. 25 mmHg; $p = 0.001$) right ventricular weight (right ventricle/left ventricle + septum, 59 vs. 23; $p = 0.001$) and pulmonary vascular remodeling (medial wall area 104% vs. 66%; $p < 0.05$) as compared to controls, with a corresponding reduction in exercise capacity (% from baseline value: 67%; $p < 0.05$). Vardenafil treatment resulted in decreased RVSP (56 mmHg vs. 95 mmHg; $p = 0.008$), right ventricular weight (41 vs. 59; $p = 0.013$), pulmonary vascular remodeling (medial wall area 64% vs. 104%; $p < 0.05$) and a significant better exercise capacity (% from baseline value: 84% vs. 67%; $p < 0.05$) compared to monocrotaline only treated animals.

In conclusion, Vardenafil exerts beneficial effects on monocrotaline-induced pulmonary hypertension in rats. Whether it is a treatment option for patients with pulmonary hypertension needs to be elucidated.

1. Introduction

Pulmonary arterial hypertension is a progressive disease leading to pulmonary vascular remodeling, right ventricular failure, functional impairment and death. Phosphodiesterase-5 (PDE5) inhibitors such as Sildenafil and Tadalafil are approved therapies for pulmonary arterial hypertension. Vardenafil is a PDE5 inhibitor which is approved for erectile dysfunction, but only little data exist regarding its effects on the pulmonary circulation. Small studies in patients with various forms of pulmonary arterial hypertension showed beneficial hemodynamic and clinical effects of Vardenafil (Jing et al., 2009, 2011).

Conflicting results have been reported in respect to the ability of the different PDE5 inhibitors to improve hemodynamics and functional class in patients with pulmonary hypertension (Buckley et al., 2010; Ghofrani et al., 2004) and to attenuate acute hypoxic pulmonary vasoconstriction in experimental animals (Tsai et al., 2006; Geiger et al., 2008).

Although Vardenafil has a more than 20-fold greater potency than Sildenafil for inhibiting purified PDE5 (Rosen and Kostis, 2003) and is the only PDE5 inhibitor with additional calcium-channel blocking properties (Toque et al., 2008), the substance has not yet been tested in established experimental models of chronic pulmonary hypertension such as chronic hypoxic or monocrotaline-induced pulmonary hypertension.

In the present study we, therefore, tested the effects of the PDE5 inhibitor Vardenafil in the model of monocrotaline-induced pulmonary hypertension in rats. We hypothesized that Vardenafil treatment ameliorates pulmonary vascular remodeling, right ventricular systolic pressure, right ventricular hypertrophy and improves exercise capacity.

2. Methods

2.1. Study design

Adult male Wistar rats (250 g; Charles River, Sulzfeld) were randomly assigned to one of the following groups: untreated animals (controls, $n = 10$), monocrotaline only (monocrotaline, Sigma, 60 mg/kg administered subcutaneously on day 1 of the study period, $n = 10$), and monocrotaline plus Vardenafil

* Corresponding author at: Department of Internal Medicine II, Pneumology, University of Regensburg, Franz-Josef- Strauß-Alle 11, 93042 Regensburg, Germany. Tel.: +49 0941 944 7281; fax: +49 0941 944 7282.
E-mail address: stephan.schroll@klinik.uni-regensburg.de (S. Schroll).

1569-9048/$ – see front matter © 2012 Elsevier B.V. All rights reserved.
http://dx.doi.org/10.1016/j.resp.2012.11.003

(monocrotaline + Vardenafil, Vardenafil 20 mg/kg, administered daily by gavage from day 1 until the end of the study period, $n = 10$).

Rats were housed in identical cages with a 12 h day–night cycle and received food and water ad libitum. Exercise testing was performed in all study animals every second day using a standard treadmill test. Four weeks after monocrotaline-administration right heart catheterisation was performed in all animals. After hemodynamic measurements rats were killed by decapitation. Hearts and lungs were removed and right ventricular weight and pulmonary vascular remodeling were analyzed.

Animal experiments were conducted according to the National Institutes of Health Guide for the Care and Use of Laboratory Animals and German laws on the protection of animals. The protocol was approved by the ethics committee of the University of Regensburg.

2.2. Exercise capacity

To evaluate maximum exercise capacity a standard treadmill for rodents with a built-in electric grid was used. The animals were educated to treadmill exercise over seven days according to a training protocol. After training all rats underwent testing for maximum exercise capacity on two consecutive days before monocrotaline-application. Exhaustion was defined as the third time the rat did no longer keep pace with the speed of the treadmill. The average of the two maximum exercise capacity tests was calculated as baseline exercise capacity. After monocrotaline-application maximum exercise capacity testing was performed every second day for four weeks at a constant slope of 12° and speed of 25 m/min. For each rat the change in maximum exercise capacity to baseline was calculated in percent. In the control group exercise testing was stopped when the animal reached baseline maximum exercise capacity.

2.3. Hemodynamic measurements

Systolic blood pressure was measured in all rats the day before the end of the study period under prewarmed conditions by the tail-cuff method (BP recorder 8005, W&W Electronics, Heifheim). At the end of the study period, right ventricular systolic pressure was measured in all rats with a closed-chest technique as a surrogate parameter for the development of pulmonary hypertension. For this purpose, each rat was taken from its cage and was immediately anesthetized with thiopental (50 mg/kg ip). The right jugular vein was cannulated, and a catheter was introduced into the right ventricle. The system was filled and flushed with <2 ml of heparin solution (1000 IU/ml). After a stable hemodynamic condition was reached, right ventricular systolic pressure was measured by using a pressure transducer (P23Db, Statham Laboratories, Hatorey, Puerto Rico).

2.4. Organ sampling

The animals were killed by decapitation. The hearts were removed and then separated in right and left ventricle and septum. The weights of the right ventricle, the left ventricle and the septum of the heart were measured separately. The lungs were separated in right and left lung. The left lung was flushed with PBS buffered 4% formalin and embedded in paraffin for histochemical analysis.

2.5. Pulmonary vascular remodeling

To analyze the different effects of pharmacological interventions on the remodeling of pulmonary arteries light microscopic analysis were performed and pulmonary medial vascular wall area

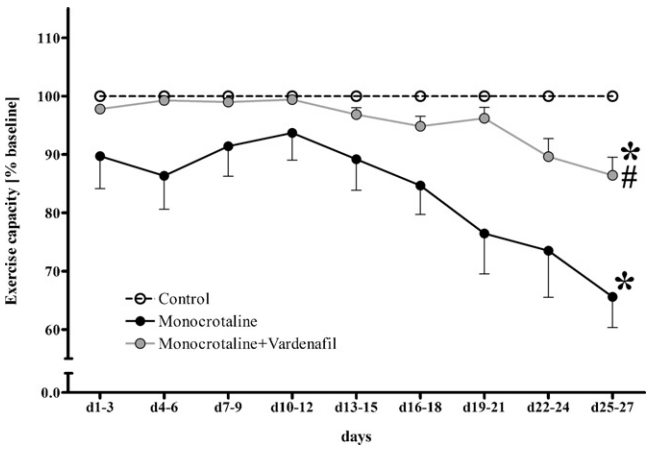

Fig. 1. Maximum exercise capacity measured as the average of two days analyzed as change to baseline in percent in controls, monocrotaline and monocrotaline plus Vardenafil-treated animals. A significant main effect of group ($p < 0.001$), a significant main effect of time ($p < 0.001$) and a significant interaction effect of time × group ($p < 0.001$) were found by linear mixed models based on ranks. For the pairwise comparisons of groups, we performed Tukey-adjusted post-hoc analyses at days 22–25. * vs. control, # vs. monocrotaline.

was determined. Microscopic slices were analyzed by using a computerized morphometric system (Meta Imaging Series Version 4.5 MetaVue Universal Imaging, Pennsylvania, USA). Total vessel area was defined as the area within the elastica externa. Medial area was defined as the area between the lamina elastica externa and the lamina elastica interna. Pulmonary arteries with an external diameter ranging from 30 to 100 μm were examined. Ten arteries per animal were measured. The average of three measurements obtained from each artery was used for calculations. Slides were analyzed by two observers who were blinded for the modality of treatment.

2.6. Statistical analysis

Parameters are expressed as means ± standard deviation. ANOVA followed by Bonferroni-adjusted post-hoc testing was used for comparisons between the different study groups for parameters that were measured once at the end of the study. For the analysis of exercise capacity a linear mixed model based on ranks with the main factors group and time was used. The covariance structure was specified as unstructured. For the last time-point, a Kruskal–Wallis-Test was performed followed by a post-hoc analysis (Wilcoxon test). Data entry and calculations were made with GraphPad Prism 4.03, linear mixed model analyses were done with SAS 9.2 by the procedure PROC MIXED.

3. Results

3.1. Exercise capacity

Monocrotaline-induced pulmonary hypertension resulted in a significant lower exercise capacity compared to control (% from baseline value at days 25–27: 67% vs. 98%; $p = 0.0006$, Fig. 1). Application of Vardenafil in monocrotaline-treated animals resulted in an improvement of exercise capacity compared to the monocrotaline only group (% from baseline value at days 25–27: 84% vs. 67%; $p = 0.0029$). There was a significance difference of exercise capacity between the control group and the monocrotaline + Vardenafil group (% from baseline value at days 25–27: 98% vs. 84%; $p = 0.0067$).