International Journal of Cardiology 216 (2016) 85–91

Contents lists available at ScienceDirect

International Journal of Cardiology

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

Soluble guanylate cyclase stimulator riociguat and phosphodiesterase 5 inhibitor sildenafil ameliorate pulmonary hypertension due to left heart disease in mice

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ARTICLE INFO

Article history:
Received 10 February 2016
Accepted 11 April 2016
Available online 16 April 2016

Keywords:
Transverse aortic constriction
Riociguat
Sildenafil
Pulmonary hypertension
Heart failure

ABSTRACT

Background: Presence of pulmonary hypertension (PH) and right ventricular dysfunction worsens prognosis in patients with chronic heart failure (CHF). Preclinical and clinical studies suggest a role for the impaired nitric oxide (NO)-soluble guanylate cyclase (sGC)-cyclic guanosine monophosphate (cGMP) signaling pathway in both PH and CHF. Hence, we examined the effects of the NO–sGC–cGMP pathway modulation by the PDE5 inhibitor sildenafil or sGC stimulator riociguat on pulmonary hemodynamics and heart function in a murine model of secondary PH induced by transverse aortic constriction.
Methods: C57Bl/6N mice were subjected to transverse aortic constriction (TAC) for 6 weeks to induce left heart failure and secondary PH and were subsequently treated with either sildenafil (100 mg/kg/day) or riociguat (10 mg/kg/day) or placebo for 2 weeks.
Results: Six weeks after surgery, TAC induced significant left ventricular hypertrophy and dysfunction associated with development of PH. Treatment with riociguat and sildenafil neither reduced left ventricular hypertrophy nor improved its function. However, both sildenafil and riociguat ameliorated PH, reduced pulmonary vascular remodeling and improved right ventricular function.
Conclusions: Thus, modulation of the NO–sGC–cGMP pathway by the PDE5 inhibitor sildenafil or sGC stimulator riociguat exerts direct beneficial effects on pulmonary hemodynamics and right ventricular function in the experimental model of secondary PH due to left heart disease and these drugs may offer a new therapeutic option for therapy of this condition.

1. Introduction

Pulmonary hypertension (PH) is a progressively fatal disorder caused by a wide array of conditions that increase pulmonary artery pressure. The PH due to left heart disease (LHD) is classified as a group 2 PH and is one of the most common forms of PH [1]. Though the true prevalence of PH in chronic heart failure (CHF) is not known, it is estimated that about 60% to 70% of patients with left ventricular (LV) dysfunction have PH [2]. Presence of PH and right ventricular (RV) dysfunction worsens prognosis in patients with CHF [3–5]. However, no approved heart failure therapies target the pulmonary vasculature of patients with CHF and no validated therapy exists for the PH in patients with CHF highlighting an enormous unmet medical need in this patient population [6].

Nitric oxide (NO)-soluble guanylate cyclase (sGC)-cyclic guanosine monophosphate (cGMP) signaling is one of the most important pathways influencing cardiovascular physiology [7]. In CHF due to chronic

Abbreviations: PH, pulmonary hypertension; LHD, left heart disease; CHF, chronic heart failure; LV, left ventricular; RV, right ventricular; NO, nitric oxide; sGC, soluble guanylate cyclase; PDE, phosphodiesterase; cGMP, cyclic guanosine monophosphate; TAC, transverse aortic constriction; LVEDD, left ventricular end-diastolic diameter; LVESD, left ventricular end-systolic diameter; PAAT, pulmonary artery acceleration time; PAET, pulmonary artery ejection time; RVSP, right ventricular systolic pressure; TAPSE, tricuspid annular plane systolic excursion; PVR, pulmonary vascular resistance; NGAL, neutrophil gelatinase associated lipocalin; TIMP-1, tissue inhibitor of matrix metalloproteinases; NM, non-muscularized; PM, partially muscularized; FM, fully muscularized.

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http://dx.doi.org/10.1016/j.ijcard.2016.04.098
0167-5273/

pressure overload, NO-derived cGMP is decreased due to uncoupling of NO synthase, sGC oxidation and increase in cGMP hydrolysis by the phosphodiesterase (PDE) isoforms 1 and 5 [8–10]. Targeting this axis suppresses and reverses pressure overload induced LV hypertrophy and dysfunction [11]. As NO–sGC–cGMP signaling pathway is impaired both in PH and CHF [12–15], we hypothesized that modulating this pathway would be beneficial in PH due to LHD. Therefore, the aim of the study was to examine if modulation of the NO–sGC–cGMP pathway by the PDE5 inhibitor sildenafil or sGC stimulator riociguat exerts beneficial effects on pulmonary hemodynamics and heart function in a murine model of secondary PH induced by transverse aortic constriction (TAC).

2. Materials and methods

2.1. Animals and experimental design

Eight to ten week old male C57BL/6N mice (Charles River Laboratories, Sulzfeld, Germany) were maintained under appropriate barrier conditions in a 12 h light–dark cycle and received food and water ad libitum. All procedures involving animals were approved by the governmental Animal Ethics Committee (Regierungspraesidium Giessen, Gi 20/10 Nr. 09/2012).

Mice were either exposed to TAC to induce pressure-overload left ventricular hypertrophy or sham surgery. Six weeks after surgery, the banded mice were randomized to receive either sildenafil (Pfizer Inc., New York, USA; 100 mg/kg/day, n = 20) in drinking water or oral riociguat (BAY 63-2521; Bayer Healthcare AG, Wuppertal, Germany; 10 mg/kg/day, n = 20) or oral placebo (1% methylcellulose, n = 20) for the following 14 days until the day of the terminal hemodynamic measurements. Sham mice (n = 20) received methylcellulose 1% by oral gavage. Transthoracic echocardiography was performed before surgery, six weeks and eight weeks after TAC.

2.2. TAC surgery

Pressure overload was induced by TAC as described [16] with modifications. Briefly, 30 min prior surgery, a subcutaneous injection of buprenorphine (Temgesic®, 0.1 mg/kg, Essex Pharma GmbH, Munich, Germany) was given to reduce pain. Surgery was performed under general anesthesia of 2% isoflurane (Forene® Abbott, Wiesbach, Germany). Small incision was made in the left second intercostal space and sternum was retracted. After identification of transverse aorta, a small titanium clip (Hemoclip®, Edward Weck, USA) was placed around transverse aorta between innominate artery and left common carotid artery to make a constriction, restricting the lumen to a diameter of 0.3 mm. The chest cavity and skin was stitched by a 6–0 polypropylene suture (Prolene®, Ethicon, Germany). In control age-matched mice, a sham operation without aorta occlusion was performed.

2.3. Echocardiography

Echocardiographic images were acquired using Vevo 770 high-resolution imaging system equipped with a 30-MHz RMV-707B scanning head (VisualSonics, Toronto, Canada). Fractional shortening of the left ventricle was calculated as (LVEDD − LVESD) / LVEDD × 100, where LVEDD — left ventricular end-diastolic diameter and LVESD — left ventricular end-systolic diameter. The measurements were obtained using the parasternal long axis view. Pulmonary artery acceleration time (PAAT) was measured as the time interval from onset of flow to peak flow and pulmonary artery ejection time (PAET) was measured as the time from onset to the end of systolic flow velocity. The ratio PAAT/PAET was calculated for a noninvasive estimation of the right ventricular systolic pressure (RVSP) [17]. Pulmonary artery flow measurements were obtained using the parasternal short-axis view at the level of the aortic root. Cardiac output was calculated as the product of the velocity-time integral of the pulsed-wave Doppler tracing of the pulmonary flow at the level of the pulmonary valve tips, the main pulmonary cross-sectional area at that level and the heart rate [18]. For assessment of the RV performance, the tricuspid annular plane systolic excursion (TAPSE) was measured. Images were then analyzed off-line by a single observer blinded to the respective treatments of mice.

2.4. Hemodynamic measurements and sample processing

Hemodynamic measurements were performed as described [19] with slight modifications. Briefly, anesthesia was induced with 3% isoflurane (Forene® Abott, Germany) in oxygen after pre-treatment with buprenorphine (0.1 mg/kg, SC) and maintained with 1.5% isoflurane. After intubation the mouse was placed supine on a homeothermic plate and connected to a small animal ventilator MiniVent type 845 (Hugo Sachs Elektronik, March-Hugstetten, Germany). The body temperature was controlled by a rectal probe connected to the control unit and was kept at 37 °C during the procedure. A high fidelity 1.4F micromanometer catheter (Millar Instruments, Houston, USA) was introduced into the right external jugular vein and then advanced into the RV to assess RVSP. Afterwards, the catheter was inserted into the aorta and the LV via the right carotid artery for measurement of pre-stenotic aortic and left ventricular pressures. Aortic pressure distal to the constriction was measured by inserting the catheter via the left carotid artery. The TAC pressure gradient was then determined by calculating the systolic pressure difference before and after constriction. Pulmonary vascular resistance (PVR) was calculated as follows: PVR = (RVSP − LVEDP) / cardiac output, where LVEDP — LV end-diastolic pressure. Data were collected and analyzed with the PowerLab data acquisition system (MPVS-Ultra Single Segment Foundation system, AD instruments, Germany) and Labchart 7 software.

At the conclusion of the measurements, blood samples were obtained. After exsanguination, the hearts and lungs were harvested, weighed and used for histological evaluation.

2.5. Histology

After flushing the lungs with saline, lungs were fixed by vascular perfusion with 3.5% formaldehyde through the pulmonary artery. Investigations were performed on 3 μm sections of paraffin embedded lungs. The degree of muscularization of pulmonary arterial vessels was assessed from lung sections that were double stained with a mouse monoclonal anti-α smooth muscle actin antibody (dilution 1:900 in 10% BSA, Sigma Aldrich, Munich, Germany) and a rabbit polyclonal anti-von Willebrand factor antibody (dilution 1:900 in 10% BSA, Dako, Hamburg, Germany) as described [20]. An antibody directed against IgG (Millipore, Schwalbach, Germany) served as a negative control. Sections were counterstained with methyl green. 80–100 vessels with an outer diameter of 20–70 μm were used for analysis. Vessels were categorized as fully- (>70% of vessel circumference is α-smooth muscle actin positive), partially- (5%–70% of vessel circumference is α-smooth muscle actin positive) or non-muscularized (<5% of vessel circumference is α-smooth muscle actin positive). Van Gieson's staining was used to measure medial wall thickness of pulmonary vessels. 80–100 vessels with an outer diameter of 20–70 μm were used for analysis. All morphometric quantifications were carried out microscopically using Leica Qwin V3 computer-assisted image analysis software (Leica Microsystem, Wetzlar, Germany).

For interstitial fibrosis determination, hearts were fixed with 3.5% paraformaldehyde. Investigations were performed on 3 μm sections of paraffin embedded hearts. Heart sections were stained with 0.1% sirius red (sirius red F3B, Niepoetter, Bürstadt, Germany) in picric acid (Fluka, Neu-Ulm, Germany). Photomicrographs were quantified to determine interstitial collagen fraction using Leica Qwin V3 computer-assisted image analysis software (Leica Microsystem, Wetzlar,