Acute effects of sildenafil and dobutamine in the hypertrophic and failing right heart in vivo

Asger Andersen,¹ Jan M. Nielsen,¹ Sivagowry Rasalingam,¹ Erik Sloth,²
Jens Erik Nielsen-Kudsk¹

¹Department of Cardiology, Institute of Clinical Medicine, Aarhus University Hospital, Skejby, Aarhus, Denmark; ²Department of Anesthesia and Intensive Care, Aarhus University Hospital, Skejby, Aarhus, Denmark

Abstract: The purpose of this study was to investigate whether acute intravenous administration of the phosphodiesterase type 5 (PDE-5) inhibitor sildenafil in a single clinically relevant dose improves the in vivo function of the hypertrophic and failing right ventricle (RV). Wistar rats ($n = 24$) were subjected to pulmonary trunk banding (PTB) causing RV hypertrophy and failure. Four weeks after surgery, they were randomized to receive an intravenous bolus dose of sildenafil (1 mg/kg; $n = 7$), vehicle ($n = 6$), or dobutamine (10 μ g/kg; $n = 6$). Invasive RV pressures were recorded continuously, and transthoracic echocardiography was performed 1, 5, 15, 25, 35, 50, 70, and 90 minutes after injecting the bolus. Cardiac function was compared to baseline measurements to evaluate the in vivo effects of each specific treatment. The PTB procedure caused significant hypertrophy, cardiac fibrosis, and reduction in RV function evaluated by echocardiography (TAPSE) and invasive pressure measurements. Sildenafil did not improve the function of the hypertrophic failing right heart in vivo, measured by TAPSE, RV systolic pressure (RVsP), and $dp/dt_{\max}$. Dobutamine improved RV function 1 minute after injection measured by TAPSE (0.1242 ± 0.03 vs. 0.1565 ± 0.04 cm; $P < 0.001$), RVsP (83 ± 11 vs. 107 ± 11 mmHg; $P < 0.001$), and $dp/dt_{\max}$ ($3,136 \pm 521$ vs. $4,489 \pm 706$ mmHg/s; $P < 0.001$). Acute administration of the PDE-5 inhibitor sildenafil in a single clinically relevant dose did not modulate the in vivo function of the hypertrophic failing right heart of the rat measured by echocardiography and invasive hemodynamics. In the same model, dobutamine acutely improved RV function.

Keywords: right heart failure, pulmonary circulation and disease, sildenafil, cardiovascular pharmacology, animal models of human disease.

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INTRODUCTION

Right ventricular (RV) function is closely correlated with mortality and morbidity in patients suffering from pulmonary arterial hypertension (PAH), chronic

thromboembolic pulmonary hypertension (CTEPH), and congenital heart disease. Most therapies are focused on unloading the RV through a direct effect on

Address correspondence to Asger Andersen, MD, Aarhus University Hospital, Skejby, Department of Cardiology, Institute of Clinical Medicine Brendstrupgaardsvej 100, 8200 Aarhus N, Denmark. E-mail: asger.andersen@ki.au.dk.

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pulmonary arteries by vasodilatation and/or reverse vascular remodeling. However, a strategy for supporting the RV may be an important supplement to the group of pulmonary vasodilatory drugs. The cyclic guanosine monophosphate pathway is a promising target in protecting the pressure-overloaded left ventricle (LV). Treatment with phosphodiesterase type 5 (PDE-5) inhibitors has been demonstrated to attenuate LV hypertrophy and improve LV function in mice,¹ but this effect was not found in rats with pressure overload-induced RV hypertrophy.²⁻⁴ Sildenafil failed to inhibit RV hypertrophy, but some improvement in RV contractile function was seen in long-term sildenafil treatment. The improved function could possibly be explained by direct inhibition of PDE-5 in RV cardiomyocytes. PDE-5 is not expressed in the healthy RV, but it is upregulated in hypertrophic RVs from rats and patients. Acute inhibition of PDE-5 increases contractility in hypertrophic RVs from rats *ex vivo* and isolated human right atrial strips.^{5,6} These findings are promising for PDE-5 inhibitors as a treatment for RV failure, but the direct acute effects of a clinically relevant dose of the PDE-5 inhibitor sildenafil have not previously been investigated in an *in vivo* model of right heart hypertrophy and failure. In this study, we determined whether PDE-5 inhibition by sildenafil, in a clinically relevant dose, improved the function of the hypertrophic dysfunctional RV *in vivo* by direct stimulation.

METHODS

Study design

Wistar rats ($n = 24$) were subjected to either pulmonary trunk banding (PTB) or sham operation (SHAM). The rats were given free access to tap water and standard rat chow (Altromin 1324, Altromin, Lage, Germany) and housed in a room with a 12L:12D light cycle and a temperature of 21°C. Four weeks after the procedure, they were anesthetized and a pressure catheter was installed in the RV. After 20 minutes of stabilization, they were randomized to receive a bolus dose of sildenafil in a clinically relevant dose (1 mg/kg; $n = 7$), vehicle ($n = 6$), or dobutamine (10 µg/kg; $n = 6$). Sham animals received only sildenafil (SHAM; 1 mg/kg; $n = 5$). Invasive RV pressures were recorded continuously, and a transthoracic echo-

cardiography (TEE) was performed 1, 5, 15, 25, 35, 50, 70, and 90 minutes after injecting the bolus. Cardiac function was compared to baseline measurements in each group to evaluate the *in vivo* effect of each specific treatment. The investigation conforms with the Guide for the Care and Use of Laboratory Animals published by the US National Institutes of Health (NIH Publication 85-23, revised 1985) and the Danish Animals Inspectorate (authorization no. 2006-561-1180).

Pulmonary trunk banding

Male Wistar rats with a weight of 140–170 g were anesthetized with an initial dose of 4% isoflurane (Forene-Isoflurane, Abbott Scandinavia, Solona, Sweden), intubated, and mechanically ventilated at 75 breaths/min and a tidal volume of 10 mL/kg with a maintenance dose of 2% isoflurane in a mixture of 50% O₂ and 50% N₂O as described earlier.^{2,7} The rats were placed on a heating pad during the procedure to maintain body temperature at 37°C. The pulmonary trunk was accessed through a thoracotomy on the left side of the sternum. A dual-view surgical microscope was used, and the pulmonary artery (PA) was separated from the aorta. A premodified horizon applier (Horizon Applier, small, HZ137081; Weck Closure Systems) was used to compress a titanium clip (Horizon Ligating Clips, 001200; Weck Closure Systems) to an inner diameter of 0.6 mm around the PA. The thoracic wall was closed in 3 separate layers. The rats were given a 10-mL/kg subcutaneous (s.c.) injection of isotonic saline to avoid dehydration and buprenorphine (Anorfin, Frederiksberg, Denmark) 0.12 mg/kg s.c. 3 times a day for 3 days to relieve postoperative pain. The SHAM animals underwent the same procedures except for banding of the PA.

Invasive pressure catheter recordings

Rats were anesthetized, intubated, and mechanically ventilated as described. The neck was shaved and a midline incision was made. Heparin, 50 IE/kg, was injected intramuscularly to avoid clotting of the catheter. The underlying tissues were dissected and the right and left jugular veins exposed. A ligature was made cranial and proximal to an incision in the right jugular vein, and a 1.4-F pressure tip catheter (Millar Instruments, Houston, TX) was inserted and guided to the RV. TEE and pressure curve morphol-

ogy were used to ensure correct placement of the catheter. To avoid fluid loss, 2 mL/h isotone NaCl was infused at a constant flow. The signal was collected through a signal-conditioning box (MPCU-200; Millar Instruments), recorded, and analyzed in a blinded fashion using Notocord Hem software (NOTOCORD Systems SAS). RVsP and pressure development over time (dp/dt) were recorded to evaluate changes in RV function after injecting the drug of interest.

Transthoracic echocardiography

A TEE was performed on the anesthetized rats using a Vivid 7 echocardiographic system (GE Healthcare, Horten, Norway) with a 10-MHz phased array pediatric transducer operating at a frame rate of about 170 Hz. Tricuspid annular plane systolic excursion (TAPSE) was measured for evaluation of RV systolic

function. A significant change in TAPSE is detectable as early as 7 days before developing clinical signs of heart failure in rats, and it correlates closely with RV ejection fraction in a clinical setting. Moreover, TAPSE has prognostic value in humans, with heart failure correlating with short- and long-term mortality.⁸⁻¹⁰ Image analysis was performed offline using EchoPac software (GE Healthcare), with the observer blinded to the source of samples. For each individual measure, 3 consecutive heart cycles were analyzed, and the mean was used as a representative value.

Drug treatment

The PDE-5 inhibitor used in this experiment was sildenafil pure substance kindly provided by Pfizer (Sandwich, UK). Sildenafil was dissolved in isotonic saline (1 mg/mL), and a single dose of 1 mg/kg was

Table 1. Baseline characteristics

	PTB: vehicle	PTB: sildenafil	PTB: dobutamine	Difference between PTB treatment groups
Anatomical measures				
RVW/LV+SW	0.61 (0.07)***	0.63 (0.11)***	0.62 (0.09)***	ns
RVW/BW 10-3	1.33 (0.17)***	1.27 (0.17)***	1.20 (0.15)***	ns
Body weight, g	309 (29)	296 (24)	289 (17)	ns
Histological measures				
Fibrosis, %	6.1 (4.1)	4.36 (3.1)	3.1 (0.8)	ns
Invasive pressures				
Heart rate, BPM	278 (60)**	329 (26)	311 (27)*	ns
RVsP, mmHg	98 (16)***	91 (18)***	93 (11)***	ns
RVPP, mmHg BPM	26,523 (7,782)***	30,255 (6,083)***	2,5251 (3,685)**	ns
$dp/dt_{\max}$, mmHg/s	3,693 (811)***	3,737 (1,070)***	3,136 (521)*	ns
$dp/dt_{\min}$, mmHg/s	-3,222 (692)**	-3,311 (836)**	-2,959 (381)*	ns
Echocardiography				
TAPSE, cm	0.11 (0.02)***	0.13 (0.02)***	0.13 (0.03)***	ns

Note: Results are expressed as mean (standard deviation). PTB, pulmonary trunk banding; RVW/LV+SW, right ventricle/left ventricle plus septum weight; RVW/BW, right ventricle/body weight; BPM, beats per minute; RVsP, right ventricular systolic pressure; RVPP, right ventricular pulse pressure product; $dp/dt_{\max}$, maximum pressure development over time; $dp/dt_{\min}$, minimum pressure development over time; TAPSE, tricuspid annular plane systolic excursion measured by transthoracic echocardiography; ns = not significant.

* $P < 0.05$.

** $P < 0.005$.

*** $P < 0.001$.

injected intravenously (i.v.) over 30 seconds to ensure clinically relevant plasma levels selective for PDE-5 inhibition.^{11,12} Dobutamine 10 µg/kg was injected i.v. over 30 seconds as a single bolus. Vehicle was administered in the same manner.

RV hypertrophy

The right ventricular weight (RVW), left ventricular plus septum weight (LV+SW), and body weight (BW) were measured at euthanization (Sartorius, Göttingen, Germany). The ratios RVW/LV+SW and RVW/BW were used as indexes of RV hypertrophy.

Histology

The RV was immersion fixated in 4% formaldehyde buffer (pH 7) for 24 hours and dehydrated in graded

ethanol. The tissue was embedded in paraffin and cut in 2-µm sections on a rotary microtome (Microm HM 360, Brock and Michelsen). The sections were stained with collagen-specific Sirius Red to visualize fibrosis in a polarized light microscope. The 4× objective (BXF50F4, Olympus, Tokyo, Japan) was used to capture RGB color images of the RV (ColorViewII, Soft Imaging Systems). To evaluate the mean area of fibrosis, 3 images of each section were randomly selected for analysis. ImageJ software (Rasband, ImageJ, NIH, Bethesda, MD; <http://rsb.info.nih.gov/ij/>) was used for the analysis. The images were converted to 8-bit grayscale, and the Otsu threshold algorithm was used for automated thresholding. Using particle analysis, the area fraction of fibrosis was determined. An observer blinded to the clinical source of the sample performed image analysis.

Figure 1. Baseline characteristics. Results are plotted as mean $\pm$ standard error of the mean. One asterisk, $P < 0.05$; three asterisks, $P < 0.001$. Numbers plotted in the columns denote the number of animals used for analysis. RVW/LV+SW, right ventricle/left ventricle plus septum weight; TAPSE, tricuspid annular plane systolic excursion measured by transthoracic echocardiography; RVsP, right ventricular systolic pressure; dp/dt_{max} , maximum pressure development over time. PTB, pulmonary trunk banding; veh, vehicle; sil, sildenafil; dob, dobutamine.

Figure 2. Acute effects of sildenafil and dobutamine. Results are plotted as mean $\pm$ standard error of the mean. Three asterisks, $P < 0.001$. Numbers plotted in the column denote the number of animals used for analysis. RVsP, right ventricular systolic pressure; TAPSE, tricuspid annular plane systolic excursion measured by transthoracic echocardiography; dp/dt_{max} , maximum pressure development over time; PTB, pulmonary trunk banding; ns, not significant.

Statistics

Quantitative data are expressed as mean $\pm$ standard deviation and plotted as mean $\pm$ standard error of the mean unless otherwise stated. $P < 0.05$ was considered statistically significant. Significance between baseline characteristics was evaluated using one-way analysis of variance (ANOVA) followed by post hoc Bonferroni analysis. Significance of the administered drug effects was evaluated with repeated-measures ANOVA followed by Dunnett's post hoc analysis comparing all measurements to baseline.

RESULTS

Baseline characteristics

The PTB procedure caused hypertrophy of the RV. The RVW/LV+SW and RVW/BW were increased more than 2-fold compared to SHAM animals. No difference in RV hypertrophy between the PTB treatment groups was recorded.

When comparing the degree of fibrosis in SHAM animals to all animals operated by PTB, there was an increase in the area fraction of fibrosis in the PTB group (SHAM $2.4\% \pm 0.5\%$ vs. PTB $4.5\% \pm 3.1\%$; $P < 0.005$). We did not record any difference in cardiac fibrosis between the PTB treatment groups.

RV function was significantly attenuated in PTB animals measured by a decrease in TAPSE compared

to SHAM (0.11 ± 0.02 vs. 0.20 ± 0.02 cm; $P < 0.001$). There were no differences in TAPSE comparing PTB treatment groups.

Invasive pressure measurements revealed a 3-fold increase in RVsP in the PTB animals compared to SHAM animals. Other invasive measurements such as $dp/dt_{max/min}$ were more than doubled in PTB animals compared to SHAM animals. No differences were observed in heart rate between PTB and SHAM animals. There were no differences in invasive pressure measurements between the PTB treatment groups (Table 1; Fig. 1)

Drug effects

The RV function of PTB animals treated by dobutamine improved 1 minute after injection measured by both TAPSE and invasive pressure measurements (Fig. 2). Five minutes after injection, all values returned to baseline level. Despite a slight decrease in dp/dt_{min} at 25 and 50 minutes, no measures differed from baseline from 5 to 90 minutes after the injection of dobutamine (Fig. 3).

Sildenafil did not improve RV function in the PTB animals (Fig. 2). During the first 50 minutes after administration, there were no changes in TAPSE or invasive measures. From 70 to 90 minutes after administration, there was a decrease in RV function as seen in the hemodynamic measures right

Figure 3. Pulmonary trunk banded animals treated by dobutamine. Hemodynamic measurements at baseline and after injection of a single bolus of dobutamine 10 $\mu\text{g/kg}$. Results are plotted as mean $\pm$ standard error of the mean. Two asterisks, $P < 0.005$; three asterisks, $P < 0.001$ compared to baseline measurement. TAPSE, tricuspid annular plane systolic excursion measured by transthoracic echocardiography; RVsP, right ventricular systolic pressure; RVPP, right ventricular pulse pressure product; dp/dt_{max} , maximum pressure development over time; dp/dt_{min} , minimum pressure development over time.

Figure 4. Pulmonary trunk banding animals treated by sildenafil. Hemodynamic measurements at baseline and after injection of a single bolus of sildenafil 1 mg/kg. Results are plotted as mean $\pm$ standard error of the mean. One asterisk, $P < 0.05$; two asterisks, $P < 0.005$; three asterisks, $P < 0.001$ compared to baseline measurement. TAPSE, tricuspid annular plane systolic excursion measured by transthoracic echocardiography; RVsP, right ventricular systolic pressure; RVPP, right ventricular pulse pressure product; $dp/dt_{\max}$, maximum pressure development over time; $dp/dt_{\min}$, minimum pressure development over time.

ventricular pulse pressure product (RVPP; RVsP beats per minute), heart rate, $dp/dt_{\max}$, and $dp/dt_{\min}$ (Fig. 4).

Vehicle treatment caused a slight increase in RVsP 25 and 35 minutes after administration. No changes were observed in TAPSE or other hemodynamic measurements (Fig. 5). Sildenafil did not improve RVsP, TAPSE, or $dp/dt_{\min}$ in SHAM animals. There was an increase in heart rate from 1 to 25 minutes, a slight increase in $dp/dt_{\max}$ at 15 and 25 minutes, and an increase in RVPP at 15 minutes (Fig. 6).

DISCUSSION

The effects of a single and clinically relevant dose of sildenafil were evaluated in vivo in the hypertrophic and dysfunctional RV myocardium. The inotropic potential of the RV in this model was validated using dobutamine. PDE-5 inhibition by sildenafil failed to show any acute effects in the hypertrophic and failing right heart of the rat.

Right heart hypertrophy and dysfunction

Our model of PTB induced RV hypertrophy and dysfunction. The weight of the RV normalized for BW and LV+SW more than doubled. Cardiac fibrosis was increased in animals subjected to PTB as an indicator of pathologic remodeling. Systolic function was attenuated measured by a TAPSE comparable to patients with the most severe degree of PAH. Compared with other previously reported PTB studies, we achieved an increased degree of fibrosis, RVsP, and RVW and a further decrease in TAPSE. This reflects the 0.6-mm clip banding to produce an increased afterload compared to the more widely used 1.3–1.7-mm ligature banding used in other studies.^{3,4,13,14} We also found an increase in $dp/dt_{\max/\min}$ despite a decrease in TAPSE. As a result of increased afterload caused by the PTB procedure, the RV dilates and hypertrophies. This causes an increase in dp/dt and RV pressures compared to SHAM animals, but these compensatory changes are not sufficient to maintain a normal cardiac output. The dp/dt is highly dependent on the loading condition of the ventricle and increases with ventricular end-diastolic volume and pressure. This is considered because of stretch of the sarcomere according to the Frank-Starling relationship and likely explains the raised $dp/dt_{\max}$ seen following PTB.

However, this increase is not enough to provide an efficient circulation as a result of the increased afterload as reflected by the decrease in TAPSE. This emphasizes that dp/dt can be increased despite a failing circulation merely indicating an increased afterload.

Acute effects of sildenafil and dobutamine

To evaluate whether there was an inotropic potential in our animal model, we administered a single dose of dobutamine. It immediately improved RV function in PTB animals. A positive inotropic response of the healthy and failing RV to dobutamine has previously been reported.¹⁵⁻¹⁷ This confirms that the observed dobutamine effects validate the potential of our model of RV hypertrophy and dysfunction to effectively display positive inotropic drug responses.

In the SHAM animals, sildenafil caused a slight increase in heart rate, $dp/dt_{\max}$, and RVPP. Sildenafil has systemic vasodilatory properties.¹⁸ This increases systemic venous return, causing an improvement in RV filling and hence RV function. This mechanism could explain the observed improvement of RV function after sildenafil administration in the SHAM animals.

A single clinically relevant dose of sildenafil did not improve the function of the hypertrophic dysfunctional RV. The lack of direct RV or LV myocardial effects of sildenafil are also indicated by different previous human and animal studies. In the LV of men with a wide range of heart diseases, no improvement in heart function was found after administration of sildenafil.¹⁹⁻²¹ In patients suffering from distal CTEPH²² and in the healthy heart of patients subjected to hypoxia,²³ acute sildenafil administration did not improve RV function.

Sildenafil did improve RV function in patients suffering from PAH in both acute and chronic settings²⁴⁻²⁶ and also LV function in patients with left heart failure.²⁷ Sildenafil has both pulmonary and systemic vasodilatory actions,¹⁸ and unloading of the heart caused by the vasodilatory action of sildenafil, and not by a direct stimulation of the cardiomyocytes, was believed to mediate the beneficial effects in these studies. Recent experimental studies in canines with pacing-induced heart failure,²⁸ patients with end-stage heart failure,²⁹ and patients with severe aorta stenosis³⁰ find beneficial effects of acute sildenafil on LV

Figure 5. Pulmonary trunk banded animals treated by vehicle. Hemodynamic measurements at baseline and after injection of a single bolus of vehicle. Results are plotted as mean $\pm$ standard error of the mean. One asterisk, $P < 0.05$ compared to baseline measurement. TAPSE, tricuspid annular plane systolic excursion measured by transthoracic echocardiography; RVsP, right ventricular systolic pressure; RVPP, right ventricular pulse pressure product; dp/dt_{max} , maximum pressure development over time; dp/dt_{min} , minimum pressure development over time.

Figure 6. Sham-oriented animals treated by sildenafil. Hemodynamic measurements at baseline and after injection of a single bolus of sildenafil 1 mg/kg. Results are plotted as mean $\pm$ standard error of the mean. One asterisk, $P < 0.05$; two asterisks, $P < 0.005$ compared to baseline measurement. TAPSE, tricuspid annular plane systolic excursion measured by transthoracic echocardiography; RVsP, right ventricular systolic pressure; RVPP, right ventricular pulse pressure product; dp/dt_{max} , maximum pressure development over time; dp/dt_{min} , minimum pressure development over time.

function. The authors mainly contribute this to unloading of the LV but do not rule out a direct effect of sildenafil on the cardiomyocytes.

PDE-5 is expressed in the hypertrophic ventricle but not the healthy RV of rats and humans. Ex vivo studies show a direct inotropic effect of PDE-5 inhibition in human atrial strips from hearts with a hypertrophic RV, in isolated atrial strips, and in hearts from rats with RV hypertrophy.^{5,6,31} A very high dose of sildenafil was used in these ex vivo studies comparable to a free plasma sildenafil concentration 40 times higher than steady state plasma concentrations in patients given a high clinical dose of 80 mg 3 times daily.³² Thus, the inotropic effects may be caused by unspecific PDE inhibition.³³ The difference in dosing likely explains the discrepancy between the present in vivo study and the previous ex vivo studies reporting a direct inotropic effect of sildenafil. In conclusion, we find that acute PDE-5 inhibition by a clinically relevant dose of sildenafil fails to improve the function of the dysfunctional hypertrophic RV in male Wistar rats subjected to PTB.

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