SILDENAFIL TREATMENT IN ESTABLISHED RIGHT VENTRICULAR DYSFUNCTION IMPROVES DIASTOLIC FUNCTION AND ATTENUATES INTERSTITIAL FIBROSIS INDEPENDENT FROM AFTERLOAD

MAJ Borgdorff, B Bartelds, MG Dickinson, P Steendijk, M de Vroomen, RMF Berger

An adapted version of this chapter is published as:
ABSTRACT

Aims
Right ventricular (RV) function is an important determinant of prognosis in congenital heart diseases, pulmonary hypertension, and heart failure. Preventive Sildenafil treatment has been shown to enhance systolic RV function and improve exercise capacity in a model of fixed RV pressure load. However, it is unknown whether Sildenafil has beneficial effects when treatment is started in established RV dysfunction, which is clinically more relevant. Our aim was to assess the effects of Sildenafil treatment on RV function and fibrosis in a model of established RV dysfunction due to fixed afterload.

Methods and Results
Rats were subjected to pulmonary artery banding (PAB) which induced RV dysfunction after 4 weeks, characterized by reduced exercise capacity, decreased TAPSE and RV dilatation. From week 4 onward, 50% of rats were treated with Sildenafil (100mg/kg/d; PAB-SIL, n=9) or vehicle (PAB-VEH, n=9). At 8 weeks, exercise capacity was assessed using cage wheels and RV function using invasive RV pressure-volume measurements under anesthesia. Sildenafil treatment, compared to vehicle, improved RV ejection fraction (44±2 vs. 34±2%, p<0.05 PAB-SIL vs. PAB-VEH), reduced RV end diastolic pressure (2.3±0.5 vs. 5.1±0.9mmHg, p<0.05), and RV dilatation (end systolic volume 468±45 vs. 643±71 μl, p=0.05). Sildenafil treatment also attenuated RV fibrosis (30±6 vs. 17±3‰, p<0.05), but did not affect end-systolic elastance or submaximal exercise capacity.

Conclusion
Sildenafil improves RV diastolic function and attenuates interstitial fibrosis in rats with established RV dysfunction, independent from afterload. These results indicate that Sildenafil treatment has therapeutic potential for established RV dysfunction.
Right ventricular (RV) failure due to pressure overload is a major determinant of outcome congenital heart diseases(24) and in pulmonary arterial hypertension(14). RV function also determines outcome in congestive heart failure(21, 34). Given the increasing incidence of heart failure as well as the quickly expanding population of grown-ups with congenital heart disease, there is a growing need for therapies that specifically support RV function. Unfortunately, despite a growing interest in the mechanisms underlying RV failure(2, 18), so far no RV specific therapy is available. Treatments successful in left ventricular failure might be beneficial in RV failure, but application could be limited due to the fact that the RV is morphologically, functionally and embryologically different from the LV(18, 31, 33).

However, recent studies have shown that –like LV failure- experimental RV failure is associated with ventricular dilatation, impaired systolic and diastolic function and adverse myocardial remodeling, including hypertrophy and interstitial fibrosis(2, 7, 31). In LV failure due to pressure load, inhibition of phosphodiesterase type 5A (PDE5A), has been proven to successfully reduce hypertrophy and interstitial fibrosis and improve diastolic function(29).

Therapeutic administration of PDE5A inhibitors (e.g. Sildenafil) is now being tested in several clinical heart failure trials(13, 26).

PDE5A inhibitors have also been successfully used in patients with pulmonary arterial hypertension(16). In these patients, the beneficial effects of PDE5A inhibition on the RV might partly be explained by decreased RV afterload, resulting from the Sildenafil effects on the diseased pulmonary vasculature. However, there is emerging evidence that Sildenafil also exerts direct beneficial effects on the pressure loaded RV. PDE5A is activated in the RV of patients with a pressure loaded RV(23). We have previously shown that Sildenafil administered from the onset of pressure load (preventive treatment) enhanced systolic RV function, attenuated ventricular dilatation and limited the decline in exercise tolerance in a rat model of fixed RV pressure overload, but also modestly increased interstitial fibrosis(7). Since RV afterload was fixed in these experiments (pulmonary artery banding is unaffected by the pulmonary vasodilatory effects of Sildenafil), these findings indicated that Sildenafil directly affected the RV myocardium.
In clinical practice however, most patients already present with RV dysfunction, which disqualifies them for preventive treatment. Therefore, if Sildenafil also has beneficial effects when started in established RV dysfunction, this would be very relevant for the clinical setting. The aim of the present study was to test whether Sildenafil could improve systolic and diastolic function (measured with echocardiography and pressure volume analysis) and attenuate fibrosis in a model of established, pressure load induced RV dysfunction. Secondary, we assessed whether changes in RV function and remodeling were associated with changes in exercise tolerance (measured as voluntarily run distance). To define the applicability of Sildenafil treatment in different phases of RV dysfunction we compared our results with those from the preventive strategy study.

**MATERIALS AND METHODS**

**Animal model and study design**

Animal care and experiments were conducted according to the Dutch Animal Experimental Act and conform to the *Guide for the Care and Use of Laboratory Animals* published by the US National Institutes of Health (NIH Publication No. 85-23, revised 1996). The Animal Experiments Committee of the University of Groningen, the Netherlands approved the experimental protocol.

To induce fixed RV pressure overload, pulmonary artery banding (PAB) was performed on Wistar rats (n=20; male; 160-180g; Charles River, the Netherlands), which leads to severe RV dysfunction in 4 weeks(7). The animals were randomly assigned to two groups; PAB-VEH and PAB-SIL. The first four weeks after PAB, both groups received regular drinking water. See figure 1A for experimental set-up. At 4 weeks after PAB, RV dysfunction was confirmed by exercise testing and echocardiography. From four weeks after PAB, the PAB-SIL group received drinking water to which Sildenafil (Pfizer Inc, New York, NY, United States of America) was added in a dose (100mg/kg/day) that specifically inhibits PDE5A(7). In the same study we have shown effective plasma levels resulting in activation of protein-kinase-G1(7). The PAB-VEH continued to receive regular drinking water. At 8 weeks after PAB, all rats were evaluated by exercise testing, echocardiography, and pressure-volume measurements.

Two animals (1 PAB-SIL, 1 PAB-VEH) died prematurely; one of severe bleeding during surgery, the other died suddenly in the second week after surgery of unknown cause, but without any sign of RV failure.
**Exercise tolerance and clinical signs of failure**

To measure voluntary exercise tolerance, running wheels were mounted in the rat cages, as described previously(7). Five days before surgery, five days before the 4 wks-mark (halfway the experiment) and 5 days before sacrifice (at 8 wks), animals were allowed to run in the cage wheel. Running distance was thus recorded daily using a digital magnetic counter (Commodoor Cycle Odometer, Commodoor, the Netherlands)(2, 7). Exercise tolerance was measured as percentage change in running distance at 4 or 8 wks compared to 0 or 4 wks respectively, for each individual animal.

Throughout the experiment, rats were examined daily for clinical signs of right ventricular failure according to a predefined ABCDE-checklist as described previously(7, 8). This ‘ABCDE-criteria’ were defined as follows: A: appearance and activity, B: bodyweight, C: cyanosis and circulation, D: dyspnea and tachypnea, E: edema and effusion. The A-symptoms were considered present when the animal had a ruffled fur, red discoloration of head and neck (due to decreased cleaning-behaviour) or was less active than previously, despite stimulation. Body weight symptom was scored if there was a change in bodyweight of more than 15 grams in <48 hours. Cyanosis was checked at exposed skin on head, paws and tail. Hampered circulation was considered present if both front paws and hind legs/tail were pale and markedly colder than previously (regarded as a consequence of decreased perfusion). Dyspnea and tachypnea were qualitatively assessed and defined as markedly increased breathing-effort and, -frequency, respectively. Edema and effusions were defined as fluid collection in thorax and/or abdomen at euthanization.

**Echocardiography**

Echocardiography was performed at 4 wks to confirm RV dysfunction and at 8 wks as described previously(7) using a Vivid Dimension 7 system and 10S-transducer (GE Healthcare, Waukesha, WI, USA). We used apical 3- and 4-chamber views and parasternal short and long axis views to measure RV and right atrial dimensions, tricuspid insufficiency, tricuspid annular plane systolic excursion (TAPSE), and continuous wave Doppler for the gradient across the PAB. Cardiac output was calculated as aorta diameter$^2 \times 3.14 \times$ velocity time integral (VTI) $\times$ heart rate, using systolic aorta diameter and pulsed wave Doppler measurements of aorta flow. Measurements from 6-12 consecutive beats were used to average out beat-to-beat variation.
Right ventricular hemodynamics

Hemodynamic characterization of the RV was performed by pressure-volume studies, obtained by right ventricle catheterization using a combined pressure-conductance catheter (SPR-869, Millar Instruments Inc., Houston, TX, USA) at 8 weeks after surgery according to a previous described protocol(7). The volume signal of the conductance catheter was calibrated for parallel conductance and slope factor in order to obtain absolute volumetric values. The parallel conductance was estimated the hypertonic saline method by infusing 10μL of hypertonic (10%) saline via the jugular vein cannula(1, 19). The slope factor was calculated as uncalibrated conductance catheter cardiac output divided by LV cardiac output, measured by echocardiography.

Load independent parameters of contractility (end systolic elastance, preload recruitable strokework) and diastolic function (end diastolic elastance) are measured during transient vena cava occlusion and could be obtained in 15 out of 19 animals (n=6/9 for PAB-VEH/PAB-SIL). We calculated RV volume at a normalized end systolic pressure of 70 mmHg (V70) as an additional measure of systolic function.

Visual inspection of the EDPVRs revealed that relations were either highly linear or clearly exponential. Subjective scoring indicated that in the untreated group 4 of 6 EDPVR were clearly exponential, whereas in the treated group only 2 of 9 EDPVRs. The presence of both linear and exponential EDPVR complicates direct statistical comparison of both groups; slope factor (end diastolic elastance) can be used for the linear EDPVRs; the exponential coefficient can be used for the exponential EDPVRs, but neither of them can be used in both. Therefore, to enable a single, objective analysis of all EDPVRs we used the following approach. Each EDPVR data set was divided into an upper and a lower volume range, separated by the median EDV. The upper and lower parts were each fitted with a linear curve, respectively: \( P_{ed} = V_{o-up} + E_{ed-up} \times V_{ed} \), and \( P_{ed} = V_{o-low} + E_{ed-low} \times V_{ed} \). For EDPVRs which are essentially linear over the full volume range Eed-up and Eed-low should be approximately equal and Eed-low / Eed-up would be expected to be close to 1. For exponential EDPVRs, Eed-up will be substantially higher than Eed-low and Eed-low/Eed-up clearly less than 1. Thus, this ratio can be used as a simple, objective linearity index, with 1 indicating linear relationships and values below 1 (increasingly steeper) exponential curves.
Organ weights, hypertrophy and fibrosis
After heart catheterization, the rats were euthanized by removing the heart from the thorax. Heart, lungs and liver were dissected. RV, interventricular septum, LV and both atria were separated and weighed. Tissue sections were fixated, trans-sectionally cut at 4μm-thick sections and stained with wheat germ agglutinin (WGA) to assess cardiomyocyte size and with Masson Trichrome to assess fibrosis. Cardiomyocyte size was measured as average surface area of cross-sectionally cut cardiomyocytes with a visible nucleus (Image-Pro, MediaCybernetics, Bethesda, MD, USA) photographed in a trans-section of the entire RV. The extent of fibrosis was quantified as the blue-stained percentage of the total tissue area, measured per whole ventricle (Image Scope 11, Aperio Technologies, Inc. Vista, CA, USA) as described previously(7).

qRT-PCR
The expression of the fetal gene program (myosin heavy chain isoforms, natriuretic pro peptides type A and B) and markers of hypertrophy (acta1, RCAN) was measured to characterize the hypertrophy response and the effects of Sildenafil. Total RNA was extracted using the RNeasy fibrous tissue kit (Qiagen), following the manufacturer’s guidelines. Data were normalized to reference gene 36B4.

Statistical analysis
All quantitative data were tested for normality and are expressed as mean±standard error of the mean (SEM). PAB-VEH versus PAB-SIL differences were evaluated using Students t-tests or Mann-Whitney U tests as appropriate. Group size is 8-9, except when specified otherwise. P<0.05 was considered significant (PASW Statistics 18 for Windows, SPSS, Chicago, Illinois).

RESULTS
Pulmonary artery banding induced RV dysfunction
At week 4, before treatment was started, PAB had induced fixed RV pressure load (PAB gradient 53±4mmHg), which led to RV dysfunction. RV dysfunction was characterized clinically by decreased exercise tolerance (running distance vs. baseline: -57±8%), Echocardiography showed low TAPSE (2.1±0.1mm, normal value ~3 mm), right ventricular dilatation (end diastolic diameter: 4.8±0.2mm,
normal value ~3.5 mm) and right atrial enlargement (maximal long-axis diameter: 4.3±0.2 mm, normal value ~3 mm(9). After this clinical and echocardiographic evaluation, the rats in the PAB-SIL group started with Sildenafil treatment. Before treatment, there were no differences in characteristics, including RV function parameters between PAB-SIL and PAB-VEH at 4 weeks (Table 1). The changes to 4 weeks of PAB were similar to those described in previous studies(7, 9). In these studies, a similar magnitude of PAB led at 4 weeks to increased contractility as measured by end systolic elastance (+164%), RV dilatation measured by an increase in end diastolic volume (+48%), as well as early phase deterioration of diastolic function measured by end diastolic elastance.

<table>
<thead>
<tr>
<th>Echocardiographic parameters</th>
<th>PAB-VEH</th>
<th>PAB-SIL</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>PAB gradient (mmHg)</td>
<td>49±6</td>
<td>56±4</td>
<td>0.33</td>
</tr>
<tr>
<td>HR (/min)</td>
<td>315±8</td>
<td>347±15</td>
<td>0.10</td>
</tr>
<tr>
<td>CO (mL/min)</td>
<td>56±5</td>
<td>75±9</td>
<td>0.12</td>
</tr>
<tr>
<td>TAPSE (mm)</td>
<td>2.1±0.2</td>
<td>2.1±0.3</td>
<td>0.94</td>
</tr>
<tr>
<td>RVEDD (mm)</td>
<td>5.0±0.2</td>
<td>4.6±0.4</td>
<td>0.48</td>
</tr>
<tr>
<td>RA diameter (mm)</td>
<td>4.6±0.2</td>
<td>4.1±0.4</td>
<td>0.34</td>
</tr>
</tbody>
</table>

Table 1 - Echocardiographic characteristics at start of Sildenafil therapy

Sildenafil beneficially affected RV dysfunction

After 8 wks, due to growth of the animals, PAB gradients had increased (74±3 vs. 77±5mmHg, PAB-VEH vs. PAB-SIL, ns). Sildenafil treatment improved ejection fraction and reduced end systolic volume (Ves) compared to untreated rats (Fig 1B,C) but did not significantly affect end diastolic volume (Table 2). In the untreated rats, RV pressure-volume loops showed ventricular dilatation (Fig 2A). In the PAB-SIL group the pressure volume-loops showed a leftward shift on the volume axis, reflecting the (non significant) reduced ventricular dilatation (Fig 2A). Contractility, assessed by end systolic elastance (Ees) and preload recruitable stroke work (PRSW), did not significantly differ between the two groups (Table 2). The RV-PA coupling, expressed as Ees/Ea, tended to increase with Sildenafil treatment although the changes failed to reach statistical
Therapeutic Sildenafil treatment in the pressure loaded RV

significance (0.68±0.15 vs. 0.82±0.15, PAB-VEH vs. PAB-SIL, p=0.054. However, Sildenafil treatment improved diastolic function: the end diastolic pressure was significantly lower in the Sildenafil treated group (Fig 1D) and the end diastolic pressure-volume relationship (EDPVR) differed markedly between the two groups (Fig 2B): in the majority of untreated animals (4/6; 66%) EDPVRs were shifted upwards and displayed a clearly exponential behavior, indicating increased ventricular stiffness. In contrast, in the Sildenafil treated animals almost all RVs had relatively low end diastolic pressures and a normal linear EDPVR (2/9; 22%) (Fig 2B). To enable comparison of linear and non-linear EDPVRs (neither end diastolic elastance nor exponential coefficient can be used in both) we divided the EDPVR data set of each animal into an upper and lower volume range, separated by the median Ved, and determined the slope (Eed) for each part with a linear fit (see Methods section). The ratio of Eed-low/Eed-up was used as an index for linearity (with 1 indicating a linear EDPVR). In the untreated rats, the linearity index was 0.35±0.12; in the Sildenafil treated rats 0.90±0.14 (p=0.01)(Fig 1D), indicating significantly improved diastolic function. The improvement in RV function by Sildenafil could not be correlated with improvements in exercise tolerance (Table 2) and symptoms of RV failure were seen in both groups (all animals in both groups were tachypneic and showed decreased cleansing behaviour); none of the rats needed to be terminated prematurely because of severe RVF symptoms.

Sildenafil attenuated RV fibrosis, but not RV hypertrophy

Sildenafil attenuated RV myocardial fibrosis (29±5‰ vs. 17±3‰, PAB-VEH vs. PAB-SIL, p<0.05). This difference was not due to severity of loading, as the amount fibrosis per mmHg RV peak pressure was also decreased by Sildenafil (Fig 3A-C). The degree of interstitial fibrosis correlated with end diastolic pressure (R²=0.587, p=0.001) (Fig 3D), suggesting interaction between fibrosis and diastolic function.

Pressure load led to hypertrophy as shown by RV weight (1.2±0.1mg/g bodyweight) (Table 2) and RV cardiomyocyte cross sectional surface area (0.13±0.01μm²)(7). The degree of RV hypertrophy in the Sildenafil treated animals did not differ from that in the untreated animals (Table 2).

No difference in expression of RV remodeling associated genes (NPPA, NPPB, acta 1, RCAN1, MYH7/MYH6) could be demonstrated between the treated and untreated groups (data not shown).
Figure 1 Functional effects of 4wks of treatment with Sildenafil at 8 wks of pulmonary artery banding.

All indices measured by pressure-conductance catheter. A Study design, a schematic illustration. B Ejection fraction. C End systolic volume (Ves). D End diastolic pressure (Ped). E Linearity index of the end diastolic pressure volume relations (Eed_low/Eed_up). Mean±SEM * p<0.05.

<table>
<thead>
<tr>
<th></th>
<th>PAB-VEH</th>
<th>PAB-SIL</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pressure-volume parameters</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>HR (/min)</td>
<td>283±12</td>
<td>283±11</td>
<td>1.00</td>
</tr>
<tr>
<td>CO (mL/min)</td>
<td>89±4</td>
<td>102±5</td>
<td>0.07</td>
</tr>
<tr>
<td>Ees (mmHg/µL) •1000</td>
<td>96±19</td>
<td>124±25</td>
<td>0.38</td>
</tr>
<tr>
<td>PRSW (mmHg)</td>
<td>39±12</td>
<td>41±7</td>
<td>0.89</td>
</tr>
<tr>
<td>Voluntary exercise</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Change at 8 wks vs. 4 wks (%)</td>
<td>-46±4</td>
<td>-45±5</td>
<td>0.94</td>
</tr>
<tr>
<td>Change at 8wks vs. baseline (%)</td>
<td>-79±10</td>
<td>-81±13</td>
<td>0.74</td>
</tr>
<tr>
<td>Organ weights</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>RV weight/BW (mg/g)</td>
<td>1.2±0.1</td>
<td>1.1±0.1</td>
<td>0.70</td>
</tr>
<tr>
<td>LV+IVS weight/BW (mg/g)</td>
<td>2.1±0.03</td>
<td>2.1±0.05</td>
<td>0.60</td>
</tr>
<tr>
<td>RA weight/BW (mg/g)</td>
<td>0.31±0.08</td>
<td>0.26±0.06</td>
<td>0.25</td>
</tr>
<tr>
<td>BW (g)</td>
<td>423±15</td>
<td>442±21</td>
<td>0.48</td>
</tr>
</tbody>
</table>

Table 2 Pressure-volume parameters, exercise and organ weights at 8 weeks

PAB-VEH= untreated pulmonary artery banding, PAB-SIL= PAB treated with Sildenafil, HR= heart rate, CO= cardiac output, Ees= End systolic elastance, PRSW= preload recruitable stroke work, RV= right ventricle, BW= bodyweight, LV= left ventricle, IVS= interventricular septum, RA= right atrium. Values are mean ±SEM.
Figure 2 Pressure-volume analysis of Sildenafil or vehicle in pulmonary artery banding.

A Representative pressure-volume loops of untreated (PAB-VEH, upper panel) and treated (PAB-SIL, lower panel) rats with a PAB during vena cava occlusion. End systolic pressure-volume relations marked by solid black lines, end diastolic pressure-volume relations marked by dashed black lines. B Overview of end diastolic pressure-volume relations obtained during vena cava occlusion in PAB-VEH (black, n=6) and PAB-SIL (gray, n=9). The pressure-volume loops in the left upper corner are shown to indicate the area of the pv-loops that is enlarged.

Figure 3 Effects of Sildenafil on fibrosis.

A Representative examples of Masson-Trichrome-stained right ventricles of untreated (PAB-VEH, n=7) and treated (PAB-SIL, n=9) rats with a PAB. Ruler is 125μm. B Permillage RV fibrosis per unit surface area. C Permillage RV fibrosis normalized for RV peak pressure (Ppeak). D Scatter plot showing correlation between fibrosis permillage and end diastolic pressure (Ped). Mean±SEM, * p<0.05
DISCUSSION

In this study in rats with fixed RV pressure overload, we show that Sildenafil treatment, initiated in established RV dysfunction, leads to reduced end diastolic pressure, altered end diastolic pressure volume relations, improved ejection fraction and attenuated RV fibrosis. These beneficial effects on the RV were not accompanied by a better voluntary exercise tolerance. These data demonstrate that Sildenafil has a direct beneficial effect on the diastolic function of the pressure-loaded RV independent from its afterload; the pulmonary vasodilatory effects of Sildenafil are negated by the pulmonary artery banding. This study is the first to show that Sildenafil may be effective in the treatment of established RV dysfunction.

Sildenafil improves diastolic dysfunction in established RV dysfunction

Under normal physiological conditions the RV is coupled to the pulmonary vascular bed, which is characterized by low resistance and high compliance. When RV afterload increases, e.g. due to pulmonary hypertension or congenital heart diseases, the RV initially responds with increased contractility and RV hypertrophy. Eventually, RV adaptation progresses into RV dysfunction and failure. Although the mechanisms of RV dysfunction are incompletely understood, diastolic dysfunction is thought to be important in the progression to RV failure(17, 25). Clinical studies show that increased right atrial pressure (as an indirect measure of diastolic RV function) is associated with poor outcome in pulmonary arterial hypertension(3). The importance of diastolic dysfunction is confirmed in experimental studies showing diastolic dysfunction while contractility (end systolic elastance, PRSW) is preserved(7, 15).

Using pressure-volume measurements, we here show that Sildenafil beneficially affects the end diastolic pressure-volume relation (EDPVR) and lowers end diastolic pressure. The EDPVR reflects intrinsic diastolic stiffness(11). In humans the RV EDPVR cannot be recorded accurately but we, and others, have previously shown that within a physiological range, the EDPVR in a normal rat RV is linear(7, 15, 19).

The observed linear-to-exponential transformation of the EDPVR reflects increased stiffness of the diseased RV. In the low volume range, compliant elastin fibers and titin-molecules are being stretched, resulting in shallow slope of the EDPVR. In the higher volume range, slack length of titin and collagen fibers is exceeded, resulting in much steeper slope of the EDPVR(11). Sildenafil
Therapeutic Sildenafil treatment in the pressure loaded RV.

Treatment prevents the linear-to-exponential transformation of the EDPVR. This in turn, results in lower end diastolic pressures, highlighting the improved diastolic function. Since diastolic dysfunction is a prominent feature of RV failure, these beneficial effects of Sildenafil on diastolic function may have therapeutic merit for patients with advanced RV dysfunction.

Figure 4 Summary of Sildenafil effects in the early (0-4 wks) and late (4-8 wks) stage of pressure load-induced RV dysfunction.

The data set of the effects of Sildenafil on rats with a PAB at week 4 are derived from our previous study(7), in which Sildenafil treatment was started at the day of surgery (preventive strategy). A End systolic elastance (Ees). B End diastolic elastance (Eed). To allow comparison of Eed between the studies, the Eed of the initial 2 mmHg end diastolic pressure drop during occlusion was used here (see Methods on characterization of diastolic function). C Permillage RV fibrosis per unit surface area. D Ejection fraction. Mean±SEM, * p<0.05

In addition to the observed improvement in diastolic performance, parameters of systolic function and ventricular-arterial coupling tended to improve with Sildenafil, although these changes failed to reach statistical significance. These slight improvements in systolic performance might have contributed to the
increased cardiac output measured in this study. An improvement of ventriculo-arterial coupling (Ees/Ea) in this PAB-model of fixed RV-afterload might be interpreted as either improved efficiency of ventricular performance at its fixed afterload or as a functional adaptation in the pulmonary trunk proximal to the PAB.

**Potential mechanisms of remodeling**

The exponential behavior of the EDPVR observed in the untreated rats, is not secondary to a shift towards a high volume range, but also occurs at relatively normal volumes. These results suggest that Sildenafil targets ventricular remodeling, rather than just preventing ventricular dilatation. Sildenafil enhances PKG1 activity, as shown in our previous study(7), which may affect relaxation via phosphorylation of titin(20). Another important component of RV dysfunction in experimental models is fibrosis(5, 8). Fibrosis plays a central role in the adaptation of the ventricle to stress in general and pressure load in particular(4, 32). In congenital heart disease and pulmonary hypertension, interstitial fibrosis has been shown to contribute to diastolic dysfunction(10, 12, 25). In the present study the reduction of fibrosis in the Sildenafil treated rats was strongly related to the reduction in end diastolic pressures (Fig 3D), suggesting an important component of the positive effects. Whether Sildenafil has additional beneficial mechanisms to explain the improved hemodynamics remains to be explored.

**Clinical Implications**

Sildenafil is increasingly being used in the treatment of various types of cardiovascular disease(28). However, the place of PDE5A inhibitors in the treatment of RV dysfunction to fixed pressure overload is yet to be determined. Comparing with the results of preventive treatment strategy(7) indicates differences and similarities in response (summarized in Fig 4). Whereas the preventive strategy primarily further enhances parameters of contractility (Ees), the reversal strategy affects diastolic function (end diastolic pressure volume relations, end diastolic pressure), although Ees/Ea also appeared to subtly improve. One explanation of the less pronounced effect on contractility could be that contractility is almost maximally enhanced already. In contrast, a prominent feature of (more progressed) RV dysfunction is diastolic dysfunction. This study
is the first to report a beneficial effect on diastolic dysfunction in a model of fixed afterload, which circumvents the potential effects on the pulmonary vasculature. Previous studies in rats have shown that the degree of RV dysfunction presented in this study (reduced cardiac output at rest, RV dilatation, reduced exercise tolerance) can be clinically tolerated for an extended period of time(6). Similarly, in patients with congenital heart diseases increased RV afterload may be clinically well tolerated (30). However, also similar to patients with a systemic right ventricle, PAB rats exhibited further decline in RV parameters and clinical function, i.e. RV dilatation and reduced exercise capacity(30).

These analyses also reveal that Sildenafil may be associated with increased fibrosis in the early stage, whereas in the later stage of the disease, when fibrosis is a more prominent feature of RV remodeling, Sildenafil attenuates fibrosis in the RV (Fig 4). In both strategies, preventive and reversal, Sildenafil limits ventricular dilatation(7) and has a positive effect on ejection fraction. The positive effects of Sildenafil in our rats with a pressure load appear to be in contrast with the recently reported negative results of the RELAX trial, studying the long term effects of Sildenafil on patients with HF-PEF(26). However, in the RELAX trial, pulmonary artery pressure was only mildly elevated (41 mmHg), hence RV dysfunction was probably also mild. Redfield et al suggested that RV dysfunction has to reach a certain limit for Sildenafil to become effective in ventricular remodeling, a dose-effect relation that has been previously suggested from studies in mice with a LV load(22). Therefore, further trials are warranted to study the long term effects of PDE5inhibition in RV and LV failure (SIL-Hf trial:(13) ; PITCH-HF trial (I.D: NCT01910389)).

It is unclear why these functional changes did not result in an improvement of exercise tolerance. Exercise tolerance as measured with voluntary cage wheel exercise can be compared with clinically often used 6-minute walking distance test (6MWT) rather than a maximal exercise test. The 6MWT is a valuable outcome parameter in many clinical trials in pulmonary hypertension or heart failure(16, 27). In all clinical trials in pulmonary hypertension, Sildenafil treatment was associated with an improvement in 6MWT, however, in all these studies the pulmonary vascular resistance or pulmonary artery (PA) pressure also decreased(16), suggesting that the effect might be due to reduced afterload, hence improved RV-PA coupling. In the current study, the RV afterload
was fixed due to the pulmonary artery banding. We suggest that the persistence of afterload explains the lack of effect on exercise tolerance, despite preserved contractility and improved diastolic function.

Limitations

Our model of rats with RV dysfunction due to a fixed afterload comes with some limitations, which should be discussed. Firstly, we did not address the effects of different dosages of Sildenafil, which should be performed in future studies. Secondly, our relatively small animal study represents hypothesis generating groundwork, based on which mechanistic studies and larger (clinical) trials can be designed. Thirdly, no invasive pressure-volume measurements were performed at 4 weeks, as the extent of this procedure precludes survival of the animals. However, data from our previous studies in this model clearly show RV dysfunction at 4 weeks(7), which is confirmed by the echocardiographic measurements we performed in the present study. Lastly, survival analysis would have provided supplemental information on the therapeutic potential of Sildenafil in this disease model. Even so, the strong effects of Sildenafil on hemodynamics and fibrosis observed in the current and previous study(7) prompt further clinical study on Sildenafil in the fixed pressure loaded RV.

Conclusion

In this study in rats with fixed RV pressure overload, we show that Sildenafil treatment, in established RV dysfunction, reduced end diastolic pressure, altered end diastolic pressure volume relations, improved ejection fraction and limits fibrosis. These data demonstrate that Sildenafil has a direct beneficial effect on the pressure-loaded right ventricle independent from effects on RV afterload. These results indicate that Sildenafil treatment has therapeutic potential for treatment of established RV dysfunction.

FUNDING

This work was supported by the Sebald foundation and a grant from the Dutch Heart Foundation (2007T068).

ACKNOWLEDGEMENTS

The authors are indebted to M. Weij for excellent surgeries and B. Boersma and M. Dokter for expert technical assistance.
Therapeutic Sildenafil treatment in the pressure loaded RV

REFERENCES


Borgdorff MA, Bartelds B, Dickinson MG, Steendijk P and Berger RM. A cornerstone of heart failure treatment is not effective in experimental right ventricular failure. *Int J Cardiol* 2013 Sep 7. doi:pii: S0167-5273(13)01685-9. 10.1016/j.ijcard.2013.08.102. [Epub ahead of print]


