In vivo model of drug-induced valvular heart disease in rats: pergolide-induced valvular heart disease demonstrated with echocardiography and correlation with pathology

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Aims Valvular heart disease (VHD), inducing valvular regurgitation, has been described in carcinoid heart disease and recently in Parkinson’s patients treated with pergolide. The aim of this study was to develop an in vivo model of drug-induced valvulopathy with pergolide in rats.

Methods and results Thirty male Wistar rats were given daily injections of either pergolide (0.5 mg/kg intraperitoneally) (n = 8), serotonin (20 mg/kg subcutaneously) (n = 8), or the vehicle only (n = 14) for 5 months. At 20 weeks, echocardiography demonstrated the presence of aortic regurgitation (AR) and/or mitral regurgitation (MR) in serotonin (86% AR, P = 0.0001; 57% MR, P = 0.006) and in pergolide animals (67% AR, P = 0.003; 67% MR, P = 0.003) compared with none in placebo. Pulmonary regurgitation (PR) and tricuspid regurgitation (TR) were found in the serotonin (71% PR, P = 0.19; 100% TR, P = 0.06 vs. placebo), pergolide (100% PR, P = 0.014; 83% TR, P = 0.35 vs. placebo), and placebo groups (36% PR; 57% TR). Tricuspid regurgitant area ratio (jet/atrium), however, was more severe in the serotonin [median 26.5 (range 17–42)%; P = 0.02] and pergolide animals [32 (17–39)%; P = 0.03] compared with placebo [12.5 (5–33)%]. We found a good correlation between valvular regurgitation and histologically assessed valvular thickness. Histological examination revealed the presence of diffusely thickened and myxoid aortic, mitral, and tricuspid valves in serotonin and pergolide animals as seen in VHD.

Conclusion We demonstrated, for the first time, that long-term pergolide administration led to VHD in rats. This small animal model will permit further in vivo investigation of drug-induced valvulopathies.

Introduction

During the last decade, several drugs have been identified to cause cardiac valvulopathy. Ergot derivatives (ergotamine, methysergide) and appetite-suppressants (fenfluramine) were the first drugs described to cause valvular heart disease (VHD).1,2 This entity is characterized by thickening of the leaflets and thickening and shortening of the subvalvular apparatus, finally leading to valvular insufficiency. Recently we described the occurrence of VHD in 26 of 78 patients with Parkinson’s disease treated with pergolide.3 The involvement of ergot-derived dopamine agonists (pergolide, cabergoline) in the development of VHD is still an important topic of investigation.4,5

Drug-induced VHD has a histological resemblance with the carcinoid syndrome showing myxoid valvular changes, fibrosis and extracellular deposits of proliferative plaque-like material containing fibroblasts, myofibroblasts, and smooth muscle cells.2,6 Although many pathophysiological mechanisms remain to be elucidated, it is clear that serotonin [5-hydroxytryptamine (5-HT)] plays a central role. Cell culture studies indicated the mitogenic effects of 5-HT on different cell types such as fibroblasts7 and aortic smooth muscle cells.8–10 A recent animal study confirmed the development of carcinoid like valvular deposits in rats after 3 months of daily subcutaneous serotonin injections.11 Deficiency of the 5-HT transmembrane transporter in mice led to valvular fibrosis, providing further support for a direct link between 5-HT and cardiac valvulopathy.12 Functional recombinant receptor assays suggested that the effects of these valvulopathic drugs are mainly mediated
by the 5-HT$_{2b}$ receptor. These data also demonstrated that pergolide potently activates the 5-HT$_{2b}$ receptor.

Until now, most of the studies were performed in vitro to evaluate the response of valvular and cardiac cells to 5-HT, ergot-related drugs, and fenfluramine derivates. Therefore, important questions such as dose dependency, reversibility, and possible protective effects of antagonists remain unanswered. Hence, an in vivo model of drug-induced VHD is needed for future studies.

The aim of this study was to develop and characterize an in vivo model of drug-induced valvulopathy. For that purpose we studied the effect of daily injections of pergolide compared with serotonin and placebo on the cardiac valves of male Wistar rats using echocardiography. At the end of the study, the animals were sacrificed and the clinical data were compared with the histological findings.

**Methods**

**Study design**

Thirty male Wistar Unilever rats (Harlan, the Netherlands) (350 ± 3 g; 11 weeks) were randomized into two placebo-controlled arms. In the first arm, eight rats received daily one injection of serotonin (20 mg/kg) subcutaneously and seven rats received the vehicle only. In the second arm, eight rats received daily one injection of pergolide (0.5 mg/kg) intraperitoneally and seven rats the vehicle only. An echocardiographic evaluation was performed at baseline and at 10 and 20 weeks, followed by necropsy and histological examination of the heart. This study protocol was approved by the Ethics Committee of the Vrije Universiteit, Brussel.

**Animals handling**

During the whole study, the animals were housed in stainless steel cages with sawdust bedding. They were kept at an average room temperature of 24°C, a relative humidity of 50%, and a 12 h day/night cycle. Food (rat maintenance diet, SAFE, France) and water were provided at libidum.

**Drugs preparation and administration**

Serotonin (5-HT Creatinin Sulphate Complex, Sigma-Aldrich) was dissolved in physiological saline at a concentration of 20 mg/mL. In order to avoid skin lesions like subcutaneous bleedings and traumatic wounds, the injection side was changed daily. An equal volume of physiological saline (1 mL/kg) was given to the placebo rats.

Pergolide mesylate (Sigma-Aldrich) was prepared in a 10% alcoholic solution at a concentration of 0.5 mg/mL. An equal volume of a 10% ethanol solution (1 mL/kg) was given to the placebo rats.

Histopathology

The animals were sacrificed 2 weeks after the last injections. The hearts were weighted and fixed in formalin for 2 h. They were embedded in paraffin, cut in an axial plane (from basis to apex) and stained with hematoxylin and eosin and alcin blue for glycosaminoglycans. Additional step sections, ranging from 1 to 3 (approximately 100 µm between sections) were carried out for those heart sections without valves. Extreme care was taken in sectioning the heart so that the valves were mainly cut transversely (with the attachment sides of the leaflets visible on both ends of the valve). Morphometry was performed by digital image analysis using a PC digital image camera (Digital Sight DS-5M, Nikon Corp, Japan) mounted on an Axiolab Zeiss light microscope (Carl Zeiss Corp, Germany) with a ×10 objective (Acroplan, Zeiss). We used the NIH Image program (Image-J 1.35d, Nation Institutes of Health, Bethesda, USA). The program was calibrated with a graduated slide. Microscopic images were used to evaluate blindly the cardiac valves and cardiomyocytes. The maximum thickness of every valve present was measured. The width of at least ten cardiomyocytes was measured on the left ventricle of each section.

**Statistical analysis**

Data are expressed as median with range or interquartile range. Comparison between groups were performed by using the Mann-Whitney U-test and Fisher’s exact test. A cut-off value for valvular thickness and correlation between valvular thickness and regurgitation was calculated using receiver operating characteristic (ROC) analysis. Data were not corrected for multiple comparisons. All P-values were calculated two-tailed. A value of $P < 0.05$ was considered significant. Statistical analysis was done with GraphPad Prism (version 4.03, San Diego, CA, USA).

**Results**

There were no differences between the two placebo groups for all measurements. In the following part, the placebo data were pooled for further analysis.

**Clinical signs**

The serotonin injections induced flushing, loose stools, drowsiness, and tachypnea persisting for several hours after the injections. The clinical signs of the pergolide injections were most pronounced in the first 2 weeks of the study and included hyperactivity, poor grooming, aggressive behaviour, and increased gnawing activity. Three treated animals died during follow-up (one serotonin at 10 weeks immediately after anaesthesia and two pergolide rats were found dead at 14 and 19 weeks without identifiable cause at necropsy).
Echocardiography

Valvular analysis
Every valve was visualized in all animals during the study. A baseline echocardiography was performed before the start of the injections. There were no valvular abnormalities.

The occurrence of valvular regurgitation in the different groups during follow-up is shown in Table 1. At 20 weeks, aortic regurgitation (AR) was present in six (86%; \( P = 0.0001 \)) animals of the serotonin group and in four (67%; \( P = 0.003 \)) of the pergolide group (Figure 1). The median colour-Doppler ratio of regurgitant jet width to the left ventricular outflow tract diameter was 35.5 (24–43) and 25 (21–50), respectively. AR was not found in the placebo group.

Mitrail regurgitation (MR) was also not found in the placebo group. At 20 weeks, MR was present in four (57%; \( P = 0.006 \)) serotonin and four (67%; \( P = 0.003 \)) pergolide-treated animals (Figure 1B). The median regurgitant area ratio were 16.5 (8–36) and 25 (14–31)% respectively.

Tricuspid regurgitation (TR) and pulmonary regurgitation (PR) were found in both the placebo and treated animals. However, TR was found in 57% of placebos, in all serotonins (\( P = 0.06 \)), and in 83% pergolide rats (\( P = 0.35 \)). PR was present in 36% of placebos, in 71% of serotonins (\( P = 0.19 \)), and in all pergolide rats (\( P = 0.014 \)). In addition, the TR was more severe in the serotonin group [regurgitant area ratio 26.5 (17–42)%; \( P = 0.02 \)] and in the pergolide group [regurgitant area ratio 32 (17–39)%; \( P = 0.03 \)] compared with the placebo group [regurgitant area ratio 12.5 (5–33)%].

M-mode measurements
A significant increase in LVEDD and a decrease in FS were observed at 20 weeks in the pergolide but not the serotonin treated animals (Table 2). Both groups had a diminished left ventricular wall thickness compared with placebo.

Pathology

Post-mortem measurements
At sacrifice, serotonin- and pergolide-treated animals had a lower body weight [400 (362–468) g; \( P = 0.0003 \)] and [467 (435–517) g; \( P = 0.001 \), respectively, compared with the placebo group [571 (483–626) g]. The heart to body weight ratio was higher in the serotonin group [2.63 (3.13–3.89) mg/g; \( P = 0.0007 \)] and in the pergolide group [3.17 (2.78–3.50) mg/g; \( P = 0.07 \)] compared with the placebo group [2.79 (2.57–3.56) mg/g].

Valvular pathology
Histological examination revealed the presence of thickened aortic, mitral, and tricuspid cusps in the serotonin- and pergolide-treated animals (Figure 2). Moreover, regurgitant valves were thicker on histology [192 (69–405) \( \mu \text{m} \); \( P = 0.0003 \)] compared with non-regurgitant valves [106 (61–278) \( \mu \text{m} \)]. Using ROC analysis, a good correlation was found between echocardiography and pathology (AUC 0.76; \( P = 0.0003 \) with 161 \( \mu \text{m} \) as cut-off for a thickened pathological valve.

Valvular thickening was because of myxoid change in the sponge layer of the leaflet (Figure 3). These

<table>
<thead>
<tr>
<th>Table 1</th>
<th>Evolution of the valvular regurgitations of the placebo, serotonin- and pergolide-treated animals at 10 and 20 weeks</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>10 weeks</td>
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<tr>
<td></td>
<td>Placebo</td>
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<tr>
<td>N</td>
<td>14</td>
</tr>
<tr>
<td>Tricuspid regurgitation (%)</td>
<td>36</td>
</tr>
<tr>
<td>Pulmonary regurgitation (%)</td>
<td>7</td>
</tr>
<tr>
<td>Mitral regurgitation (%)</td>
<td>0</td>
</tr>
<tr>
<td>Aortic regurgitation (%)</td>
<td>0</td>
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</tbody>
</table>

Percentage of rats with valvular regurgitation. *\( P < 0.05 \), **\( P < 0.01 \), and ***\( P < 0.001 \), compared with placebo of the same age.
glycosaminoglycans deposits were also observed in the placebo group, but were smaller and localized at the distal free edge of the valve leaflet. In contrast, the serotonin- and pergolide-treated animals showed diffuse thick myxoid changes throughout the valves reaching the base of the cusps. Several areas of chondroid metaplasia were noted at the basal septum between the attachment sites of the aortic and mitral leaflets in the serotonin- (50%; $P = 0.28$), pergolide- (60%; $P = 0.24$), and placebo-treated animals (18%). True valvular fibrosis with dense collagen was not observed.

### Left ventricular pathology

The left ventricular cardiomyocytes were hypertrophic in both serotonin- [width 15.4 (12.4–17.4) μm; $P = 0.01$] and pergolide-treated animals [16.1 (13.7–18.1) μm; $P = 0.003$] compared with placebo-treated animals [12.4 (10.5–14.5) μm]. Macroscopically, left ventricular cavities were more dilated in both the serotonin and pergolide groups.

**Table 2** M-mode parameters of the left ventricle (LV) (parasternal short-axis view) of the placebo animals and the treated animals at 10 and 20 weeks

<table>
<thead>
<tr>
<th></th>
<th>Placebo</th>
<th>IQR</th>
<th>Serotonin</th>
<th>IQR</th>
<th>$P$-value</th>
<th>Pergolide</th>
<th>IQR</th>
<th>$P$-value</th>
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<tr>
<td>10 Weeks</td>
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<td>$N$</td>
<td>14</td>
<td>7</td>
<td>8</td>
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<tr>
<td>Anterior wall diastole (cm)</td>
<td>0.20</td>
<td>0.17–0.23</td>
<td>0.21</td>
<td>0.17–0.24</td>
<td>0.79</td>
<td>0.19</td>
<td>0.17–0.20</td>
<td>0.39</td>
</tr>
<tr>
<td>Inferior wall diastole (cm)</td>
<td>0.18</td>
<td>0.17–0.22</td>
<td>0.17</td>
<td>0.16–0.19</td>
<td>0.26</td>
<td>0.17</td>
<td>0.16–0.18</td>
<td>0.09</td>
</tr>
<tr>
<td>LV enddiastolic diameter (cm)</td>
<td>0.71</td>
<td>0.68–0.76</td>
<td>0.73</td>
<td>0.65–0.76</td>
<td>0.91</td>
<td>0.73</td>
<td>0.69–0.75</td>
<td>0.89</td>
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<tr>
<td>LV endsystolic diameter (cm)</td>
<td>0.45</td>
<td>0.39–0.47</td>
<td>0.42</td>
<td>0.38–0.45</td>
<td>0.68</td>
<td>0.45</td>
<td>0.41–0.48</td>
<td>0.56</td>
</tr>
<tr>
<td>Fractional shortening (%)</td>
<td>40</td>
<td>38–45</td>
<td>42</td>
<td>36–44</td>
<td>0.48</td>
<td>38</td>
<td>34–40</td>
<td>0.19</td>
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<tr>
<td>20 Weeks</td>
<td></td>
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<td>$N$</td>
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<td>7</td>
<td>6</td>
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<tr>
<td>Anterior wall diastole (cm)</td>
<td>0.20</td>
<td>0.18–0.22</td>
<td>0.17</td>
<td>0.16–0.18</td>
<td>0.01</td>
<td>0.17</td>
<td>0.15–0.19</td>
<td>0.01</td>
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<tr>
<td>Inferior wall diastole (cm)</td>
<td>0.20</td>
<td>0.19–0.23</td>
<td>0.16</td>
<td>0.15–0.18</td>
<td>0.004</td>
<td>0.16</td>
<td>0.15–0.17</td>
<td>0.008</td>
</tr>
<tr>
<td>LV enddiastolic diameter (cm)</td>
<td>0.72</td>
<td>0.69–0.79</td>
<td>0.75</td>
<td>0.72–0.80</td>
<td>0.26</td>
<td>0.80</td>
<td>0.73–0.85</td>
<td>0.03</td>
</tr>
<tr>
<td>LV endsystolic diameter (cm)</td>
<td>0.43</td>
<td>0.39–0.47</td>
<td>0.44</td>
<td>0.40–0.48</td>
<td>0.65</td>
<td>0.52</td>
<td>0.48–0.55</td>
<td>0.009</td>
</tr>
<tr>
<td>Fractional shortening (%)</td>
<td>41</td>
<td>37–44</td>
<td>40</td>
<td>36–52</td>
<td>0.91</td>
<td>35</td>
<td>32–39</td>
<td>0.02</td>
</tr>
</tbody>
</table>

Values are expressed as median and interquartile range (IQR). $P$-values are compared with placebo of the same age.

**Discussion**

In this study, we presented and characterized for the first time an *in vivo* animal model of pergolide-induced valvulopathy. We showed that long-term pergolide administration led to VHD in these rats. This was demonstrated by serial *in vivo* echocardiographic assessment of valvular changes during the course of the experiment. Moreover, there was an excellent correlation between these echocardiographic findings and histological analysis. We finally describe the pathological lesions in this model of drug-induced valvulopathy.

Drug-induced valvulopathy was demonstrated by means of echocardiographic illustration of valvular regurgitations. AR and MR were found only in the pergolide- and serotonin-injected animals, whereas PR and TR were also present in the placebo group. This illustrates that regurgitations are also present in normal rats and one should take this fact into account when studying VHD in rodents. Right-sided valvular regurgitations could be a physiological manifestation of the natural aging process of rats and might be
more pronounced under anaesthesia. More research is needed to clarify this observation. On the other hand, this can explain the absence of significant difference regarding TR in the pergolide-treated animals compared with controls by echocardiography. However, the severity of TR was more pronounced in both pergolide- and serotonin-treated animals compared with placebo. This might be because of the higher pulmonary resistance in these rodents. Moreover, histological analysis confirmed the tricuspid involvement in both pergolide- and serotonin-treated animals. This was not the case for the pulmonary valves. Although speculative, this can be explained by the more difficult imaging of the pulmonary valves by echocardiography and pathology in clinical and preclinical research in rodents. On the other hand, the pulmonary valve might present a different density of the 5-HT<sub>2B</sub> receptor. This needs to be addressed in future research. As in our clinical study with pergolide, left- and right-sided heart valves were affected. Carcinoid heart disease is mainly limited to the right side since serotonin is broken down by monoaminoxidase in the lungs, but the left side can also be affected. In our study, left-side involvement was also found in the serotonin-treated animals and is probably because of the relatively high dosage injected. Similar findings were observed in the study of Gustafsson.

Our study also demonstrated an excellent correlation between echocardiographic valvular regurgitation and valvular thickness as measured by histological examination. With exception of the pulmonary valve, all valves were significantly thicker in the pergolide and serotonin groups. Since no data exist about normal values of valvular thickness in rats, we calculated 161 μm as a best cut-off for a pathologically thickened valve in this study.

The histological lesions consisted of myxoid thickening of the valvular sponge layer in this animal model as observed by others. These endocardial myxoid changes have been described in normal aging rats, but the deposits are smaller and mainly situated at the distal free edges of the valvular leaflets. On the other hand, 5-HT<sub>2B</sub> receptor agonists such as pergolide could lead to an increased biosynthesis of collagen and glycoaminoglycans which accumulate in the valvular sponge layer in a diffuse pattern.
Besides these typical histological findings, we also describe the presence of chondroid metaplasia at the attachment sides of the aortic and mitral valves. This could be an age-related manifestation aggravated by over-stimulation of the 5-HT receptor as reported in studies of human calcified valves.\textsuperscript{23,24} This phenomenon was also described in 5-HT transmembrane transporter knockout mice.\textsuperscript{12} In these mice, fibrotic lesions were found in the valves and in the heart with left ventricular dilatation and decreased fractional shortening. Although we did not observe valvular and myocardial fibrosis, the pergolide group also developed a pronounced, diluted cardiomyopathy with thinning of the left ventricular wall and decreased fractional shortening. Chronic AR and MR might lead to volume overload and eventually to the development of a dilated cardiomyopathy, but this might also be because of a direct toxic effect on the cardiomyocytes although we did not find necrosis of the cardiomyocytes. In the serotonin group, the left chamber dilatation was not so marked. In addition, histological examination showed hypertrophy of the cardiomyocytes in both groups, possibly reflecting the 5-HT receptor overstimulation, as described by others.\textsuperscript{25,26}

Medications interacting with the serotonergic system are becoming increasingly common in clinical practice, i.e. in the treatment of migraine (5-HT\textsubscript{1A} receptor agonists), chemotherapy-induced emesis (5-HT\textsubscript{3} receptor antagonists), irritable bowel syndrome (serotonin agonists and antagonists), depression (selective serotonin reuptake inhibitors), and Parkinson’s disease ( pergolide and cabergoline).\textsuperscript{4,5}

With the development of this model of pergolide-induced valvulopathy, it may also be possible to study other specific drug-induced diseases for preclinical research, including drugs with a low affinity for the 5-HT receptor. This in vivo animal model permits to answer urgent questions in the field of drug-induced valvulopathies. Exposure to higher cumulative doses of serotonergic drugs could lead to more pronounced valvular lesions, whereas recovery of valvular function might occur after cessation of these drugs. Furthermore, the addition of 5-HT receptor antagonists could reduce or inhibit the development of valvular lesions. These issues will require further investigation. Because echocardiography showed to be a very useful tool to study in vivo drug-induced valvulopathy in this study, it will be the method of choice for the serial assessment of cardiac valves during these interventions.

Conclusions

We described the first pergolide-induced valvulopathy animal model and proved also that in vivo ultrasound imaging of VHD is feasible and correlates well with pathology. This opens the door towards research in the field of drug-induced VHD for answering important remaining questions such as dose-dependency, reversibility and influence of inhibitory drugs.

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Conflict of interest: none declared.

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