Combined pulmonary stenosis and insufficiency preserves myocardial contractility in the developing heart of growing swine at midterm follow-up

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Kuehne, Titus, B. Kelly Gleason, Maythem Saeed, Daniel Turner, Jochen Weil, David F. Teitel, Charles B. Higgins, and Phillip Moore. Combined pulmonary stenosis and insufficiency preserves myocardial contractility in the developing heart of growing swine at midterm follow-up. J Appl Physiol 99: 1422–1427, 2005. First published June 23, 2005; doi:10.1152/japplphysiol.00324.2005.—This study was conducted to determine the effects of chronic combined pulmonary stenosis and pulmonary insufficiency (PSPI) on right (RV) and left ventricular (LV) function in young, growing swine. Six pigs with combined PSPI were studied, and data were compared with previously published data of animals with isolated pulmonary insufficiency and controls. Indexes of systolic function (stroke volume, ejection fraction, and cardiac functional reserve), myocardial contractility (slope of the end-systolic pressure-volume and change in pressure over time-end-diastolic volume relationship), and diastolic compliance were assessed within 2 days of intervention and 3 mo later. Magnetic resonance imaging was used to quantify pulmonary insufficiency and ventricular volumes. The conductance catheter was used to index the stenotic cardiac functional reserve, diastolic compliance, and myocardial contractility from pressure-volume relations acquired at rest and under dobutamine infusion. In the PSPI group, the pulmonary regurgitant fraction was 34.3 ± 5.8%, the pressure gradient across the site of pulmonary stenosis was 20.9 ± 20 mmHg, and the average RV peak systolic pressure was 70% systemic at 12 wk follow-up. Biventricular resting cardiac outputs and cardiac functional reserves were significantly limited (P < 0.05), LV diastolic compliance significantly decreased (P < 0.05), but RV myocardial contractility significantly enhanced (P < 0.05) compared with control animals at 3-mo follow-up. In the young, developing heart, chronic combined PSPI impairs biventricular systolic pump function and diastolic compliance but preserves RV myocardial contractility.

MANY PATIENTS WITH CONGENITAL heart disease involving right ventricular (RV) outflow tract obstruction, such as tetralogy of Fallot, have residual pulmonary stenosis and insufficiency (PSPI) after surgical repair. Individually, these abnormalities, if severe, can lead to right heart failure. In these patients, timing of pulmonary valve replacement is crucial to extend the time window for repetitive cardiac surgery without increased risk of irreversible RV damage. In this setting, recent studies focused on the evaluation of the effects of pulmonary insufficiency (PI) on ventricular function and propose a less restrictive management of pulmonary valve replacement (9, 17, 25, 26, 28, 29). However, the combined effect of PSPI on biventricular function remains not well investigated. Although some reports suggest that increased RV afterload modulates the degree of PI, the effect of combined PSPI on biventricular systolic and diastolic function is inadequately understood (4, 21, 24). This lack of understanding limits our ability to develop an optimal treatment strategy that minimizes intervention yet maximizes long-term biventricular function.

The aim of this study was to create chronic pressure and volume overload of the RV in a growing pig model to determine the effect on biventricular systolic pump function, diastolic compliance, and myocardial contractility. The data were compared with previously published data on controls and animals with isolated PI (13). Cardiac chamber size and systolic pump function were assessed by magnetic resonance imaging (MRI). Hemodynamic data were obtained by using standard catheterization techniques, and parameters of RV and left ventricular (LV) myocardial contractility and passive diastolic compliance were measured by using the conductance catheter technique.

MATERIALS AND METHODS

Six pigs were studied and compared with data obtained on five controls. The mean animal weight at the initial procedure was 14 ± 0.8 kg, which corresponds approximately to an age of 5–6 wk. All animals underwent four experimental procedures: cardiac catheterization with or without stent implantation, MRI study within 2 days of catheterization, cardiac catheterization with conductance pressure-volume loop analysis ~12 wk thereafter, followed within 2 days by repeat MRI study. We defined the follow-up study at 12 wk as midterm follow-up. All procedures were performed in accordance with the National Institutes of Health Guide for Care and Use of Laboratory Animals and with the approval of the Laboratory Animal Research at our institution. For all procedures, anesthesia was initiated with telazol-ketamine-xylsol (0.025 mg/kg im) and then maintained with 1–2% inhaled isofluorane. Heart rate, pulse oximetry, and systemic pressures were monitored continuously during each procedure. At the end of the last procedure, the animals were euthanized with pentobarbital sodium. The hearts were harvested, dissected, and weighed. The membrane stent was inspected for patency and position.

Intervention

After baseline hemodynamics, an 18 × 20-mm self-expanding nitinol stent (Memotherm), fitted with a thin intraluminal Teflon membrane (WL Gore), was deployed across the pulmonary valve through an 8-F custom delivery system under fluoroscopic guidance. The membrane was cut to the diameter of the stent and sutured through an 8-F custom delivery system under fluoroscopic guidance. Magnetic resonance imaging was used to confirm position, and hemodynamic measurements were repeated.
Hemodynamic Study

Pressures were measured in the right atrium, RV, main pulmonary artery proximal and distal to the membrane stent, and LV using fluid-filled catheters connected to a transducer at the time of stent placement and 12 wk later.

MRI Study

MRI was performed by using a GE 1.5-T magnetic resonance (MR) imager (Signa 5, General Electric Medical Systems) with a standard body coil. Pulmonary flow volumes were assessed by using a velocity-encoded cine (VEC) MRI sequence in a plane perpendicular to the dominating flow direction in the main pulmonary artery (14, 29). Instantaneous flow volumes were summed to give total forward and regurgitant flow per cardiac cycle. The following acquisition parameters were used for VEC MRI: repetition time/echo time = 257/7 ms, slice thickness = 5 mm, flip angle = 30°, field of view = 24 × 24 cm, matrix = 256 × 192, number of excitations = 2, and VEC = 200 cm/s.

A cine MRI sequence in the cardiac short-axis plane was used to assess RV and LV chamber volumes (14). End-systolic and end-diastolic volumes were measured by manually tracing the area of the endocardial and epicardial surfaces. Ventricular chamber volumes were computed as the sum of RV and LV volumes of all short-axis view slices containing RV and LV chambers. The following imaging parameters were used: repetition time/echo time = 8/5 ms, slice thickness = 10 mm, spacing = 0, flip angle = 20°, field of view = 24 × 24 cm, matrix = 256 × 128, number of excitations = 2, and phases = 16. Two independent observers analyzed cine MR and VEC MR images.

Conductance Catheter Study

A 5- to 7-F dual-field combination pressure-conductance catheter (Millar Instruments) was used to generate RV and LV loops, with the catheter tip placed at the ventricular apex. Vascular access was gained from the jugular vein and carotid artery. The catheter was connected to a Sigma-5 signal conditioner processor (Leycom) that computed time-varying segmental conductance and generated analog output. The analog output was digitized at 200 Hz by an analog-to-digital data card (National Instruments) connected to a Power Macintosh computer (Apple). The data were analyzed on LabView 5.2 software (National Instruments).

Pressure and volume data were obtained during skeletal muscle relaxation and with the ventilator held at end expiration. Data were acquired simultaneously in both ventricles before and during inferior vena cava balloon occlusion. Measurements were made in the resting state and during infusion of dobutamine at a rate of 3 μg·kg⁻¹·min⁻¹.

Calculations

General hemodynamics. The pressure difference across the membrane stent was calculated by subtracting the peak systolic pulmonary arterial pressure distal to the stent from the peak systolic RV pressure.

Quantification of pulmonary regurgitant fraction. Pulmonary flow volumes were generated from the VEC MRI sequences. The volume of insufficiency per beat was calculated as the retrograde flow in the main pulmonary artery in diastole. Total RV stroke volume was calculated as the antegrade flow in systole in the main pulmonary artery. These flow volumes previously have been shown to be accurate in the presence of a stent in the pulmonary artery (14). The regurgitant fraction was calculated as the ratio of insufficiency to total RV stroke volume.

Turbulent blood flow in the presence of vascular stenosis can cause spin dephasing and in turn affect the accuracy of VEC MRI techniques. Therefore, internal validation of VEC MRI-derived pulmonary regurgitant fraction comprised comparison with RV volume analysis by using cine MRI (14). Cine MRI-derived regurgitant fraction was calculated as stroke volume divided by end-diastolic volumes (14).

Systolic pump function. Resting indexes of systolic pump function were derived from cine MRI at rest and from conductance measurements during dobutamine infusion. Cardiac functional reserve was defined as the ability to increase effective stroke volume during dobutamine infusion. It was calculated by dividing the increase in ventricular stroke volume during dobutamine infusion by the stroke volume at rest and is presented as a percentage increase over baseline.

All measurements were obtained at an increase in heart rate of <10% compared with rest.

Myocardial contractility. Indexes of RV and LV myocardial contractility were derived from pressure-volume loop analyses during preload reduction. Pressure was differentiated during the prejection period, and the slope of the maximum change in pressure over time-end-diastolic volume relationship was calculated (8, 18). End systole was defined as the point of maximal elastance using the iterative method (2). From the end-systolic point derived from each loop, the slope of the end-systolic pressure vs. end-systolic volume relationship was calculated. See Fig. 2.

Diastolic performance. Investigation of diastolic performance was limited to the evaluation of passive compliance. Active relaxation could not be calculated in our model because PI precludes isovolumic data points in the RV. Passive ventricular compliance was derived from the end-diastolic pressure-volume relationships of both ventricles, where the stiffness coefficient (κ) was calculated as previously reported (12, 13).

Pulmonary forward flow, as assessed by VEC MRI, was used as an indirect measure of passive RV diastolic compliance.

Statistical Analysis

Data measured at rest and during dobutamine infusion (conductance catheter-derived indexes of ventricular function) were analyzed by analysis of covariance. Variables were coded for animal numbers and the presence or absence of dobutamine. Data obtained only in the resting state (MRI, hemodynamic data, and weights) were analyzed by either unpaired or paired Student’s t-test with Bonferroni correction for multiple comparison. A value of P ≤ 0.05 was considered significant. Data are expressed as means ± SD. Bland-Altman test was used to determine the agreement between VEC and cine MRI-derived pulmonary regurgitant fraction.

RESULTS

Control and PSPI pigs were of similar weight at the initial intervention and at the final study (Table 1). All animals
showed throughout the study normal activity and feeding habits. As expected, hemodynamic data were normal and similar in the two groups before stent implantation.

**Global Cardiovascular Effects**

See Table 1. At 12 wk, the PSPI group had a pulmonary regurgitant fraction of 34.0 ± 7.1%, an average gradient across the pulmonary outflow of 20.9 ± 20.0 mmHg, and a significantly increased RV mass of 52 ± 4 g at 12 wk. Control pigs had no pulmonary regurgitation and no gradient across the pulmonary outflow and an average RV mass of 27 ± 3 g. RV end-diastolic pressures were significantly elevated in the PSPI animals (12.3 ± 1.6 vs. 7.8 ± 0.7 mmHg, P < 0.05), as were RV end-systolic and end-diastolic volumes (P < 0.05, Table 1). LV peak systolic and diastolic pressures were similar in both groups (control = 72.4 ± 5.6 and 6.8 ± 1.6 mmHg vs. PSPI = 73.2 ± 5.3 and 8.4 ± 2.4 mmHg, P > 0.05). LV end-systolic volumes of the PSPI group were at control level; however, end-diastolic volumes were significantly decreased (P < 0.05, Table 1).

Inter- and intraobserver variability of VEC MRI-derived pulmonary regurgitant fraction was 2.9 and 2.7%, respectively. Inter- and intraobserver variability of cine MRI-derived ventricular volumes was 5.8 and 3.1%, respectively. Bland-Altman analysis showed good agreement between pulmonary regurgitant fraction as measured with VEC and cine MRI method, with a bias of +2.2%.

**Systolic Pump Function**

See Tables 1 and 2. After 12 wk of PSPI, the animals had significantly increased RV total stroke volume (P < 0.05), whereas the effective cardiac output was significantly de-

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**Table 1. Global cardiovascular effects**

<table>
<thead>
<tr>
<th></th>
<th>Initial</th>
<th>PSPI</th>
<th>Control</th>
<th>PSPI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Body weight, kg</td>
<td>13.8±0.9</td>
<td>14.2±0.6</td>
<td>32.6±1.8</td>
<td>33.8±2.2</td>
</tr>
<tr>
<td>Cardiac mass, g/m²</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>RV free wall</td>
<td>NA</td>
<td>NA</td>
<td>41.9±6.8</td>
<td>86.3±7.3*</td>
</tr>
<tr>
<td>LV free wall</td>
<td>NA</td>
<td>NA</td>
<td>89.9±7.9</td>
<td>87.1±8.5</td>
</tr>
<tr>
<td>Septum</td>
<td>NA</td>
<td>NA</td>
<td>37.1±9.3</td>
<td>39.9±5.6</td>
</tr>
<tr>
<td>Total weight</td>
<td>NA</td>
<td>NA</td>
<td>168.9±8.7</td>
<td>213.2±7.4*</td>
</tr>
<tr>
<td>Ventricular volumes by cine MRI, ml/m³</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>RV-EDV</td>
<td>78.1±7.3</td>
<td>86.8±6.6*</td>
<td>72.4±7.6</td>
<td>102.4±8.1*</td>
</tr>
<tr>
<td>RV-ESV</td>
<td>33.1±4.6</td>
<td>34.4±3.1</td>
<td>30.5±4.3</td>
<td>54.6±4.0*</td>
</tr>
<tr>
<td>LV-EDV</td>
<td>75.6±6.5</td>
<td>71.7±3.5</td>
<td>73.5±5.3</td>
<td>68.3±7.5*</td>
</tr>
<tr>
<td>LV-ESV</td>
<td>32.1±4.9</td>
<td>30.7±4.6</td>
<td>30.8±4.7</td>
<td>31.6±3.9</td>
</tr>
<tr>
<td>Ventricular pressures, mmHg</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>RV (peak systolic/end diastolic)</td>
<td>26.2±2.8/6.8±1.6</td>
<td>27.1±3.1/6.1±1.5</td>
<td>28.1±4.7/7.8±0.7</td>
<td>50.3±16.4*/12.3±1.6*</td>
</tr>
<tr>
<td>LV (peak systolic/end diastolic)</td>
<td>71.1±5.3/9.7±1.4</td>
<td>70.6±5.9/8.3±1.7</td>
<td>72.4±5.6/6.8±1.6</td>
<td>73.2±5.3/8.4±2.4</td>
</tr>
<tr>
<td>Pulmonary regurgitant fraction by VEC MRI, %</td>
<td>0.9±0.8</td>
<td>28.2±1.8*</td>
<td>0.6±1.1</td>
<td>34.3±5.8*</td>
</tr>
<tr>
<td>Effective RV cardiac output, l/min</td>
<td>1.7±0.5</td>
<td>1.6±0.5</td>
<td>3.4±0.6</td>
<td>3.1±0.7*</td>
</tr>
<tr>
<td>Heart rate, beats/min</td>
<td>102.3±5.3</td>
<td>105±19.8</td>
<td>92.5±7.4</td>
<td>93.7±7.3</td>
</tr>
<tr>
<td>Catheterization at rest</td>
<td>109.4±12.2</td>
<td>105.3±9.8</td>
<td>83.8±6.8</td>
<td>89.3±9.0</td>
</tr>
</tbody>
</table>

Values are means ± SD. PSPI, pulmonary stenosis and insufficiency; RV, right ventricular; LV, left ventricular; MRI, magnetic resonance imaging; EDV, end-diastolic volume; ESV, end-systolic volume; VEC, velocity-encoded cine; NA, not applicable. *P < 0.05, control vs. PSPI analyzed by unpaired Student’s t-test.
Diastolic Performance

See Table 3. After 12 wk of PSPI, the diastolic compliance was significantly decreased in both the RV and LV. The RV systolic function was also attenuated, however, and went along with decreased RV myocardial contractility and increased RV compliance. In addition, PSPI resulted in a smaller regurgitant fraction, more RV hypertrophy, and a smaller increase in RV volumes than did isolated PI (Table 4).

Stenosis, in addition to insufficiency, seems to have been beneficial by promoting hypertrophy, limiting RV dilatation, and enhancing myocardial contractility. This enhanced contractility is supported by recent animal data that showed an increased RV contractility in the presence of both acutely and chronically (8 wk) increased RV afterload with isolated pulmonary stenosis (PS) (5, 6, 15, 19). These beneficial effects of short- to intermediate-term stenosis on RV myocardial function were not observed in a similar model (13). In this study, systolic pump function was associated with decreased RV diastolic compliance but enhanced RV myocardial contractility. These findings contrast markedly with the effects of isolated PI observed in a similar model (13). In this study, systolic pump function was also attenuated, however, and went along with decreased RV myocardial contractility and increased RV compliance. In addition, PSPI resulted in a smaller regurgitant fraction, more RV hypertrophy, and a smaller increase in RV volumes than did isolated PI (Table 4).

Table 4. Comparison between PSPI and isolated PI model at 12-wk follow-up

<table>
<thead>
<tr>
<th>Control</th>
<th>PSPI</th>
<th>Isolated PI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Regurgitant fraction, %</td>
<td>0.6±1.1</td>
<td>34.3±5.8*</td>
</tr>
<tr>
<td>RV free wall thickness, g/m²</td>
<td>41.9±6.8</td>
<td>86.3±7.3*</td>
</tr>
<tr>
<td>RV ESV, ml/m²</td>
<td>30.5±4.3</td>
<td>54.6±4.0*</td>
</tr>
<tr>
<td>RV SV, ml</td>
<td>30.2±2.8</td>
<td>39.9±19.5*</td>
</tr>
<tr>
<td>RV EF, %</td>
<td>56.7±3.7</td>
<td>46.7±2.6*</td>
</tr>
<tr>
<td>RV (increase in %)</td>
<td>22±12.3†</td>
<td>5.2±3.2*</td>
</tr>
<tr>
<td>RV Mdp, mmHg/ml</td>
<td>25.8±5.9</td>
<td>32.2±2.1*</td>
</tr>
<tr>
<td>+ dobutamine</td>
<td>52.8±13.5†</td>
<td>73.5±9.9†</td>
</tr>
<tr>
<td>RV Emax, mmHg/ml</td>
<td>2.6±0.3</td>
<td>3.9±0.7*</td>
</tr>
<tr>
<td>+ dobutamine</td>
<td>3.5±0.4†</td>
<td>5.5±0.9*</td>
</tr>
<tr>
<td>κ RV</td>
<td>0.17±0.04</td>
<td>0.21±0.05*</td>
</tr>
<tr>
<td>κ LV</td>
<td>0.10±0.03</td>
<td>0.13±0.04*</td>
</tr>
</tbody>
</table>

Values are means ± SD. PI, pulmonary insufficiency; EF, ejection fraction; RV, stroke volume. *P < 0.05, control vs. PSPI or control vs. PI analyzed by unpaired Student’s t-test; †P < 0.05, relative change with dobutamine analyzed by ANCOVA.
tility raise many questions in light of the known adverse effects of long-term severe PS on RV function. This also brings into question the long-held belief that stenosis, even mild, is detrimental to ventricular function while insufficiency is well tolerated.

Despite the potential beneficial effects of stenosis on myocardial contractility, the systolic pump performance was decreased in the PSPI animals, similar to that seen with isolated PI. These results are in accordance with reports from a clinical study involving patients with pulmonary valve insufficiency and/or stenosis (27). This suggests that different mechanisms in these two conditions lead to similar global effects. A possible explanation may be that, in the presence of enhanced RV myocardial contractility (PSPI group), the inability to increase output in response to positive inotropic stimulation is due to abnormal diastolic loading of the RV. In contrast, with isolated insufficiency, compensatory hypertrophy is not adequate, resulting in myocardial contractile dysfunction, whereas RV diastolic compliance is (at 3-mo follow-up) not decreased.

At the initial study, pulmonary regurgitant fraction was not significantly different between the combined PSPI and isolated PI group (28.2 ± 1.8 vs. 33.1 ± 4.1, P = 0.28). However, after 3-mo follow-up, regurgitant fraction differed significantly between the two groups (34.3 ± 5.8 vs. 49.2 ± 5.9, P < 0.01, Table 4). These data are indicative that, at least in the presence of proximal PS, RV hypertrophy might limit the degree of PI. However, the course of PI in the presence of increased pulmonary arterial resistance due to distal PS or hypoplastic pulmonary arterial bed cannot be extrapolated from the present data but should be subject to future research. Furthermore, isoflurane inhalation can modulate pulmonary arterial resistance, and that should be taken into account when interpreting the present data.

LV cardiac reserve was reduced in both experimental groups (PSPI and PI). This finding could be explained by impaired LV diastolic filling and, in turn, decreased LV systolic ejection, whereas LV filling may be altered by 1) limited RV effective outputs (serial coupling between the RV and LV) and 2) decreased LV diastolic compliance. The latter is possibly mediated by parallel coupling between the RV and LV through the interventricular septum in the presence of RV eccentric and/or concentric hypertrophy. In both the PI and PSPI group, MR images showed changes in RV configuration with a flat “D”-shaped interventricular septum.

Cardiac reserve is considered to be a good measure of the quality of life and prognosis in a patient with impaired ventricular function. In adults, peak exercise oxygen consumption, as a measure of cardiac reserve, has been used to estimate the prognosis of patients with decreased ejection fraction (1, 10). Besides peak exercise oxygen consumption, dobutamine stress was used for assessment of the functional status of the heart and to differentiate decreased function, resulting from abnormal loading conditions from true myocardial dysfunction (7, 16, 20, 22, 23). Our study suggests that cardiac reserve, as assessed by dobutamine loading, may be a poor marker for contractile dysfunction of the RV in the setting of both volume and moderate pressure loads on the RV. A decreased cardiac reserve seems to be substantially mediated by the effects of changed RV configuration and may not reflect RV contractile dysfunction.

Limitations

The conductance catheter has been shown to assess accurately relative volume changes of the RV; however, care must be taken for analysis of absolute volumes and the distribution of the measuring field of the catheter in the RV infundibulum (3, 5–7, 11, 15). Day-by-day changes of stroke volume have to be taken into account when comparing MRI with conductance catheter data, as both measures were acquired in our study within 2 days of each other. The impact of heart rate-velocity and muscle mass-velocity interaction was taken into account in this study, but it cannot be fully excluded. Care must be taken in extrapolating these animal data to human populations with congenital heart disease. The hemodynamic abnormalities in this model are homogeneous and of short duration compared with patients with repaired RV outflow tract obstruction. In addition, confounding effects on cardiac and myocyte function due to chronic cyanosis, cardiopulmonary bypass associated with surgical repair, and the effects of noncompliant synthetic material in the RV outflow tract that alters pulse propagation are not taken into consideration in this study. Finally, in patients, various degrees of isolated and/or combined PSPI are found, and their balance must be considered as a major determinant for the course of RV function. The present study provides valuable insight into a subset of possible RV overload conditions; however, more research is needed to cover a broader range of PSPI.

In summary, using MRI and conductance catheter techniques, we showed in an animal model that chronic PSPI causes biventricular systolic and diastolic dysfunction, despite preservation of LV and enhanced RV myocardial contractility. Changes in RV configuration and their serial and parallel coupling effect with the LV appear to play a major role in this dysfunction. At midterm, PS combined with PI preserves RV myocardial contractility, compared with isolated PI. Our physiological findings need to be confirmed in long-term studies and for different degrees of PSPI.

REFERENCES


