Fetal Cardiac Morphology of Tetralogy of Fallot With Absent Pulmonary Valve in the Rat

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Fetal in situ cardiovascular and bronchial morphologies were studied in rats with tetralogy of Fallot and absent pulmonary valve to clarify the prenatal pathology of this complex. There were 42 fetal rats with this complex among 300 fetuses treated with \(N,N'\)-bis-(dichloroacetyl)-1,8-octamethylenediamine (bis-diamine) (200 mg) on the 10th day of pregnancy. After undergoing rapid whole-body freezing on the 21st day, they were studied by means of serial cross-sectional photographs of the frozen thorax. Fetuses with normal heart treated with bis-diamine served as control. Tetralogy of Fallot and absent pulmonary valve in the fetus was associated with absence of the ductus arteriosus, enlargement and hypertrophy of both ventricles, and enlargement of the pulmonary arteries that compressed the bronchial trees. These abnormalities were inversely correlated with the degree of pulmonary stenosis. Milder stenosis (pulmonary valve ring >50% of control, \(n=28\)) was associated with larger pulmonary arteries (250±12% of control) (mean±SEM) and larger ventricles (volume, 200±15%; mass, 130±4%). Severe stenosis (pulmonary valve ring <50% of control, \(n=14\)) was associated with mildly enlarged pulmonary arteries and mild bronchial compression. We concluded that enlargement of the pulmonary arteries and bronchial compression develop in fetal life and are associated with mild pulmonary stenosis in tetralogy of Fallot and absent pulmonary valve. (Circulation 1990;82:1343–1351)

Tetralogy of Fallot with absent pulmonary valve in its most severe form is associated with severe respiratory symptoms secondary to bronchial compression by the huge pulmonary arteries in early infancy.\(^\text{1-5}\) This complex is difficult to treat, and its prognosis is generally poor.\(^\text{1-5}\) Bronchial compression is assumed to develop in fetal life. Recently, some cases with this complex were diagnosed by echocardiography in fetal life.\(^\text{3-5}\) Bronchial and cardiac changes in this fetal complex, however, still need much clarification. In the past, good animal models of this complex were not available. Recent discovery of potent cardiac teratogens\(^\text{6,7}\) and development of methods of studying in situ cardiovascular morphology of fetal rats\(^\text{8,9}\) made it possible to study cardiac pathology in rat fetuses with congenital heart disease. This is a report of in situ cardiovascular and bronchial morphology of near-term rat fetuses with tetralogy of Fallot and absent pulmonary valve.

Methods

Animals

Virgin Wistar rats (pregnancy period, 21.5 days) were mated overnight from 5:00 PM to 9:00 AM, and the presence of sperm on vaginal smears fixed the zero day of pregnancy. These rats were fed commercial solid food and water. Treatment of these rats conformed to the guiding principles of the American Physiological Society.

Drug

\(N,N'\)-Bis-(dichloroacetyl)-1,8-octamethylenediamine (No. F-8378, Fertilysin, Sigma Chemical, St. Louis, Mo.) (bis-diamine) was blended with 1% aqueous suspension of gum tragacanth to give a mixture of 100 mg/ml. A single 200-mg dose of this suspension was given by gastric tube to 40 pregnant rats on the 10th day of pregnancy.

Freezing, Cutting, and Photographing

Fetal cardiac morphology was studied on the 21st day of pregnancy by using the rapid whole-body freezing technique as previously reported.\(^\text{8,9}\) Briefly,
Figure 1. Photographs showing angled coronal cross-section of the control fetus (top panel) and a fetus with tetralogy of Fallot and absent pulmonary valve (bottom panel). Cross-sections through the aortic valve are shown. Note subaortic ventricular septal defect, overriding of the aorta over the ventricular septum, and a huge main pulmonary artery compressing the left atrium in the bottom panel. Clockwise rotation around the cardiac long axis and dilatation of the ventricles are present in the bottom panel. Ao, aorta; DA, ductus arteriosus; E, esophagus; LA, left atrium; LPA, left pulmonary artery; LSVC, left superior vena cava; LV, left ventricle; PA, pulmonary artery; RA, right atrium; RPA, right pulmonary artery; RV, right ventricle; SVC, superior vena cava; T, trachea.
the rats were killed by cervical dislocation, and the fetuses were delivered quickly by cesarean section and quickly frozen by immersion in acetone cooled by dry ice to $-80^\circ$ C. The frozen fetuses were weighed, and the thoraxes were trimmed and sectioned on a freezing microtome (Komatsu Solidate, Tokyo, Japan) at either the cardiac short-axis plane, angled coronal or cardiac long-axis plane, transverse plane, or the left sagittal plane encompassing the whole heart. The short-axis plane was defined as the plane 45 degrees to the transverse plane and 45 degrees to the sagittal plane. The long-axis or angled coronal plane was defined as the plane 45 degrees to the transverse plane and perpendicular to the sagittal plane. Cardiac cross-sections were photographed with a binocular stereoscopic microscope (Wild M 400 Photomakroscope, Wild Heerbrugg, Heerbrugg, Switzerland) using color film (Reala, Fuji Film, Tokyo, Japan). Cardiac cross-sections were photographed serially at every 500-$\mu$m thickness for morphological study and for measurement of chamber volumes and ventricular masses. Additional crossections were photographed to record the origin of the pulmonary arteries and the morphology of the right ventricular outflow tract. Numbered section paper (1×1 mm) was photographed for a scale, and the pictures were printed on paper.

**Measurement**

Diameters of the pulmonary valve ring, main pulmonary artery, right and left pulmonary arteries, ascending aorta, and bronchi were measured on the photographs. The thickness of the pericardial fluid was measured around the ventricle at the level of the left ventricular papillary muscles. To study the volumes of 4 cardiac chambers and ventricular masses of each heart, areas of each chamber on the photographs were cut and weighed, and then calculated by using the weight of the photograph of section paper as a scale. The volumes and the muscle mass were calculated by area multiplied by thickness (0.5 mm). The accuracy of this method has been tested and reported in another study.9

**Cardiac Anomalies**

Of the 335 fetuses from 40 litters, 16 were either dead or resorbed, and the survival rate was 95%. The diagnoses of 300 fetuses included 160 fetuses with tetralogy of Fallot with pulmonary valve, 42 fetuses with tetralogy of Fallot with absent pulmonary valve, 55 fetuses with persistent truncus arteriosus, eight fetuses with simple ventricular septal defect, and 35 fetuses without major cardiac anomalies. The 42 fetuses with tetralogy of Fallot and absent pulmonary valve were included in this study. Of these fetuses, 31 were sectioned and photographed in the cardiac short-axis plane, 7 fetuses in the angled coronal plane, 2 fetuses in the transverse plane, and 2 fetuses in the left sagittal plane. The 35 fetuses without major cardiac anomalies were compared as the control.

**Statistical Analysis**

Experimental results are presented as mean±SEM. Comparisons between the two groups were subsequently submitted to Student's t test at a confidence level of 95%.

**Results**

**Cardiac Cross-sectional Morphology**

Large subaortic ventricular septal defect was present in the hearts with this complex. Aortic overriding on the ventricular septum was commonly present and was most apparent in the angled coronal section (Figure 1). Fibrous continuity was present between the aortic valve and the mitral valve. The ductus arteriosus was absent in all hearts. The pulmonary valve was either completely absent (35 fetuses) or partially absent with a small residual ridge (seven fetuses). There was pulmonary stenosis of varying degrees, and the pulmonary valve ring was smaller than in the control. In 29 of 42 fetuses, a narrow infundibulum constituted pulmonary stenosis in addition to a narrow pulmonary valve ring (Figure 2, lower left panel). In the remaining 13 fetuses, the infundibular septum was absent (Figure 2, upper right panel).

**Pulmonary Arteries and Bronchi**

The main pulmonary artery and the right and left pulmonary arteries were enlarged (Figures 1, bottom panel; 3, bottom panel; 4, bottom panel). In each fetus, the most prominent enlargement occurred at the right hilum. Enlarged pulmonary arteries compressed the adjacent bronchi (Figures 3, bottom panel; 4, bottom panel). The diameter of the right pulmonary artery and the diameter of the adjacent bronchi were inversely related, as shown in Figure 5. The enlarged main pulmonary artery compressed the left atrium (Figure 1, bottom panel).

Diameters of the pulmonary valve ring and diameters of the maximum right pulmonary artery were positively correlated (Figure 6). Those fetuses with only mildly hypoplastic pulmonary valve rings (ring diameter >500 $\mu$m, or 50% of control) were associated with larger pulmonary arteries. Those fetuses with severely hypoplastic pulmonary valve rings (ring diameter <500 $\mu$m, or 50% of control) were associated with only modestly enlarged pulmonary arteries.

The inner diameters of the pulmonary valve ring, the main pulmonary artery, and maximum diameters of the right and left pulmonary arteries are listed in Table 1. In this table, the fetuses were divided into two groups according to the degree of pulmonary stenosis. Those with milder stenosis and a pulmonary valve ring larger than 50% of the control had a more prominent enlargement of the pulmonary arteries than those fetuses with more severe stenosis.

**Right and Left Ventricles**

Both the right and left ventricles were enlarged in the fetuses with mild pulmonary stenosis (Table 1).
Ventricular enlargement was associated with an increase in ventricular mass (Table 1). Right ventricular enlargement was associated with enlargement of the right atrium. Left atrial volume was diminished, but left atrial volume was also diminished in tetralogy of Fallot without absent pulmonary valve (unpublished data). No significant change was observed in the ventricular volumes of those fetuses with severe pulmonary stenosis (Table 1).

Noncardiovascular Malformations

Noncardiovascular malformations included hypoplasia of the thymus, as shown in Table 1, and craniofacial anomalies. The thymus was commonly hypoplastic and completely absent in some fetuses. Craniofacial anomalies included median facial cleft, cleft palate, and open eyelids.

Discussion

Tetralogy of Fallot and Absent Pulmonary Valve

In this animal model induced by bis-diamine in rats, a high incidence of absent pulmonary valve associated with tetralogy of Fallot is a unique feature and was not reported previously. Etiology of the absent pulmonary valve is not known. Several studies suggest that the teratogenic effects of bis-diamine work as inhibitors of the normal action of neural crest cells in cardiovascular and other organogenesis. It is possible that neural crest cells are important in the formation of the pulmonary valve, and that bis-diamine works to inhibit its effect in pulmonary valve formation. Emmanouilides et al proposed another hypothesis regarding absent pulmonary valve associated with tetralogy of Fallot, that is, that the absence of the ductus arteriosus, which is frequently associated with this complex, hemodynamically induces absence of the pulmonary valve. In our rat models induced with bis-diamine, all fetuses with tetralogy of Fallot and absent pulmonary valve were associated with absent ductus arteriosus, supporting the hypothesis by Emmanouilides et al.

Bronchial Compression

The present study showed that enlargement of the pulmonary arteries and compression of the bronchi were already present in near-term fetuses. These compressions and deformities of the bronchi probably occur in fetal life in the clinical situation as well, and persist even after surgical correction of the heart disease.
FIGURE 3. Photographs showing angled coronal cross-sections of the control fetus (top panel) and tetralogy of Fallot with absent pulmonary valve (bottom panel). Note huge pulmonary arteries compressing bronchi in the bottom panel, in contrast to small pulmonary arteries and widely open bronchi in a control fetus in the top panel. Ao, aorta; B, bronchus; E, esophagus; HAV, hemizygos vein; IVC, inferior vena cava; LPA, left pulmonary artery; LSVC, left superior vena cava; LV, left ventricle; RA, right atrium; RPA, right pulmonary artery; RV, right ventricle; PV, pulmonary vein.
FIGURE 4. Photographs showing short-axis cross-section of the control fetus (top panel) and tetralogy of Fallot with absent pulmonary valve (bottom panel) at the right hilum. Note the huge right pulmonary artery compressing the bronchi in the bottom panel, in contrast to the small right pulmonary artery and widely open bronchi in a control fetus in the top panel. Ao, aorta; B, bronchus; E, esophagus; HAV, hemizygous vein; L SVC, left superior vena cava; PV, pulmonary vein; RA, right atrium; RPA, right pulmonary artery; SVC, superior vena cava.
Ventricular Dilatation

Ventricular dilatation associated with this complex indicates diastolic load due to massive regurgitation from the pulmonary artery in fetal rats. Ventricular function in these fetal rats was assumed to be fairly well maintained because the pericardial fluid was not increased and the right atrial volume was only minimally increased. This probably reflects a large compensatory potential of the fetal heart, and the increase in ventricular mass supports this speculation.

Acknowledgment

The editorial help of Miss Barbara Levene is highly appreciated.

Table 1. Morphometric Study of Fetal Rats With Tetralogy of Fallot and Absent Pulmonary Valve

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Mild (PVR &gt; 50%)</th>
<th>Severe (PVR &lt; 50%)</th>
<th>Control (absolute values)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Body weight</td>
<td>92 ± 2 (27)*</td>
<td>79 ± 4 (14)*</td>
<td>5.3 ± 0.2 g (17)</td>
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<tr>
<td>Pericardial fluid</td>
<td>116 ± 10 (27)</td>
<td>94 ± 6 (13)</td>
<td>57 ± 5 μm (16)</td>
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<td>Thymus mass</td>
<td>87 ± 10 (17)</td>
<td>47 ± 8 (12)*</td>
<td>3.8 ± 0.4 mm³ (23)</td>
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<td>Vascular diameters</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Pulmonary valve ring</td>
<td>61 ± 2 (27)*</td>
<td>35 ± 2 (14)*</td>
<td>1030 ± 30 μm (15)</td>
</tr>
<tr>
<td>Main pulmonary artery</td>
<td>112 ± 3 (27)*</td>
<td>61 ± 6 (14)*</td>
<td>830 ± 14 μm (31)</td>
</tr>
<tr>
<td>Right pulmonary artery</td>
<td>256 ± 12 (25)*</td>
<td>151 ± 7 (13)*</td>
<td>430 ± 30 μm (33)</td>
</tr>
<tr>
<td>Left pulmonary artery</td>
<td>237 ± 11 (27)*</td>
<td>134 ± 11 (13)*</td>
<td>350 ± 20 μm (33)</td>
</tr>
<tr>
<td>Chamber volumes</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Right ventricle</td>
<td>196 ± 11 (17)*</td>
<td>106 ± 13 (12)</td>
<td>5.3 ± 0.6 mm³ (29)</td>
</tr>
<tr>
<td>Left ventricle</td>
<td>202 ± 15 (17)*</td>
<td>93 ± 12 (12)</td>
<td>4.1 ± 0.4 mm³ (29)</td>
</tr>
<tr>
<td>Right atrium</td>
<td>116 ± 5 (17)*</td>
<td>104 ± 5 (12)</td>
<td>28.1 ± 1.0 mm³ (26)</td>
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<tr>
<td>Left atrium</td>
<td>67 ± 4 (17)*</td>
<td>59 ± 4 (12)*</td>
<td>13.6 ± 0.4 mm³ (28)</td>
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<tr>
<td>Ventricular mass</td>
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<td></td>
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<tr>
<td>Right ventricle</td>
<td>136 ± 4 (17)*</td>
<td>105 ± 5 (12)</td>
<td>16.3 ± 0.4 mm³ (28)</td>
</tr>
<tr>
<td>Left ventricle</td>
<td>119 ± 4 (17)*</td>
<td>85 ± 3 (12)*</td>
<td>12.0 ± 0.2 mm³ (29)</td>
</tr>
</tbody>
</table>

Mean ± SEM in percent vs. control value. Number of fetuses are shown in parentheses.
PVR, pulmonary valve ring.
*Statistically significant values vs. control.
References


KEY WORDS • bisdiamine • teratogen • cardiac anomaly • bronchial anomaly • tetralogy of Fallot
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