High Rate of Ventricular Septal Defects in WKY Rats

Michel Slama, Dinko Susic, Jasmina Varagic, Edward D. Frohlich

Abstract—In 1979, we first reported occurrence of biventricular hypertrophy in the original normotensive Wistar-Kyoto (WKY) strain obtained from the National Heart, Lung, and Blood Institute, which was derived directly from the Kyoto laboratory of Okamoto. At that time, we recommended that both ventricles be weighted when WKY are studied so that invalid conclusions are not made. Because no paper confirmed these findings for almost 20 years, heart weights were reported in only a few WKY studies, and the cause of this biventricular hypertrophy remained unknown, we re-evaluated this problem in commercially available rats. We, therefore, investigated WKY rats using transthoracic echocardiography to define the congenital heart defect. Up to 28% of commercially available WKY rats demonstrated severe congenital cardiac abnormalities associated with biventricular hypertrophy. Ventricular septal defect with pulmonary regurgitation was the most commonly encountered cardiac defect; other abnormalities included patent ductus arteriosus, and valvular defects. Pathologic and invasive hemodynamic studies confirmed these echocardiographic findings. Because this defect occurs in a large number of WKY rats obtained commercially from 2 different sources, investigators using this strain must carefully measure both ventricular weights to be certain that inappropriate and invalid conclusions are not derived therefrom. (Hypertension. 2002;40:175-178.)

Key Words: rats, inbred WKY ■ hypertrophy ■ ventricular function ■ hypertension, essential ■ echocardiography

In studies of inbred genetic animal models of human disease, development of an appropriate comparator control group remains a most important consideration. The normotensive Wistar-strain rat from Kyoto (WKY), which was developed by Okamoto and Aoki in 19631 and is presently available commercially, represents the most appropriate normotensive counterpart to the spontaneously hypertensive rat (SHR). Almost 25 years ago, a hemodynamic and cardiac functional abnormality was detected in a sizable component of the normotensive WKY rats directly obtained from the National Heart Institute. Our studies suggested that it was most likely the result of a left to right cardiopulmonary shunt.2 No confirmatory reports emerged, apparently no efforts were made to exclude this defect in other studies using WKY, and the cause of this shunt remained unknown. Unless this problem is investigated by measuring ventricular weights in each study in which the WKY is employed, erroneous conclusions may be derived, not only from the WKY, but also from the SHR. Because no further attention has been focused on this problem, we reinvestigated it using transthoracic echocardiographic and Doppler techniques (TTE), not only to define the cardiac disease, but also to elaborate on the associated hemodynamic changes.3–6

Methods

Rats

Twenty WKY rats of both genders were obtained from Charles River Breeding Laboratories Inc (Wilmington, Mass), as well as additional 20 male WKY rats from Harlan Laboratories (Indianapolis, Ind). The rats were 7 to 12 weeks of age at the time of purchase. In addition, we studied 8 one-year-old WKY already present in our laboratory, which were purchased from Charles River. All rats were housed in plastic cages and allowed free access to food (PMI Nutrition International) and tap water. The animal facility was temperature- and humidity-controlled with a 12-hour light-dark cycle. All rats were handled in strict accordance with National Institutes of Health guidelines.

Echocardiographic Examination

In all rats, after intraperitoneal injection of pentobarbital (50 mg/kg), we performed TTE examination in the left lateral decubitus position using a commercially available echocardiographic system (Sonos® 4500 with an 8 to 12 MHz transducer, Agilent Technologies) to detect any anatomic cardiac defects. In brief, the TTE probe was placed to obtain short axis, long axis, and 4- and 5-chamber apical cardiac views. Two-dimensional (2D)-guided M-mode pulsed, continuous, and color Doppler images were obtained to define any cardiac abnormality.6 The left and right ventricular cavities and wall thicknesses were measured, and left ventricular mass was calculated in the 20 male rats obtained from Charles River.3,4 The relative wall thickness of the left ventricle (%) was calculated as 2×PW/LVEDD, where PW indicates the left ventricular posterior wall and LVEDD, the left ventricular end-diastolic diameter. From the 4-chamber apical view, right and left ventricular maximal diameter (RVDDa, LVDDa) and area (RVA, LVA) in diastole were measured. Cardiac output was calculated using the velocity time integral of aortic and pulmonary flows.6 In those rats with ventricular septal defect (VSD), maximal velocity of the shunt flow was recorded and systolic pulmonary pressure was calculated.7 Maximal and minimal velocity of pulmonary pressure and maximal velocity of associated tricuspid regurgitation was recorded using continuous wave Doppler. Then the pulmonary systolic, diastolic, and mean pressures were calculated.5,9

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From the Hypertension Research Laboratories, Ochsner Clinic Foundation, New Orleans, La.
Correspondence to Michel Slama, Research Division, Ochsner Clinic Foundation, New Orleans, LA 70121. E-mail MSlama0508@aol.com
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175
Contrast echocardiography, using a mixed 0.6 mL of saline solution and 0.1 mL of air, was performed to confirm intracardiac shunting when a high-velocity signal was detected within the right ventricle.

Invasive Hemodynamic Examination
In 7 rats with echocardiographically demonstrated VSD, we measured the fraction of left ventricular output returning to the lungs and compared these findings with those data obtained from 10 WKY without cardiac defects. After intraperitoneal injection of pentobarbital (50 mg/kg), the right carotid was cannulated, and the catheter (PE-50), connected to a pressure transducer (P23 dB, Statham Instrument), was advanced into the left ventricle in a retrograde fashion. Arterial pressure was recorded using a catheter (P-50) placed into the descending aorta throughout the left femoral artery. After a 15-minute stabilization period, pressures were recorded at a paper speed of 100 mm/s using a multichannel physiograph (R612, Sensor Medics). The right jugular vein was cannulated with P-50 catheter through a preshaped P-100 catheter and advanced into the right ventricle to measure the right ventricular pressures. Finally, approximately 100 000 radiolabeled (113 Sn) microspheres (15±1 μm in diameter, Dupont), suspended in 0.045 mL saline containing Tween 80 (<0.01%), were injected into the left ventricle, followed by a warm saline (0.5 mL) flush. The reference blood sample was withdrawn from the femoral artery catheter using a Harvard infusion/withdrawal pump (Harvard Apparatus) at a rate of 0.45 mL/min over 60 seconds, starting 20 seconds before microsphere injection. This formula was used to calculate cardiac output:

\[
\text{Cardiac output (mL/min)} = \frac{n \times \text{injected radioactivity (counts/min)}}{\text{reference sample radioactivity (counts/min)}/\text{sampling rate (mL/min)}}
\]

The cardiac index was calculated from cardiac output and body weight and expressed in mL/min per kilogram. At the end of each study, each rat was killed with an overdose of pentobarbital. Immediately thereafter, the lungs were removed and their radioactivity was measured in a deep gamma well-type scintillation counter. Immediately thereafter, the lungs were removed and their radioactivity was measured in a deep gamma well-type scintillation counter. The right and left ventricles were weighted (to the nearest milligram) and expressed in terms of body weight (milligrams of ventricle weight per gram of body weight).

Pathologic Study
After the heart was removed, the atria were dissected free from the ventricles and discarded, and the free wall of the right ventricle was separated carefully from the left ventricle. The heart was carefully examined for the presence of a VSD, patent ductus arteriosus, and any other defect. The right and left ventricles were weighted (to the nearest milligram) and expressed in terms of body weight (milligrams of ventricle weight per gram of body weight).

Statistical Analysis
All data are presented as mean±standard error. A Mann-Whitney nonparametric analysis was performed to compare normal and abnormal rats. A probability value of 5% was considered to be statistically significant.

Results
In the WKY rats examined, 28% had cardiac lesion (15% to 40%). In 15 WKY, echocardiographic study demonstrated biventricular hypertrophy related to a VSD, which is usually associated with pulmonary regurgitation (Figures 1 through 3). In 9 of these 15 rats, the lesions were isolated, but 6 were associated with other abnormalities (Table 1). In 7 rats with VSD, fractional distribution of blood flow to the lungs was 46±14% (range 4.8% to 90%) compared with 1±0.01% in normal rats. Right ventricular systolic pressure was measured in 3 rats and found to be increased (35, 63, and 83 mm Hg).

The echocardiographic findings of 20 12-week-old male WKY rats are presented in the Table 2. The VSD was in a perimembranous position in 6 rats and in a muscular position in 2. Severe pulmonary regurgitation was detected in all rats with VSD. The right ventricle was hypertrophied and dilated, and pulmonary arterial pressures were high. Right ventricle output was significantly greater than the left in the VSD rats (due to both VSD and pulmonary regurgitation) (Table 3). Right, but not left, ventricular weight was greater in rats with septal defect than in normal rats (Table 4). On pathological examination, we confirmed a VSD in 7 WKY.

Discussion
In this study, severe congenital cardiac defect was demonstrated in 28% of commercially available normotensive WKY rats, which explains a biventricular hypertrophy that was described approximately 25 years ago.2 Using complete echocardiographic and Doppler examination, the biventricular hypertrophy was shown to result from a VSD associated

![Figure 1. Visualization of the ventricular septal defect (between arrows) using color Doppler. LV indicates left ventricle; RV, right ventricle.](image)

![Figure 2. Visualization of the ventricular septal defect using continuous-wave Doppler. Vmax indicates the maximum velocity of the flow through the ventricular septal defect.](image)
with pulmonary regurgitation and pulmonary hypertension, and has not been reported in this strain of WHY. The present report appears to differ in several respects from our first report describing cardiac functional abnormalities. In the initial report using electromagnetic flowmetry, cardiac output was found to be increased, with a left-to-right shunt demonstrated using microspheres. Therefore, the postulated shunt must have been distal the ascending aorta and patent ductus arteriosus; AR, aortic regurgitation.

The present findings in normotensive WKY rats present a serious problem for careful investigators. Thus, this study techniques have been used to analyze cardiac structure in rats: De Simone et al. and Pawlush et al. have demonstrated a strong correlation between echocardiographic measurement of left ventricular mass and pathologic measurement. More recently, these techniques were used to assess cardiac function accurately and noninvasively. Very recently, Ono et al. demonstrated a good correlation between endocardial fractional shortening and LV peak +dP/dt, and cardiac output measurement was validated against the thermodilution technique. Derumeaux et al. and Dent et al. used and validated the Doppler technique for the assessment of left ventricular diastolic function.

**Perspectives**

The present findings in normotensive WKY rats present a serious problem for careful investigators. Thus, this study

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### Table 1. Echocardiographic Findings

<table>
<thead>
<tr>
<th>Company</th>
<th>n</th>
<th>Gender</th>
<th>Age (wk)</th>
<th>Normal Rats</th>
<th>VSD and PR</th>
<th>VSD, PR, and Other Defects</th>
<th>Other Defects</th>
<th>Abnormal Rats n (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Charles River</td>
<td>20</td>
<td>M</td>
<td>12</td>
<td>12</td>
<td>5</td>
<td>3</td>
<td>0</td>
<td>8 (40)</td>
</tr>
<tr>
<td>Charles River</td>
<td>20</td>
<td>F</td>
<td>12</td>
<td>15</td>
<td>2</td>
<td>1</td>
<td>2 (PS)</td>
<td>5 (25)</td>
</tr>
<tr>
<td>Harlan</td>
<td>20</td>
<td>M</td>
<td>12</td>
<td>17</td>
<td>2</td>
<td>0</td>
<td>1 (PDA)</td>
<td>3 (15)</td>
</tr>
<tr>
<td>Charles River</td>
<td>8</td>
<td>M</td>
<td>52</td>
<td>5</td>
<td>0</td>
<td>2</td>
<td>1 (AR)</td>
<td>3 (15)</td>
</tr>
<tr>
<td>Total</td>
<td>68</td>
<td></td>
<td></td>
<td>49 (72)</td>
<td>9 (12)</td>
<td>6 (9)</td>
<td>4 (7)</td>
<td>19 (28)</td>
</tr>
</tbody>
</table>

VSD indicates ventricular septal defect; PR, pulmonary regurgitation; PS, pulmonary stenosis; PDA, patent ductus arteriosus; AR, aortic regurgitation.
TABLE 3. Noninvasive Hemodynamics

<table>
<thead>
<tr>
<th></th>
<th>Normal WKY Rats</th>
<th>WKY Rats With VSD</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(n=12)</td>
<td>(n=8)</td>
</tr>
<tr>
<td>sPAPpr, mm Hg</td>
<td>46±8</td>
<td>NA</td>
</tr>
<tr>
<td>dPAPpr, mm Hg</td>
<td>14±0.8</td>
<td>NA</td>
</tr>
<tr>
<td>mPAPpr, mm Hg</td>
<td>25±3</td>
<td>NA</td>
</tr>
<tr>
<td>sPAPvsd, mm Hg</td>
<td>51±6</td>
<td>NA</td>
</tr>
<tr>
<td>Pulmonary CI, mL/min/kg</td>
<td>288±27</td>
<td>734±120*</td>
</tr>
<tr>
<td>Aortic CI, mL/min/kg</td>
<td>312±14</td>
<td>312±14</td>
</tr>
<tr>
<td>Pulmonary CI/Aortic CI</td>
<td>1±0.25</td>
<td>1.78±1.3*</td>
</tr>
</tbody>
</table>

Ci indicates cardiac index; VTI, velocity time integral; sPAPpr, systolic pulmonary arterial pressure on pulmonary regurgitation; dPAPpr, diastolic pulmonary arterial pressure on pulmonary regurgitation; mPAPpr, mean pulmonary arterial pressure on pulmonary regurgitation; sPAPvsd, systolic pulmonary arterial pressure on VSD. *P<0.05.

presents another important cautionary note to investigators when using WKY. We strongly suggest that investigations conducted with WKY should not be reported without at least weighing both ventricles after each study.15 Moreover, a study of WKY rats using echocardiography would be necessary to breed normal rats free of the congenital abnormalities described here. Alternatively, we also suggest that rats with VSD may be useful for those workers interested in congenital cardiac lesions.

In conclusion, using echocardiographic techniques, we demonstrated VSD and other congenital heart defects responsible for biventricular hypertrophy in 28% of commercially obtained normotensive WKY rats. These findings suggest the necessity for evaluating all WKY rats after each study to exclude related physiological, biochemical, or other inadequate conclusions.

TABLE 4. Weight of Hearts

<table>
<thead>
<tr>
<th></th>
<th>Normal WKY Rats</th>
<th>Abnormal WKY Rats</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(n=12)</td>
<td>(n=8)</td>
</tr>
<tr>
<td>Right ventricle, mg/g</td>
<td>0.63±0.03</td>
<td>1.77±0.11*</td>
</tr>
<tr>
<td>Left ventricle, mg/g</td>
<td>2.37±0.02</td>
<td>2.56±0.13</td>
</tr>
<tr>
<td>Biventricular weight, mg/g</td>
<td>3.00±0.04</td>
<td>4.33±0.26*</td>
</tr>
</tbody>
</table>

*P<0.05.

Acknowledgment

We give very special thanks to Agilent Technologies (Philips Medical Systems), which provided the echocardiographic machine.

References

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