Right Ventricular Concentric Hypertrophy and Left Ventricular Dilatation by Ductal Constriction in Fetal Rats

Kazuo Momma and Atsuyoshi Takao

Fetal cardiac changes due to ductal constriction by maternal ingestion of nonsteroidal anti-inflammatory drugs were studied morphologically in near-term rats as an animal model, and results were compared with values of control 1 (C1, twenty-first day) and control 2 (C2, twenty-second day). The fetal ductus was constricted (−70%) (p<0.05) by maternal administration of 10 mg/kg indomethacin. Dilatation of the right ventricle and evidence of congestive heart failure including increased pericardial effusion (+200%) (p<0.05) and an increase in water content in the abdominal wall were present at 1, 4, and 8 hours after drug administration. At 24 hours after drug administration, concentric right ventricular hypertrophy was shown by a diminished right ventricular cavity (−36% vs. C2) (p<0.05), increased right ventricular wall thickness (+70% vs. C2) (p<0.05), and increased right ventricular mass (+31% vs. C1) (p<0.05). Left ventricular dilatation was indicated by an increased cavity volume (+87% vs. C2) (p<0.05) and increased muscle mass (+29% vs. C1 [p<0.05] or +9% vs. C2 [p>0.05]). Both the wet and dry weights of the ventricles were increased. In conclusion, fetal ductal constriction caused right ventricular hypertrophy, diminished right ventricular cavity, and left ventricular dilatation and hypertrophy at 24 hours after drug administration in rats after initial congestive failure. (Circulation Research 1989;64:1137–1146)

It has been shown experimentally1–3 as well as clinically4–5 that nonsteroidal anti-inflammatory drugs such as aspirin and indomethacin constrict the fetal ductus arteriosus. Persistent pulmonary hypertension of the newborn may develop as a consequence of fetal ductal constriction.4–5 Very little is known about fetal cardiac changes caused by ductal constriction. This prompted us to study in situ morphology of the fetal heart and great vessels at the time of fetal ductal constriction in rats. Our second purpose in this study was to establish an animal model of the fetal right ventricular hypertrophy that develops in response to pressure overload.6

Materials and Methods

General Experimental Methods

Wistar rats were raised in separate cages and fed commercially obtained solid foods. Animals were mated overnight from 4 PM, and vaginal smears were checked at 9 AM the next day. Pregnancy day 0 was defined by the presence of sperm in the vaginal smear.

Three to 10 pregnant rats were used in each experimental group. Control studies were done on the twenty-first day (control 1, C1) and twenty-second day (control 2, C2) of pregnancy without administration of indomethacin. Pure indomethacin (Sumitomo Chemical, Osaka, Japan) was diluted 40 times with lactose. Indomethacin (10 mg/kg) was suspended in 2 ml water and administered through an orogastric tube to the pregnant rat on the twenty-first day. The fetuses were studied at 1, 4, 8, or 24 hours after administration of indomethacin; these fetuses were designated as 1-1, 1-4, 1-8, and 1-24, respectively. The ratio of the inner diameter of the fetal ductus to that of the main pulmonary artery (DA/PA=1.04±0.03 [mean±SEM] [n=24] in controls) diminished to 0.46±0.04 (n=22) at 1 hour, 0.33±0.05 (n=43) at 4 hours, 0.30±0.05 (n=16) at 8 hours, and 0.33±0.05 (n=16) at 24 hours, as reported previously.7,8 These diameters were measured on frontal sections. The morphology of the constricted ductus changed markedly, as reported previously,8 but the minimum diameters of the ductus did not change significantly from 1 hour to 24 hours. Preg-
niant animals were killed by cervical dislocation, and fetuses were delivered quickly by cesarean section.

The fetal heart was studied by three methods: 1) semimicroscopic morphological study, 2) volume and mass measurements, and 3) weight study (Table 1).

**Semimicroscopic Morphological Study**

A gross morphological study of the cross sections of the heart and great vessels was done as follows: Fetuses were fixed immediately after delivery by the whole-body freezing technique with acetone cooled to -80°C with dry ice. The frozen thorax of the fetus was trimmed and sectioned on a freezing microtome (Komatsu Solidate Co, Tokyo, Japan) in one of three planes. The sagittal plane, or right ventricular long axis plane, was defined as the plane parallel to the long axis of the right ventricle. The anterior and inferior right ventricular wall, the outflow tract of the right ventricle, the main pulmonary artery, the descending aorta, and the left superior vena cava were seen in this section (Figure 1). In fetal rats, this plane, as defined, was deviated to the left by about 20° from the anatomical sagittal plane. The four-chamber plane was defined as the plane that cut the midpoint of the interatrial and interventricular septa (Figure 2). In fetal rats, this plane had angles of about 20° to the right-to-left axis and about 45° to the long axis. The short axis plane was defined as the plane perpendicular to the ventricular septum; it was about 45° to the right-to-left axis and about 45° to the long axis in the fetal rat. Reproducible photographic recording of these planes was possible by meticulous placement of the frozen thorax on the freezing table of the microtome and photographing of multiple planes after serial parallel sections 30-μm thick were sliced. The cross sections were photographed with a binocular stereoscopic microscope (Wild M 400 Photomakroscope, Wild Heerbrugg Ltd, Heerbrugg, Switzerland) and Kodak color film (Kodachrome 40 film 5070 [Type A], Eastman Kodak Co, Rochester, New York). Numbered section paper (1x1 mm) was photographed for a scale.

Right ventricular size, the diameters of the inlet portion and the infundibulum, the thickness of the right ventricular wall, the breadth of pericardial effusion, and the inner diameter of the left superior vena cava and the descending aorta were measured on the photographs of the right ventricular sagittal section (Figure 1). Usually these right ventricular parameters, the diameter of the left superior vena cava, and the breadth of the pericardial space were measured on one section. However, maximum diameters of the main pulmonary artery, the ductus

![Figure 1. Sagittal cross section through right ventricular long axis, main pulmonary artery, ductus arteriosus, and descending aorta of control 1 fetal rat. Points of morphometries and widely patent ductus are shown. In this section, ductus was cut slightly left to true center and its diameter looks smaller than that of main pulmonary artery. A, anteroposterior length; B, supero-inferior length; C, inlet diameter; D, infundibular diameter; E, anterior wall thickness; F, inferior wall thickness; G, breadth of pericardial effusion; H, diameter of main pulmonary artery; I, diameter of ductus arteriosus; J, diameter of descending aorta; K, diameter of left superior vena cava.](http://circres.ahajournals.org/...
FIGURE 2. Four-chamber view cross sections of fetal rats. Compared with control 1 (A), fetus 24 hours after administration of indomethacin (B) shows thick-walled, small-cavity right ventricle and enlarged left ventricle. Atrial septum of fetal rat is thin and is hard to recognize on control 1 (A). A small amount of pericardial effusion is usually present in control fetal rats. DAo, descending aorta; LA, left atrium; ISVC, left superior vena cava; LV, left ventricle; PE, pericardial effusion; RA, right atrium; RV, right ventricle.

arteriosus, and the descending aorta were measured on several separate parallel sections of one fetus. The anterior wall thickness was measured at three levels—at one quarter, at the middle, and at three quarters of the supero-inferior length—and then averaged. The inferior wall thickness was also averaged from three values at the three points dividing it into four equal lengths. The amount of pericardial effusion was estimated from the breadth of the pericardial space adjacent to the corner of the cross section of the left superior vena cava (Figure 1).

Volume and Mass Measurements

Because the gross morphological study revealed initial biventricular dilatation at 1, 4, and 8 hours and subsequent concentric hypertrophy of the right ventricle at 24 hours after administration of indomethacin, ventricular volumes and masses were studied at 1, 4, 8, and 24 hours. Fetuses were delivered at 1, 4, 8, or 24 hours after administration of indomethacin and frozen immediately. The frozen chest was trimmed and sectioned in the cardiac short axis plane. Then, 0.5-mm-thick sections were cut serially from the cardiac apex to the cranial end of the atria. The cross sections of the heart and great vessels were photographed serially on Fuji color film (Fujicolor Super HR 100, Fuji Film Co, Tokyo, Japan). About 15 sections were recorded in the individual fetal heart. Numbered section paper (1x1 mm) was also photographed and was used for the scale. The pictures were printed in color on paper. The volumes and masses of each slice were added together from top to bottom of the individual heart to get these volumes and masses of ventricles and atria.

For the study of ventricular muscle mass, the septum was divided to the right and left ventricles in the same proportion as the right and left ventricular free-wall thicknesses (Figure 3). The pictures of the cavities of the four chambers and the ventricular masses were cut and weighed, and areas were calculated using the weight of the photograph of the section paper as a scale. The volumes and muscle mass were calculated by area times thickness (0.5 mm). The accuracy of this method has been tested and reported in another paper. Briefly, frozen ventricular muscle mass of 10 newborn rats was measured both with the method using photographs of serial cross sections (volume 1) and with the weight and specific gravity (volume 2). These two volumes are expected to be equal if these two methods are accurate. In our study, the ratio of volume 1 to volume 2 was 0.987±0.040 (mean±SD) (n=10).

Weight Study

A weight study was performed for documentation of the water content of tissues as an index of
FIGURE 3. Ventricular cross sections in short axis. Compared with cross section of control 2 (A), ventricle of fetal rat 24 hours after administration of indomethacin (B) shows right ventricle with thick wall and small cavity and enlarged left ventricle. Dotted lines show dividing line of ventricular septum into right and left parts. LV, left ventricle; RV, right ventricle.

congestive heart failure and of ventricular hypertrophy. The water content of the whole body was studied as follows: The delivered fetus was killed by intraperitoneal injection of 0.05 ml 5% solution of phenobarbital and weighed. The body was dried in a dryer at 120°C for 20 hours and was weighed for determination of the dry weight. The weights of the heart, liver, and abdominal wall were studied as follows: The newborn rat was weighed and killed by decapitation within 30 minutes after delivery, and the heart, liver, and abdominal wall were dissected with scissors and put in physiological saline solution. The cardiac ventricles were isolated from the atria and great vessels with scissors under the stereoscopic microscope. These tissues were kept in saline for prevention of drying until they were weighed. The heart and other tissues were measured with a microbalance (Mettler, Zurich, Switzerland), which was sensitive to 0.1 mg. Wet weight of these organs was measured after placement of the organ on a filter paper for a few seconds to absorb excessive saline. After they were weighed, the tissues were dried at 120°C for 20 hours in a dryer and were weighed again for determination of the dry weight. The dry-to-wet weight ratio was calculated as an index of the water content.

Data Analysis

All experimental results were presented as mean±SEM except the aforementioned results of the accuracy test on our new method. Serially measured values of each parameter were tested by analysis of variance, and the differences between the means were tested by Duncan's multiple range test. The acceptable level of significance was 5%. The values at 1, 4, and 8 hours after administration on the twenty-first day were compared with C1. The values at 24 hours after administration were compared with both C1 and C2.

Results

Semimicroscopic Morphology

In control fetuses, the long axis cross section of the right ventricle revealed a fetal channel that was composed of the infundibulum, the main pulmonary artery, the ductus arteriosus, and the descending aorta. These four components were approximately in the same plane and could be cut in a single cross section, as shown in Figure 4. At 1, 4, and 8 hours after administration of indomethacin, the right ventricle was dilated and pericardial and peritoneal fluids were increased (Figure 4C). At 24 hours after administration of indomethacin, the right ventricular cavity was diminished and its wall was thick (Figure 4D), although in some hearts the right ventricle was dilated and not hypertrophied. In the most extreme form, the infundibulum was almost completely closed, but the inflow portion was always patent. Serial measurement of the diameter
FIGURE 4. Sagittal cross sections of right ventricle. These four cross sections were recorded in the same magnification. In control heart (A), ductus widely patent, right ventricle is not dilated, and small amount of pericardial effusion is present. At 1 hour after administration of indomethacin (B), ductus is moderately constricted and right ventricle is dilated. At 8 hours after administration (C), ductus is severely constricted and pericardial, pleural, and peritoneal effusions are increased. At 24 hours (D), right ventricle has a thick pale wall and a small cavity. Ductus has a membranous constriction at distal end with a small central opening. DA, ductus arteriosus; DAo, descending aorta; LA, left atrium; ISVC, left superior vena cava; mPA, main pulmonary artery; PE, pericardial effusion; RV, right ventricle.

The results of the morphometry on these cross sections of the right ventricular long axis are shown in Figure 5. At 1 hour after administration of indomethacin, the right ventricle was dilated and the pericardial effusion was increased. At 24 hours after administration, the infundibular cavity had diminished and the right ventricular wall was thicker. The pericardial effusion showed less increase at 24 hours than at 8 hours.

The cross section of the four-chamber view in control fetal rats revealed approximately equal cavity sizes and wall thicknesses of both ventricles. At 24 hours after administration, the right ventricular cavity had diminished and its wall was thicker than before. The left ventricular cavity was enlarged, mainly in the sinus portion (Figure 2).

Mass and Volume
The results of the study on the right and left ventricular masses revealed an increase in both ventricular masses at 24 hours after administration of indomethacin. The ventricular masses and ventricular masses per body weight are shown in Table 2 and Figure 6, respectively. The increase in ven-
The right ventricular muscle mass increased at 24 hours by 31% vs. C1 (p<0.05) and by 10% vs. C2 (p>0.05). Left ventricular mass increased by 29% vs. C1 (p<0.05) and 9% vs. C2 (p>0.05).

The study of the cavity volumes revealed an initial increase and late diminishment of the right ventricular cavity and an increase of the left ventricular cavity, as shown in Table 2 and Figure 7. The decrease in the right ventricular volume at 24 hours was the most remarkable, and the difference between the volumes at 8 and 24 hours was highly significant. The right ventricular volume at 24 hours was smaller than C2 by 36% (p<0.05).

The volume-to-mass ratios were calculated. The right ventricular ratios increased at 4 and 8 hours after administration but decreased at 24 hours. In contrast, the left ventricular ratios increased at 8 and 24 hours.

The volumes of the right and left atria did not change except at 24 hours, when the right atrial volume decreased slightly and insignificantly.

Cardiac and Other Tissue Weight

The study of the wet and dry weights showed changes of body water content at 1, 4, 8, and 24 hours and increased wet and dry ventricular weights at 24 hours after administration of indomethacin (Figures 8 and 9). The dry-to-wet weight ratios of the whole body and three tissues showed a general tendency to decrease with development of edema, but there were some differences in the time course. The ratio was minimum at 8 hours in the whole body and the heart. In the liver and abdominal wall, the ratio was minimum at 24 hours.

Discussion

It is evident that nonsteroidal anti-inflammatory drugs such as aspirin and indomethacin constrict...
the fetal ductus arteriosus. However, the clinical sequelae are not clear, and only sporadic cases of persistent pulmonary hypertension of the newborn have been reported as sequelae. Hemodynamic changes in the fetal heart after ductal constriction are assumed as follows: Because the ductus is a large channel through which about 60% of the total cardiac output passes in the fetus, its severe constriction is assumed to cause pulmonary and right ventricular hypertension, decreased right ventricular output, rise in right atrial pressure, increased blood flow through the foramen ovale, and increased preload or volume load to the left ventricle. Although these hemodynamic parameters were not measured in this study, morphologic changes at 1, 4, and 8 hours after administration of indomethacin were compatible with these hemodynamic changes. In addition, our study revealed evidence of fetal hydrops such as increased pericardial effusion and increased water content of organs and tissues. This is probably evidence of fetal cardiac failure, although the effect of indomethacin on the fetal water content and transplacental water exchange are not known and, therefore, cannot be ruled out as the cause of increased water content in the fetus.

A diminished right ventricular cavity associated with hypertrophy at 24 hours after administration of indomethacin in this study is a unique feature in the fetal ventricular reaction to the pressure overload and decreased output. Contribution of tissue edema was ruled out by the results of the dry-weight study. In contrast, the apparent increase in right ventricular mass per body weight at 8 hours was associated with an increased water content and was presumed to be due to edema and not to hypertrophy. In the experiments with postnatal animals, a chronic increase in afterload caused only mild ventricular hypertrophy without diminution of the cavity.

The apparent increase in right ventricular volume at 4 and 8 hours after administration of indomethacin may be partly due to tricuspid regurgitation, which frequently accompanies right ventricular hypertension clinically. One possible explanation for the subsequent decrease in right ventricular volume at 24 hours may be the decrease in tricuspid regurgitation, because constricted ductal morphology changes from the hourglass type to the membranous type and ductal obstruction may be less severe at 24 hours.

### Table 2. Ventricular Masses and Volumes in Control Fetal Rats and After Administration of Indomethacin

<table>
<thead>
<tr>
<th>Group</th>
<th>Number of animals</th>
<th>Body weight (gm)</th>
<th>RV mass (mm³)</th>
<th>LV mass (mm³)</th>
<th>RV volume (mm³)</th>
<th>LV volume (mm³)</th>
</tr>
</thead>
<tbody>
<tr>
<td>C1</td>
<td>14</td>
<td>5.4±0.1</td>
<td>15.6±0.5</td>
<td>12.6±0.4</td>
<td>3.7±0.5</td>
<td>3.0±0.3</td>
</tr>
<tr>
<td>C2</td>
<td>15</td>
<td>5.6±0.5</td>
<td>18.5±1.0</td>
<td>14.9±1.0</td>
<td>4.8±0.6</td>
<td>3.2±0.5</td>
</tr>
<tr>
<td>1-1</td>
<td>23</td>
<td>5.3±0.1</td>
<td>14.6±0.5</td>
<td>11.9±0.4</td>
<td>4.2±0.5</td>
<td>3.2±0.4</td>
</tr>
<tr>
<td>1-4</td>
<td>15</td>
<td>5.6±0.1</td>
<td>16.8±0.5</td>
<td>13.6±0.7</td>
<td>5.9±0.7</td>
<td>3.9±0.4</td>
</tr>
<tr>
<td>1-8</td>
<td>12</td>
<td>5.1±0.2</td>
<td>17.9±0.5</td>
<td>14.3±0.5</td>
<td>6.4±0.8*</td>
<td>5.5±0.6*</td>
</tr>
<tr>
<td>1-24</td>
<td>21</td>
<td>5.5±0.2</td>
<td>20.4±0.7*</td>
<td>16.2±0.6*</td>
<td>3.1±0.4t</td>
<td>6.2±0.8t</td>
</tr>
</tbody>
</table>

C, control; I, indomethacin; LV, left ventricle; RV, right ventricle.

*p<0.05 vs. C1. tp<0.05 vs. C2. Values are mean±SEM.

### Figure 6. Ventricular masses calculated from serial sections in short axis divided by body weight. Both right and left ventricular masses increased progressively, and those of fetal hearts 24 hours after administration of indomethacin were significantly larger than either control 1 or control 2. LV, left ventricle; RV, right ventricle.
Right ventricular hypertrophy and diminished cavity are interesting from the clinical as well as the academic point of view. Clinically, such a right ventricle is supposed to have decreased diastolic compliance and decreased volume after birth and is supposed to promote the right-to-left shunt through the foramen ovale, which is the main hemodynamic feature of persistent pulmonary hypertension of the newborn.

Right ventricular hypertrophy and diminished cavity observed in this study are interesting because this combination is present in some congenital heart diseases such as isolated atresia or stenosis of the pulmonary valve.\(^{13}\) In these congenital heart diseases, stenosis or atresia of the pulmonary valve is assumed to occur at some stage of fetal life after the completion of cardiac morphogenesis,\(^{14}\) and diminished cavity is assumed to be the consequence of decreased output due to excessive afterload to the right ventricle. This assumption about the morphogenesis of diminished right ventricle is supported by the present study; although the levels of the obstruction in the right ventricular outflow tract are different, there is no ejection in pulmonary atresia with intact ventricular septum, and changes are acute in the present animal model.

Several earlier studies have been reported on the experimental pulmonary artery banding and consequent right ventricular hypertrophy in fetal lambs.\(^{15-18}\) Massive right ventricular hypertrophy was observed at 40-50 days after pulmonary arterial banding in fetal lambs in these experiments.\(^{15-18}\) In addition to the right ventricular hypertrophy, increased weight of the left ventricle was con-
Diminished right ventricular cavity was reported in a preliminary study. Our present study supported the results of these early studies, showed the presence of the early phase of congestive heart failure, and showed the time course of the development of these cardiac changes in response to ductal constriction in the fetal rat.

The present study showed that the ventricular hypertrophy in the rat fetus developed within 24 hours. Such a rapid development of hypertrophy is not surprising because development of the fetal rat is very rapid. The cardiac morphogenesis is completed on the fourteenth day of gestation in the rat. The gestational period of the rat is 21-22 days. The fetus develops in the last 7 or 8 days, and the fetal body weight doubles in 2 days, from the nineteenth to the twenty-first day. Therefore, rapid growth of the whole body and the heart in the fetal rat is presumably the basis of the rapid development of the right ventricular hypertrophy observed in this study.

Our study showed that fetal ductal constriction by maternally administered nonsteroidal anti-inflammatory drugs and subsequent right ventricular hypertrophy are unique animal models of fetal cardiac-pressure overload and cardiac hypertrophy. This animal model does not need surgical intervention of the fetus and is easy to produce. Excessive ventricular hypertrophy, such as that associated with congenital pulmonary stenosis or pulmonary atresia, is difficult to produce in postnatal animal models but may be produced in fetal rats, as shown in this study. Generally, for the better understanding of adaptive cardiac hypertrophy in congenital heart diseases, the study of animal models produced by fetal cardiac manipulation is mandatory. The present study shows that such an animal model may be easily produced.

Definite evidence of cardiac failure due to fetal ductal constriction in near-term rats was also shown in our study. Clinically, only persistent fetal circulation or persistent pulmonary hypertension of the newborn has been documented as a consequence of maternal ingestion of anti-inflammatory drugs in the last trimester of pregnancy. Two mechanisms may explain the apparent lack of fetal hydrops in the clinical situation. The first mechanism is compensation by the left ventricle. Because of the presence of the widely open foramen ovale in the fetus, the decrease in right ventricular output is associated with increased flow through the foramen ovale to the left atrium and left ventricle, and left ventricular output increases as a compensation. However, this compensation is probably not sufficient because of incomplete development of cardiac reserve in the fetus.

The second mechanism is based on our recent study on preterm fetal rats, which showed that constriction of the fetal ductus due to maternal ingestion of indomethacin or flurbiprofen was transient and disappears at 24 hours, although the plasma concentration of indomethacin is still sufficiently high. Therefore, the ductus of the fetal rat is open in the presence of indomethacin after initial constriction by the drug. If this phenomenon is present in the human fetus, the ductal constriction and its cardiac effects may be transient even if the anti-inflammatory drug is ingested by the mother for a long period and evidence of fetal ductal constriction may be absent at birth.

In conclusion, fetal ductal constriction caused right ventricular hypertrophy, diminished ventricular cavity, and left ventricular dilatation at 24 hours after administration of indomethacin in rats. At the same time, fetal hydrops was evident. These results underscore the risks of fetal ductal constriction due to maternal ingestion of anti-inflammatory drugs.
This is a unique animal model of congenital heart disease of right ventricular pressure overload.

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References


KEY WORDS • fetal circulation • ductus arteriosus • ventricular hypertrophy • indomethacin
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