Induction of Right Ventricular Hypertrophy in Neonatal Guinea Pigs by Monocrotaline

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The purpose of this study was to develop an experimental model of neonatal right ventricular hypertrophy which was similar to human congenital heart disease associated with pulmonary hypertension. Monocrotaline (200 mg/kg), a pyrrolizidine alkaloid, was injected into neonatal Hartley guinea pigs on the day of delivery. The occurrence of pulmonary hypertension and right ventricular hypertrophy was confirmed by pressure studies and a determination of the right ventricular wet weight and myocyte diameter on the seventh day after delivery. Right ventricular systolic pressure was significantly increased at 7 days after monocrotaline treatment compared with the untreated control group. The ratio of right ventricular systolic pressure to left ventricular systolic pressure, an indicator of pulmonary hypertension, was significantly elevated from 0.32±0.02 in the controls to 0.59±0.03 in the monocrotaline group. Right ventricular wet weight was also significantly increased, indicating right ventricular hypertrophy. The diameter of cardiac myocytes was significantly increased in the right ventricle, and was decreased in the left ventricle and interventricular septum in the monocrotaline group. Neonatal guinea pigs developed pulmonary hypertension and marked right ventricular hypertrophy within 1 week after treatment with monocrotaline. This simple experimental model may have features similar to those of human congenital heart disease associated with pulmonary hypertension.

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Since the number of infants with complex cardiac anomalies requiring corrective surgery is increasing, myocardial protection in the neonatal heart has become an important issue. However, the effectiveness of cardioplegic solution during open-heart surgery in neonates remains controversial. We previously found that crystalloid cardioplegic solution is less effective in the neonatal heart than in the adult heart in guinea pigs, and that oxygen radicals are one cause of this inadequate protective effect.1—3 Since our experiments used normal hearts, one problem was the potential difference between healthy animals and patients with congenital heart disease. Congenital heart disease is often accompanied by various abnormalities of pulmonary circulation, eg, increased pulmonary blood flow in ventricular septal defect with pulmonary hypertension and decreased blood flow in tetralogy of Fallot. The purpose of this study was to use monocrotaline, a pyrrolizidine alkaloid, to develop an experimental model of neonatal pulmonary hypertension that resembled human congenital heart disease associated

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with pulmonary hypertension. It is well known that a single dose of monocrotaline produces pulmonary hypertension after approximately 4 weeks.\(^6\)\(^7\) However, this lapse of 4 weeks allows neonatal animals to become mature. Therefore, we applied higher doses of monocrotaline to induce pulmonary hypertension within 1 week.

**MATERIALS AND METHODS**

Experiments were conducted on neonatal Hartley guinea pigs. All animals received humane care in compliance with the “Guide for the Care and Use of Laboratory Animals” prepared by the Institute of Laboratory Animal Resources and published by the US National Institutes of Health (NIH publication No. 85-23, revised 1985), and the “Guide for the Animal Experimentation in Niigata University” formulated by Niigata University. Neonatal guinea pigs were divided into a control group (n=20) and a monocrotaline-treated group (n=20). Monocrotaline (Sigma Chemical Company, St Louis, USA) was dissolved in saline at a concentration of 20 mg/ml. On the day of delivery, all of the neonatal guinea pigs were weighed and 10 ml/kg of saline was subcutaneously injected into the backs of the control group, while 200 mg/kg of monocrotaline was injected into the same site in the monocrotaline group. On the seventh day after delivery, the animals were weighed again and anesthetized with pentobarbital sodium. Median sternotomy was then rapidly performed and the chest was opened. The systolic pressure of the right ventricle (RVSP) and that of the left ventricle (LVSP) were measured with catheters placed at the right ventricular outflow tract and the left ventricular apex, respectively. The ratio of RVSP to LVSP (RVSP/LVSP ratio) was calculated as a parameter of pulmonary hypertension. After the various measurements were obtained, the animals were sacrificed with an overdose of pentobarbital sodium. The animals in each group were divided into 2 sub-groups (n=10 in each); in one group the dry and wet weights of the heart were measured, and the other group was used for microscopic examination. In the subgroup used to measure the dry and wet weights of the heart, the atria were removed from the heart, and the remaining tissue was divided into the left ventricle plus the interventricular septum (LV+S) and the right ventricle (RV) according to the method of Fulton et al.\(^8\) After clots and debris were removed, the wet weight was measured, and the dry weight was measured after 24 h in a drying oven. In the subgroup used for microscopic examination, the hearts were excised and fixed in 10% neutral formaldehyde for 48 h. The ventricles were then divided into 2 parts as mentioned previously. The wet weight of these ventricular portions was also measured. Specimens of myocardium were embedded in paraffin and stained with hematoxylin and eosin for microscopy. Myocardial specimens were obtained in both the longitudinal and transverse directions with respect to muscle fiber orientation, and muscle fiber diameters were measured in photographs of the cross-sectional specimens using a pen digitizer computer system (Oscan, Ohsawa Company, Tokyo, Japan). The diameter was defined as the maximal span of a cross-section of the muscle fiber perpendicular to its long axis. The diameters of 200 muscle fibers were measured sequentially, a histogram of fiber diameter was constructed, and the median value was calculated for fibers containing nuclei.

All data except cardiac myocyte diameters are expressed as the mean±standard error, while myocyte diameters are expressed as the median±standard error. Statistical analysis was performed using Student's t-test and differences were judged to be significant at p<0.05.

**RESULTS**

The body weight on the day of delivery and on the seventh day of life, as well as the weight gain during this 1-week period are indicated in Table I. Although there was no significant difference in body weight between the 2 groups, the monocrotaline group showed significantly less weight gain than the control group.

**LVSP, RVSP, and RVSP/LVSP Ratio**

The data on LVSP, RVSP, and the RVSP/LVSP ratio are also shown in Table I. No significant difference in LVSP was observed between the 2 groups. However,
TABLE I BODY WEIGHT AND PRESSURE DATA

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>Monocrotaline</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Body weight (g)</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Day of delivery</td>
<td>96.6±4.1</td>
<td>102.3±4.5</td>
</tr>
<tr>
<td>7th. day</td>
<td>125.2±4.2</td>
<td>120.0±5.0</td>
</tr>
<tr>
<td><strong>Weight gain (g)</strong></td>
<td>28.6±1.6</td>
<td>18.3±1.3*</td>
</tr>
<tr>
<td>LVSP (mmHg)</td>
<td>62.6±1.5</td>
<td>65.1±2.3</td>
</tr>
<tr>
<td>RVSP (mmHg)</td>
<td>19.7±0.9</td>
<td>38.1±2.0***</td>
</tr>
<tr>
<td>RVSP/LVSP</td>
<td>0.32±0.02</td>
<td>0.59±0.03***</td>
</tr>
</tbody>
</table>

LVSP: left ventricular systolic pressure.
RVSP: right ventricular systolic pressure.
* ** ***: significant difference from the control group (p<0.05, 0.001, respectively).

TABLE II VENTRICULAR WEIGTHS

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>Monocrotaline</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>LV+S (g)</strong></td>
<td>0.2956±0.0153</td>
<td>0.2675±0.0140</td>
</tr>
<tr>
<td>RV (g)</td>
<td>0.1019±0.0068</td>
<td>0.1244±0.0069*</td>
</tr>
<tr>
<td>RV/LV+S</td>
<td>0.3452±0.0170</td>
<td>0.4733±0.0206***</td>
</tr>
<tr>
<td>(LV+S)/BW (×10^-3)</td>
<td>2.32±0.08</td>
<td>2.27±0.06</td>
</tr>
<tr>
<td>RV/BW (×10^-3)</td>
<td>0.81±0.04</td>
<td>1.04±0.04**</td>
</tr>
<tr>
<td>Dry wet ratio (LV+S)</td>
<td>0.220±0.001</td>
<td>0.217±0.002</td>
</tr>
<tr>
<td>Dry wet ratio (RV)</td>
<td>0.218±0.003</td>
<td>0.212±0.002</td>
</tr>
</tbody>
</table>

LV+S: left ventricle plus the interventricular septum.
RV: right ventricle.
BW: body weight.
* ** ***: significant difference from the control group (p<0.05, 0.01, 0.001, respectively).

RVSP and the RVSP/LVSP ratio were both significantly higher in the monocrotaline group indicating the development of pulmonary hypertension. The highest RVSP was 42 mmHg and the corresponding RVSP/LVSP ratio was 0.67.

Ventricular Wet Weights

The wet weight of the right ventricle (RV) and the left ventricle plus the interventricular septum (LV+S) as well as the ratio of these 2 parameters (RV/(LV+S) ratio) are shown in Table II. Monocrotaline treatment increased the right ventricular wet weight by 22% above the control weight, and the RV/body weight ratio was also significantly higher in the monocrotaline group. In addition, monocrotaline decreased the LV+S weight and the LV/body weight ratio, but these changes were not significant. There were no differences in the dry/wet ratio between the 2 groups.

Myocyte Diameter

As shown in Fig 1, the median LV+S myocyte diameter in the control group (6.84±0.19 µm) was significantly greater than that in the monocrotaline group (6.10±0.13 µm). In contrast, the right ventricular myocyte diameter in the monocrotaline group was significantly greater than that in the control group (6.39±0.33 µm vs 4.85±0.14 µm).

DISCUSSION

The present study showed that treatment of guinea pigs with monocrotaline on the day of delivery resulted in an increase in RVSP and the RVSP/LVSP ratio. The right ventricular wet weight also increased, while that of the left ventricle plus the interventricular septum decreased. These changes in ventricular wet weight resulted from changes in cardiac myocytes. Although we did not measure pulmonary artery pressure directly in this study, other authors have reported that RVSP closely reflects pulmonary artery pressure. Thus, monocrotaline treatment caused pulmonary hypertension, and induced right ventricular hypertrophy within
1 week. The elevated RVSP in the monocrotaline group was associated with right ventricular hypertrophy, as shown by the significantly increased right ventricular wet weight and RV/(LV+S) ratio. Congenital heart disease associated with pulmonary hypertension exhibits cardiac anomaly, right ventricular hypertrophy, and pulmonary hypertension. Monocrotaline-treated animals showed no cardiac anomaly, and the morphological changes in the pulmonary artery were slightly less than those in congenital heart disease associated with pulmonary hypertension in humans. However, the resulting pathological state of the heart, ie, right ventricular hypertrophy and increased pulmonary resistance, seemed to be similar to that of congenital heart disease associated with pulmonary hypertension.

Right ventricular hypertrophy has 2 main histological component: hypertrophy of cardiac myocytes and changes in the interstitial space such as edema or fibrosis. To determine the main factor involved in ventricular hypertrophy, some authors have examined the dry/wet ratio of cardiac muscle. Ghodsi and Will reported that monocrotaline increased the right ventricular dry weight and that the increase in ventricular mass was not attributable to an increase in water. We cannot draw a definite conclusion, since the condition of the interstitial space in right ventricular hypertrophy was not determined in our study. However, a significant increase in the diameter of right ventricular myocytes was noted.

Although several methods for measuring myocyte diameter have been reported, only a few can provide reliable histometric data. We used a pen digitizer computer system and measured 200 muscle fibers per specimen. The median diameter was then determined from a histogram of the measured values. This method has the advantages of accuracy and simplicity. The instrumental error is less than 1 μm and variation between examiners and specimens is less than 0.1%. Thus, the reproducibility of this method is extremely good.

The dose of monocrotaline used in this study (200 mg/kg) was determined based on pilot studies in which higher doses (400 and 300 mg/kg) resulted in 100% mortality within 3 days. In contrast, 200 mg/kg of monocrotaline caused no deaths throughout the experiment.

We detected right ventricular hypertrophy in neonates within 1 week after treatment with monocrotaline, which is much less time than is required in adults. This difference was probably related to our use of neonatal animals, since Sawada et al examined the influence of age on monocrotaline-induced pulmonary hypertension and found that right ventricular hypertrophy developed sooner at a younger age among adult rats. In addition, we used a higher dose of monocrotaline (4–6 times greater than previously reported doses), and this may be associated with the early induction of right ventricular hypertrophy and pulmonary hypertension.

The mechanism by which monocrotaline causes pulmonary hypertension remains unknown. After administration, monocrotaline is washed out within 24 h, but a single exposure to monocrotaline causes pulmonary angitis, which results in the development of pulmonary hypertension. Several studies have indicated that the severity of pulmonary injury is dose-dependent, with high doses of monocrotaline inducing acute pulmonary edema and death, while lower doses cause delayed and sustained pulmonary hypertension. Most studies have shown the progression of pulmonary hypertension along with pulmonary vascular changes including hypertrophy of the arterial walls, endothelial proliferation, and perivascular inflammation. Reindel et al noted minimal arterial changes on day 8 after monocrotaline injection in adult rats. Indeed, most studies have used adult animals rather than neonates. During the time it takes for pulmonary hypertension to develop, neonates mature to become adult animals and the pathophysiology of the heart is altered. For this reason, few neonatal models of right ventricular hypertrophy have been developed.

In conclusion, a high dose of monocrotaline induced pulmonary hypertension in neonatal guinea pigs within 1 week after birth. Our method is simple and produces an experimental model with features similar to human congenital heart disease associated with pulmonary hypertension. This model may be useful not only for the investigation of neonatal myocardial protection in
cardiovascular surgery, but also for hemodynamic research in the field of pediatric cardiology.

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REFERENCES


