Effects of a Pure α/β-Adrenergic Receptor Blocker on Monocrotaline-Induced Pulmonary Arterial Hypertension With Right Ventricular Hypertrophy in Rats

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Background: It is unclear how much the sympathetic nervous system is involved in the development of pulmonary arterial hypertension (PAH). The present study examined whether or not a pure α/β-adrenergic receptor blocker (arotinolol) could prevent the development of PAH and right ventricular hypertrophy (RVH) in a rat model of monocrotaline (MCT)-induced PAH.

Methods and Results: The heart rate, arterial blood pressure (BP), left ventricular pressure, pulmonary artery pressure (PAP), and right ventricular pressure (RVP) were measured after administration of arotinolol or saline for 2 weeks. Ventricular weight and myocyte size were also measured. Mean PAP was increased less in the arotinolol group (n=6), (53±9 vs 21±2 mmHg in the control (n=6); P<0.01). Systolic RVP was also less in the arotinolol group (41±3 vs 91±14 mmHg in the control, P<0.05) without differences in BP. It also significantly reduced the RV/body weight ratio (0.58±0.01 vs 0.77±0.04 mg/g; P<0.01). Furthermore, the myocyte width was significantly decreased in the arotinolol group.

Conclusions: The pure α/β-blocker arotinolol prevented the progression of MCT-induced PAH and RVH in rats, suggesting that sympathetic nervous activation might play a role in the development of PAH. (Circ J 2009; 73: 2337–2341)

Key Words: α/β-adrenergic receptor blocker; Pulmonary arterial hypertension; Sympathetic nervous system

Pulmonary arterial hypertension (PAH) is a serious chronic disorder, similar to chronic kidney disease, which has varied causes and pathogenesis. PAH can potentially lead to right ventricular hypertrophy (RVH) and cardiovascular death related to RV failure. Among the types of PAH, idiopathic PAH has an especially poor prognosis and is difficult to manage clinically. Idiopathic PAH involves multiple factors, such as vasoconstriction, remodeling of the pulmonary vessels, and thrombosis, but it is unclear to what extent the sympathetic nervous activation plays a role in its development. Certainly, sympathetic nervous activity is reported to be increased in PAH; however, it is unclear whether or not inhibition of sympathetic activity can prevent or reverse PAH. The benefits of inhibiting sympathetic nervous activation in PAH remain uncertain, although there have been several reports regarding the effects of α- and/or β-adrenergic receptor blockers, especially carvedilol, although it is not a pure α/β-adrenergic receptor blocker. Arotinolol (Dainippon Sumitomo Pharma Co, Ltd, Osaka, Japan) is a nonselective α/β-adrenergic receptor blocker lacking local anesthetic, membrane-stabilizing or intrinsic sympathomimetic properties, which means that arotinolol is a pure α/β-adrenergic receptor blocker.2,3

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within less than 10% from baseline, and it was 0.25 mg·kg\(^{-1}\)·day\(^{-1}\), which we used in the present study. All rats were kept in a clean room and allowed water and food. Two weeks after administration of MCT, rats were anesthe-
tized by intraperitoneal injection of sodium pentobarbital (50 mg/kg) and a 1.4Fr Millar catheter (Millar Instruments, Houston, TX, USA) was inserted into the RV and pulmo-
ary artery via the right jugular vein and right atrium (RA). The catheter was connected to a pressure transducer (Nihon Koden Corporation, Tokyo, Japan) for measurement of pul-
monary hemodynamics, heart rate (HR), arterial pressure (AP), pulmonary artery pressure (PAP), and right ventricu-
lar pressure (RVP). The aortic pressure and left ventricular pressure (LVP) were measured via the carotid artery. End-
diastole was defined as the initial point of positive dP/dt. After completing these measurements, the rats were killed and their hearts were removed immediately. Each heart was cut into 2 parts (right ventricular free wall [RV] and left

### Table. Effects of Arotinolol on Hemodynamics

<table>
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<tr>
<th></th>
<th>n</th>
<th>Heart rate (beats/min)</th>
<th>LVSP (mmHg)</th>
<th>LVEDP (mmHg)</th>
<th>Mean BP (mmHg)</th>
<th>Mean RAP (mmHg)</th>
<th>RVSP (mmHg)</th>
<th>RVEDP (mmHg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>6</td>
<td>441±6</td>
<td>131±5</td>
<td>19±2</td>
<td>122±6</td>
<td>18±1</td>
<td>91±14</td>
<td>18±1</td>
</tr>
<tr>
<td>Arotinolol</td>
<td>6</td>
<td>432±15</td>
<td>132±6</td>
<td>15±1</td>
<td>115±11</td>
<td>12±2*</td>
<td>41±3*</td>
<td>11±1†</td>
</tr>
</tbody>
</table>

Values are mean±standard error of mean. *P<0.05, †P<0.01.

LVSP, left ventricular systolic pressure; LVEDP, left ventricular end-diastolic pressure; BP, blood pressure; RAP, right atrial pres-
sure; RSVP, right ventricular systolic pressure; RVEDP, right ventricular end-diastolic pressure.

**Figure 1.** Representative waveforms of pul-
monary arterial pressure (PAP) and right ven-
tricular pressure (RVP) in control (**Right**) and
arotinolol-treated (**Left**) rats.

**Figure 2.** Systolic and diastolic pulmonary arterial pressures (sPAP and dPAP, respectively) and mean PAP (mPAP) are shown. (Closed boxes) Arotinolol group, (open boxes) control group. In the arotinolol group, sPAP, dPAP, and mPAP are significantly lower compared with the control values. *P<0.05, †P<0.01.
ventricle plus septum (LV+S) according to the method of Fulton et al., and each part was weighted. The ratio of the weight of either ventricle to the body weight (BW) was calculated as an index of LVH or RVH.

Myocyte Preparation and Measurement
In several samples, cross-sectional specimens were immersed in 10% solution of formaldehyde. Masson-trichrome staining was performed to create representative images from both groups. To confirm precisely any differences in the degree of hypertrophy between the 2 groups, we examined the size of myocytes from the RV. After the physiological experiments, the hearts were rapidly removed and perfused with oxygenated (95% O$_2$–5% CO$_2$) calcium-free Tyrode’s solution (mmol/L: NaCl 130, KCl 4.8, MgCl$_2$ 1.2, HEPES 10, glucose 5; pH=7.4) at 37°C and then with Enzyme 1 Solution (type 2 collagenase 33 mg, hyaluronidase 21 mg, Tyrode’s solution 100 ml; Sigma Chemical Co, St Louis, MO, USA). Next, the RV was removed from each heart and minced with scissors in a separate vessel containing Enzyme 2 Solution (trypsin 20 mg and Enzyme 1 Solution 20 ml; Sigma). The tissue pieces were bubbled with 95% O$_2$–5% CO$_2$ for 10 min, filtered through a nylon mesh (300 μm), and washed with 20 ml of washing solution (Tyrode’s solution 15 ml and experimental solution 15 ml). The tissue pieces were centrifuged at 550 rpm for 3 min at room temperature. The supernatant was discarded and the cells were resuspended on top of an albumin solution (bovine serum albumin 1.2 g and Tyrode’s solution 20 ml). Isolated myocytes were placed in a cell chamber on the stage of an inverted microscope (Olympus, IX-70) that was continuously superfused at 37°C with the experimental solution (mmol/L: 130 NaCl, 4.8 KCl, 1.2 MgCl$_2$, 5 glucose, 10 HEPES, 1 CaCl$_2$). Cell length was measured using a SoftEdge video-based edge-detection system (IonOptix Corporation, Milton, MA, USA).

Statistical Analysis
All data are expressed as the mean±standard error of mean. The effects of arotinolol were assessed by comparison with saline using Student’s t-test. A P value <0.05 was considered to indicate statistical significance. All statistical analyses were performed with the Statistical Package for Social Sciences version 11.0 (SPSS Inc, Chicago, IL, USA).

Results
Hemodynamic Parameters (Table, Figures 1, 2)
The number of rats in each group was six. There were no significant differences in HR, systolic LVP, or mean AP between the 2 groups. LVEDP was higher in the control group, but the difference was not significant. Mean RAP and systolic RVP were significantly (P<0.05) higher in the control group compared with the arotinolol group. The differences in RVEDP between groups were similar to those for mean RAP. In the arotinolol group, systolic PAP, diastolic PAP, and mean PAP were 32±2, 18±2, and 21±2 mmHg, respectively, which were significantly lower values than in the control group (73±8, 43±9, and 53±9 mmHg, respectively).

Evaluation of Ventricular Hypertrophy (Figures 3, 4)
Representative images of the cross-sectional specimens and histology are shown in Figure 3. Body weight was not significantly different between the 2 groups. There were no significant differences in LV+S/BW between the groups, but the RV/BW ratio of the arotinolol group was significantly lower than that of the control group (0.58±0.01 vs 0.77±0.04 mg/g, P<0.01), suggesting that RVH was significantly prevented by arotinolol. Arotinolol also significantly prevented the increase in the maximum short-axis length of myocytes to 24.7±0.8 μm vs 30.9±1.1 μm in the control group (P<0.01), but not the maximum long-axis length.
and RV failure, which suggests that increased sympathetic activity might be related to the progression of RVH.\textsuperscript{13,14} In patients with isolated RV failure because of primary PAH, Bristow et al found selective $\beta_1$-receptor downregulation and decreased basal adenylyl cyclase activity.\textsuperscript{15} Nervous stimulation of the ventricles and abnormalities of the adrenergic system are involved, because cardiac denervation prevents the progression of sympathetic abnormalities and cardiac dysfunction.\textsuperscript{16} In the present study, we could not clarify the mechanism leading to the prevention of RVH, but it is possible that early inhibition of the progression of PAH might have delayed progression of RVH and inhibition of cardiac sympathetic activity may have also had a direct preventive effect.

Another possibility for the prevention of the progression of PAH could be the indirect antiplatelet activity of $\alpha/\beta$-adrenergic receptor blockers. Platelet activation increases the release of platelet-derived growth factor and serotonin into the circulation. Increased availability of thromboxane, fibrinopeptide A, and plasminogen activator inhibitor-1 creates a pro-coagulant state in the pulmonary circulation, after which vasoconstriction, cell proliferation, and thrombosis combine to cause deleterious pulmonary vascular remodeling. The $\beta$-adrenergic receptor blockers have an inhibitory effect on platelet function.\textsuperscript{17} Therefore, the indirect antiplatelet action of $\beta$-adrenergic receptor blockers might be a mechanism, but this should be confirmed by further studies.

One more possibility for the prevention of PAH is direct inhibition of the effects of MCT by arotinolol. It is known that MCT is metabolized in the liver and changed into pyrrole derivatives, which are toxic and cause vascular endothelial and smooth cell proliferation, obliteration of arterioles in the lung and induce RV hypertrophy with increased RV pressure.\textsuperscript{18} Therefore, it is possible that arotinolol can directly inhibit the effects of all pyrrole derivatives of MCT immediately after administration.

\textbf{Study Limitations}

The present study did not directly examine pulmonary arterial wall hypertrophy, but we expect that PA remodeling would have been reduced in the arotinolol-treated rats because of the significant prevention of PAH. The number of cardiac $\alpha$- and $\beta$-adrenergic receptors differs between rats and humans,\textsuperscript{19,20} and such differences could have influenced the results of the present study. Therefore, we also need to examine the preventive effect of arotinolol in PAH patients.
We examined only a 2-week treatment period in the present study. It is possible that arotinolol treatment delayed the onset of PAH rather than attenuated the disease. In the present study, the aim was to reveal hemodynamic changes during the development of PAH and RVH. It is reported that carvedilol improves survival rate in MCT-induced PAH rats. Therefore, we need to perform further study to clarify the effect of arotinolol on the long-term outcome.

We performed the experiments under anesthesia with pentobarbital, which reportedly elicits autonomic functions in rats. Therefore, our data would be influenced by pentobarbital. However, differences between the 2 groups would still be manifested even under these conditions and it is extremely difficult to measure in conscious rats all the parameters investigated in the present study.

In conclusion, the present study demonstrated that the pure α/β-adrenergic receptor blocker, arotinolol, could prevent the progression of MCT-induced PAH and RVH in rats, suggesting that sympathetic nervous activation plays a role in the development of PAH.

References