Beneficial Acute Effects of Rho-Kinase Inhibitor in Patients With Pulmonary Arterial Hypertension

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Background  Pulmonary arterial hypertension (PAH) is a poor prognostic disease with limited treatment options. Rho-kinase is involved in the pathophysiology of several diseases underlying smooth muscle hypercontraction, so the purpose of this study was to investigate the efficacy of fasudil, a Rho-kinase inhibitor, in patients with PAH.

Methods and Results  Fasudil 30 mg was intravenously injected over 30 min in 8 patients (all female, mean ± SD, 41 ± 11 years) with PAH. The lowest total pulmonary resistance (TPR) time was within 30–60 min after administration. Administration of fasudil decreased TPR from 1,069 ± 573 dyne·s·cm⁻⁵ to 809 ± 416 dyne·s·cm⁻⁵ (p<0.005) and mean pulmonary arterial pressure from 41.3 ± 12.8 mmHg to 37.9 ± 14.6 mmHg (p<0.05). The cardiac index was increased from 2.42 ± 0.73 L·min⁻¹·m⁻² to 2.84 ± 0.79 L·min⁻¹·m⁻² (p<0.02). Systemic vascular resistance and systolic systemic arterial pressure (SAP) were decreased (p<0.005, p=0.09, respectively), but the decrease in SAP was small (−6.4 ± 9.1 mmHg).

Conclusion  These results suggest that Rho-kinase is involved in the pathogenesis of human PAH and that fasudil is a novel therapeutic agent.  (Circ J 2006; 70: 174–178)

Key Words:  Hemodynamics; Hypertension; Lungs; Rho-kinase inhibitor

The prognosis of patients with severe pulmonary arterial hypertension (PAH) is poor, and the treatment options are limited. The goals of long-term therapy are to reduce the pulmonary vasoconstriction, induction regression of vascular remodeling and prevent thrombosis. Although major advances in our understanding of the mechanism of disease development and in the treatment of PAH have been achieved over the past decade, substantial gaps still remain. Available agents, such as calcium-channel blockers and bosentan, have limited efficacy, and epoprostenol is expensive and associated with significant complications. Recently, sildenafil, a phosphodiesterase type 5 inhibitor, was reported to be potentially useful for the treatment of PAH but insufficient data are available to define its role in the treatment of PAH.

Rho-kinase had been identified as one of the downstream targets of a small GTPase RhoA, and plays a pivotal role in Ca²⁺ sensitization of smooth muscle contraction through inhibition of myosin phosphatase. Rho/Rho-kinase signaling has been revealed to play a central role not only in Ca²⁺ sensitization of smooth muscle contraction but also in various diseases underlying enhanced vasoconstriction, such as hypertension and coronary artery spasm. The dramatic effects of Rho-kinase inhibitor for correcting hypertension in several hypertensive rat models were the first striking evidence linking Rho-kinase to these diseases. In addition, Rho/Rho-kinase signaling has been revealed to be involved in various pathologic conditions, including vascular remodeling and cardiac hypertrophy. Interestingly, fasudil, an agent that has been clinically used in Japan for the treatment of vasospasm after arachnoidal hemorrhage, has been identified as a Rho-kinase inhibitor and it has also been reported as effective for the treatment of vasospastic angina in humans.

Recently, it was reported that Rho/Rho-kinase signaling mediates increased pulmonary vasoconstriction and remodeling in pulmonary hypertensive rat models, including chronically hypoxic rats and monocrotaline-treated rats. Therefore, we investigated the effects of fasudil on the hemodynamics of patients with PAH.

Methods  The short-term effects of 30 mg fasudil were measured in 8 patients (all female, 41 ± 11 years old (mean ± SD)) with PAH (mean pulmonary arterial pressure (mPAP) >25 mmHg). Of these patients, 5 had idiopathic PAH (IPAH) and 3 had PAH associated with collagen vascular disease (CPAH). Exclusion criteria included congenital heart disease, pulmonary venous hypertension (pulmonary capillary wedge pressure (PCWP) >15 mmHg), lung disease, pregnancy, or other significant systemic disease. The underlying causes of pulmonary hypertension were evaluated using immunological laboratory analysis, chest X-ray, echocardiography, pulmonary function tests, ventilation/perfusion lung scan, high-resolution computed tomography of the lungs, right-heart catheterization, pulmonary angiography, and intravascular ultrasound according to the European Society of Cardiology and American College of Cardiology guidelines. The New York Heart Association functional class was II or III and patient characteristics are shown in Table I. The Ethics Committee of Mie University Hospital approved the study, and written informed consent was obtained.
consent was given by each enrolled patient.

The patients were fasted and no vasodilator was given for at least 12 h before administration of fasudil. A 7-French sheath was placed in the right internal jugular vein under local anesthesia, and a Swan-Ganz catheter (Swan-Ganz CCombo V; Edwards Lifescience, Irvine, CA, USA) was advanced into the pulmonary artery under fluoroscopy. A 20-gauge catheter was then placed in the radial artery for continuous arterial pressure monitoring. After the lines were placed, patients rested for at least 60 min, and then the fol-

### Table 1 Baseline Clinical Characteristics and Hemodynamics of the 8 Patients With Pulmonary Arterial Hypertension

<table>
<thead>
<tr>
<th>Case no.</th>
<th>Age (years)</th>
<th>Sex</th>
<th>Diagnosis</th>
<th>NYHA Class</th>
<th>Duration of PAH (months)</th>
<th>Height (cm)</th>
<th>Weight (kg)</th>
<th>BSA (m²)</th>
<th>Treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>56</td>
<td>F</td>
<td>IPAH</td>
<td>III</td>
<td>30</td>
<td>149</td>
<td>37</td>
<td>1.25</td>
<td>Beraprost, sildenafil, warfarin, O₂</td>
</tr>
<tr>
<td>2</td>
<td>29</td>
<td>F</td>
<td>IPAH</td>
<td>III</td>
<td>1</td>
<td>154</td>
<td>58</td>
<td>1.60</td>
<td>None</td>
</tr>
<tr>
<td>3</td>
<td>35</td>
<td>F</td>
<td>IPAH</td>
<td>III</td>
<td>14</td>
<td>152</td>
<td>46</td>
<td>1.40</td>
<td>Beraprost, sildenafil, warfarin</td>
</tr>
<tr>
<td>4</td>
<td>41</td>
<td>F</td>
<td>IPAH</td>
<td>III</td>
<td>216</td>
<td>153</td>
<td>50</td>
<td>1.45</td>
<td>Beraprost, O₂</td>
</tr>
<tr>
<td>5</td>
<td>26</td>
<td>F</td>
<td>IPAH</td>
<td>III</td>
<td>2</td>
<td>161</td>
<td>56</td>
<td>1.58</td>
<td>None</td>
</tr>
<tr>
<td>6</td>
<td>45</td>
<td>F</td>
<td>CPAH</td>
<td>II</td>
<td>120</td>
<td>165</td>
<td>60</td>
<td>1.66</td>
<td>Amlodipine, warfarin</td>
</tr>
<tr>
<td>7</td>
<td>53</td>
<td>F</td>
<td>CPAH</td>
<td>II</td>
<td>1</td>
<td>152</td>
<td>47</td>
<td>1.44</td>
<td>None</td>
</tr>
<tr>
<td>8</td>
<td>41</td>
<td>F</td>
<td>CPAH</td>
<td>III</td>
<td>1</td>
<td>154</td>
<td>46</td>
<td>1.40</td>
<td>None</td>
</tr>
</tbody>
</table>

NYHA, New York Heart Association; PAH, pulmonary arterial hypertension; IPAH, idiopathic PAH; CPAH, PAH associated with collagen vascular disease.

Fig 1. Change in the hemodynamic parameters from baseline to after fasudil administration (fasudil: the values at maximal reduction of TPR after fasudil infusion) in each patient.

SAP, systolic systemic blood pressure (mmHg); SVR, systemic vascular resistance (dyne·s·cm⁻²); mPAP, mean pulmonary arterial pressure (mmHg); TPR, total pulmonary resistance (dyne·s·cm⁻²); RAP, right arterial pressure; CI, cardiac index (L·min⁻¹·m⁻²); HR, heart rate (beats/min); PaO₂, partial pressure of arterial oxygen (Torr); NS, not significant; IPAH, idiopathic pulmonary arterial hypertension; CPAH, pulmonary arterial hypertension associated with collagen vascular disease.
lowing hemodynamic measurements were recorded while the patients were recumbent and defined as the baseline measurements: systolic systemic arterial pressure (SAP), pulmonary arterial pressure (PAP), PCWP, right atrial pressure (RAP), heart rate (HR), and cardiac output, using the thermodilution method. PCWP was not able to be measured in all patients because of technical reasons. Cardiac index (CI), systemic vascular resistance (SVR), pulmonary vascular resistance (PVR), and total pulmonary resistance (TPR) were calculated using the standard formulas.

**Protocol**

Each patient received fasudil (1 mg/min for 30 min) by continuous intravenous infusion. Hemodynamic monitoring was performed continuously and recorded every 15 or 30 min, for up to 60 min after administration. Arterial blood gas was measured at baseline and at the maximal point of TPR reduction. Patients were asked about symptoms indicating adverse drug action throughout the investigation.

**Statistical Analysis**

The data are expressed as mean±SD. Changes in hemodynamic parameters and in oxygenation during the investigation were compared using Student’s 2-sided, paired t-test. A p-value <0.05 was considered significant. Although the investigators performing the hemodynamic studies were not unaware of the drug’s administration, the investigators performing the statistical analysis from computerized data spreadsheets were blinded.

**Results**

Intravenous administration of fasudil significantly decreased the TPR, and in most patients, its effect peaked at 30 min and subsided 60 min after the initiation of the infusion. The changes in the parameters from baseline to after fasudil administration in each patient are indicated in Fig 1. TPR was maximally reduced from 1,069±573 dyne·s·cm–5 to 809±416 dyne·s·cm–5 (−23.9±10.1% of baseline, p<0.02), though HR (+2.9±11.5% of baseline, NS) was not significantly changed. SAP tended to decrease (−6.4±9.1 mmHg). SVR was significantly decreased from 2,119±654 dyne·s·cm–5 to 1,738±604 dyne·s·cm–5 (−18.9±11.7% of baseline, p<0.005), but the TPR/SVR ratio tended to decrease (−5.4±11.1% of baseline, NS).

Although the number of subjects was limited, TPR decreased more prominently in patients with IPAH than those with CPAH. Moreover, changes in the parameters that prescribe TPR, namely, CI and mPAP, tended to differ between the 2 groups. CI tended to be increased in patients with IPAH, and mPAP was decreased in patients with CPAH. The partial pressure of arterial oxygen (PaO2) (+5.6±6.3% of baseline, NS) was not significantly changed.

There were no major side effects: facial flushing (2 patients) and headache (1 patient) were reported.

**Discussion**

PAH is a debilitating disease characterized by an increase in PVR leading to right ventricular failure and death. Although advances in medical therapies of PAH over the past decade have been remarkable, limited treatment options are available. In this study, we demonstrated that fasudil, a Rho-kinase inhibitor, decreased mPAP and TPR, and increased CI without reducing the PaO2 in PAH, suggesting that Rho-kinase is involved in the pathogenesis of human PAH and that fasudil is a novel and useful agent for managing patients with PAH. The efficacy of fasudil in both IPAH and CPAH is consistent with data showing that Rho-kinase inhibitor corrected pulmonary hypertension in hypoxic pulmonary hypertensive rat models. In those chronically hypoxic rats the administration of Rho-kinase inhibitors markedly reduced mPAP, but caused only a small reduction of mPAP in normoxic rats. Rho-kinase inhibitor has been also shown to be effective in various hypertensive rat models, linking to results that RhoA activation was commonly observed in the aortas. These results strongly indicate that a common point in downstream signaling and a critical component of hypertension is the acti-
inhibitor. Therefore, the acute vasodilation effect of Rho-kinase inhibitors on PAH might be primarily caused by inhibition of actin–myosin interaction. The phosphorylation level of MLC is determined by the balance between MLC kinase and myosin phosphatase, inhibition of myosin phosphatase could increase MLC phosphorylation and lead to smooth muscle contraction. Indeed, a Rho-kinase inhibitor inhibits serotonin-induced contraction of human arteries. In vivo, the oral administration of a Rho-kinase inhibitor has dramatically and rapidly (within 1 h) corrected hypertension in various hypertensive rat models. In a hypoxic rat model, elevated PAP returned to an almost normal level 10 min after the intravenous injection of a Rho-kinase inhibitor. Therefore, the acute vasodilation effect of Rho-kinase inhibitors on PAH might be primarily caused by inhibition of actin–myosin interaction through the inhibition of Rho-kinase. In addition, decreased endothelial nitric oxide synthase expression by the activation of Rho-kinase has been reported to play a role in the pathologic condition of hypoxia-induced pulmonary hypertension.

To control PAH, a treatment that induces sustained mPAP and TPR reduction without significant change in blood oxygen and SAP is desirable. Vasodilators usually decrease oxygen in the arterial blood by increasing shunt flow in the lungs. As fasudil did not decrease arterial oxygen, it may be a novel and useful agent for managing patients with PAH. Although pulmonary severity and hypoxia-induced pulmonary hypertension rat models. Inhalation may be a more desirable route of clinical fasudil administration in hypotensive PAH patients.

Although the sample size was too small to discuss different effects between the 2 groups, our results showed that fasudil tended to decrease TPR more apparently in patients with IPAH than in those with CPAH whose baseline TPR was higher; however, the hemodynamic values at baseline differed between the 2 groups. Rho-kinase inhibitor might strongly influence increased vascular tone. In addition, CI tended to be increased in IPAH, and mPAP was decreased in CPAH. It is uncertain whether this tendency was related to the hemodynamic status at the baseline, or the underlying disease.

Several lines of evidence have shown the involvement of Rho-kinase in vascular remodeling via cell motility and proliferation and recent reports show that RhoA/Rho-kinase activation plays a pivotal role in pulmonary artery remodeling of chronic hypoxia in mice. Long-term treatment with Rho-kinase inhibitor improved pulmonary hypertension, right ventricular hypertrophy and pulmonary vascular remodeling, resulting in markedly improved survival of monocrotaline-induced pulmonary hypertensive rats. These results indicate that activation of RhoA/Rho-kinase-mediated signaling is substantially involved in the pathogenesis of PAH, and Rho-kinase inhibitor might be a novel therapeutic agent not only for reducing pulmonary artery hypercontraction but also for the regression of the vascular remodeling in PAH.

**Study Limitations**

These include the small sample size, only female subjects and no controls, so pathophysiological studies are required to clarify this important issue and these results should be regarded as preliminary. The safety of fasudil in terms of the systemic hemodynamics of patients with severe PAH, as well as its long-term efficacy, should be further investigated. As fasudil is short acting and needs continuous infusion, a long-active oral or inhaled form of Rho-kinase inhibitor is desirable. An oral form of fasudil has been developed and its efficacy and adequate safety profile for the treatment of stable effort angina in a multicenter phase II study has been reported. In the near future, it will be available for clinical use, providing a new class of drugs for PAH.

**Conclusions**

Rho-kinase is involved in the pathology of human PAH. Fasudil, a Rho-kinase inhibitor, induces acute pulmonary vasodilation in patients with PAH, and might be a new treatment.

**References**


