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Acute vasodilator effects of inhaled fasudil, a specific Rho-kinase inhibitor, in patients with pulmonary arterial hypertension

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Abstract We have previously demonstrated that long-term inhibition of Rho-kinase ameliorates pulmonary arterial hypertension (PAH) in animal models. In the present study, we examined acute vasodilator effects of inhaled fasudil, a specific Rho-kinase inhibitor, as a more feasible option to locally deliver the drug for PAH. We examined 15 patients with PAH (13 women and 2 men, 45 ± 4 years old), including idiopathic PAH (n = 5), PAH associated with connective tissue disease (n = 6), PAH with congenital heart disease (n = 3), and portal PAH (n = 1). In those patients, we performed right heart catheterization with a Swan–Ganz catheter in the two protocols with inhalation of nitric oxide (NO) (40 ppm, 10 min) and fasudil (30 mg, 10 min) with a sufficient interval (>30 min). Both NO and fasudil inhalation significantly reduced mean pulmonary arterial pressure (PAP) (NO: $P < 0.01$, fasudil: $P < 0.05$) and tended to decrease pulmonary vascular resistance (NO: $P = 0.07$, fasudil: $P = 0.1$), but did not affect cardiac index. The ratio of pulmonary to systemic vascular resistance was significantly reduced both in NO and fasudil inhalation (NO: $P < 0.01$, fasudil: $P < 0.05$), indicating that both NO and fasudil inhalation selectively affect lung tissues. Interestingly, there was no correlation in the vasodilator effects between NO and fasudil, and a positive correlation with serum levels of high-sensitivity C-reactive protein was noted for fasudil but not for NO. These results suggest that inhalation of fasudil is as effective as NO in patients with PAH, possibly through different mechanisms.

Key words Rho-kinase · Inhalation · Pulmonary arterial hypertension · Pulmonary arterial pressure · Pulmonary vascular resistance

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Introduction

Pulmonary arterial hypertension (PAH) is characterized by progressive elevation of pulmonary artery pressure and vascular resistance with poor prognosis, which is defined as a mean pulmonary arterial pressure greater than 25 mmHg at rest or greater than 30 mmHg during exercise.[1] The pathological changes in the disorder include endothelial injury of hypertensive pulmonary arteries, proliferation and hypercontraction of vascular smooth muscle cells (VSMC), and migration of inflammatory cells.[2–4] Although anticoagulant agents, vasodilators, and lung transplantation are currently used for the treatment of PAH, more effective treatments, such as local delivery of drugs, need to be developed.[4]

The Rho/Rho-kinase pathway has recently attracted much attention in the cardiovascular research field for several reasons. First, the Rho/Rho-kinase pathway plays an important role in various cellular functions that are involved in the pathogenesis of cardiovascular diseases.[5,6] Second, this intracellular signaling pathway is substantially involved in the effects of many vasoactive substances that are implicated in the pathogenesis of cardiovascular diseases.[5,6] Indeed, we and others have recently demonstrated the roles of the Rho-kinase pathway in the pathogenesis of PAH.[4,7–14] We and others have previously reported the acute effects of intravenous administration of a selective Rho-kinase inhibitor, fasudil, in patients with severe PAH.[11,13] However, local administration of the drug seems to be more favorable for avoiding systemic side effects in this chronic disorder. In this study, we thus examined the acute vasodilator effects of fasudil inhalation compared with those of nitric oxide (NO) inhalation in patients with pulmonary hypertension (PH).

Materials and methods

The Ethical Committee of the Tohoku University Hospital approved this study, and all patients provided informed consent. The authors had full access to the data and take

Table 1. Patients' characteristics

Case	Age (years)	Sex	Diagnosis		WHO class	Duration of PAH (months)	Medication	UA (mg/dl)	BNP (pg/ml)	hsCRP (mg/dl)
1	25	F	IPAH		II	11	Beraprost, warfarin	8.3	472.0	1.000
2	24	F	IPAH		II	26	Epoprostenol, sildenafil	3.9	3.8	0.023
3	53	F	IPAH		II	51	Epoprostenol	4.8	5.0	0.956
4	55	F	IPAH		II	77	Epoprostenol	5.8	6.5	0.356
5	53	F	IPAH		II	9	Bosentan, warfarin	5.1	40.1	0.014
6	42	M	IPAH		II	216	Beraprost, warfarin, amlodipine	4.4	31.1	0.031
7	41	F	PAH-CTD	MCTD	II	29	Beraprost, warfarin, amlodipine	5.9	16.7	2.000
8	75	F	PAH-CTD	CREST	III	1	None	6.3	75.0	1.000
9	40	F	PAH-CTD	RA	III	48	Epoprostenol	3.5	60.5	0.018
10	48	F	PAH-CTD	SSc	III	24	Warfarin	10.0	270.7	0.864
11	43	F	PAH-CTD	MCTD	III	74	Epoprostenol, bosentan	6.2	338.0	1.000
12	38	F	PAH-CHD	ASD	I	65	Epoprostenol	5.9	291.9	0.111
13	37	F	PAH-CHD	ASD	II	156	Sildenafil, warfarin	2.2	60.2	0.042
14	31	M	PAH-CHD	VSD	III	379	Amlodipine	4.5	69.9	0.956
15	72	F	Portal PH	LC, HCV	II	48	Bosentan, beraprost, amlodipine	4.3	53.9	0.214

ASD, atrial septal defect; BNP, brain natriuretic peptide; CREST, CREST (Calcinosis; Raynaud phenomenon; Esophageal motility disorders; Sclerodactyly; and Telangiectasia) syndrome; HCV, hepatitis C virus; hsCRP, high-sensitivity C-reactive protein; IPAH, idiopathic PAH; LC, liver cirrhosis; MCTD, mixed connective tissue disease; PAH, pulmonary arterial hypertension; PAH-CHD, PAH associated with congenital heart disease; PAH-CTD, PAH associated with connective tissue disease; PH, pulmonary hypertension; RA, rheumatoid arthritis; SSc, systemic sclerosis; UA, uric acid; VSD, ventricular septal defect; WHO, World Health Organization

full responsibility for its integrity. All authors have read and agreed to the article as written.

Study population

Fifteen patients (mean age of 45 years; range, 24–75; 13 women and 2 men) with severe PH (Table 1) were prospectively enrolled to examine the acute effects of inhaled NO and fasudil hydrochloride on pulmonary hemodynamics at the Tohoku University Hospital. Six patients had idiopathic PAH (IPAH), 5 PAH associated with connective tissue disease (PAH-CTD), 3 PAH associated with congenital heart disease (PAH-CHD), and 1 portopulmonary hypertension (portal PH) (Table 1). Five patients had World Health Organization functional class III, 9 had class II, and 1 had class I (Table 1). Thirteen patients had been treated with vasodilators and/or warfarin for more than 9 months (Table 1).

For each patient, baseline demographic, clinical, procedural, and outcome data were collected by use of a standardized data collection. Serum or plasma levels of uric acid (UA) and B-type natriuretic peptide (BNP), and high-sensitivity C-reactive protein (hsCRP) were measured before cardiac catheterization.

Connective tissue disease and liver disease were diagnosed clinically and by blood tests.[1,3] Congenital heart disease was diagnosed by echocardiography, and the presence of thromboembolic pulmonary disease was examined by both ventilation–perfusion scans and computed tomography (CT).[1,3] Pulmonary function tests, arterial blood gases, chest X-ray, or CT scan were used to diagnose lung disease and hypoxia. In the patients diagnosed with idiopathic PAH, the abnormalities described above were ruled out.

Study protocol

All patients underwent right heart cardiac catheterization while they continued all medications, including oral or intravenous prostaglandin I_2 and calcium antagonists. Cardiac catheterization was performed in the afternoon. On the day of the examination, they received bosentan and amlodipine after breakfast, and beraprost and/or sildenafil after breakfast, and at noon. The dose of continuous epoprostenol was 10–68 ng/kg per minute. A 5–6-F sheath was placed in the jugular or basilic vein under local anesthesia and a Swan–Ganz catheter was advanced into the pulmonary artery under fluoroscopy. A 4-F sheath was then placed in the right radial artery under local anesthesia. We measured systemic and pulmonary vascular resistance (SVR and PVR), systemic and pulmonary arterial blood pressure (BP and PAP), mean pulmonary capillary wedge pressure (mPCWP), central venous pressure (CVP), systemic and pulmonary arterial blood oxygen saturation (SaO_2 and SvO_2), cardiac output (CO) by Fick method, and heart rate (HR). With the catheter in the pulmonary artery, the patients breathed NO (40 ppm for 10 min) via a continuous mask under constant flow of air before a complete hemodynamic measurement was repeated. Nitric oxide application was continued during the hemodynamic measurements. After the confirmation of hemodynamic recovery at an interval of more than 30 min, the patients inhaled aerosolized fasudil hydrochloride (30 mg for 10 min), which was generated by a jet nebulizer set at an airflow rate of 10 l/min. Fasudil application was also continued during these hemodynamic measurements. The order of the NO and fasudil inhalation was fixed partly because the half-life of NO is very short compared with fasudil and partly because fasudil could affect the responses to NO when used before NO.[4]

Table 2. Acute vasodilator effects of nitric oxide and fasudil inhalation on pulmonary hemodynamics in patients with pulmonary hypertension

	Baseline	NO	*P* value	Baseline	Fasudil	*P* value
PCWP (mmHg)	7.9 ± 0.6	8.1 ± 0.7	NS	7.3 ± 0.6	7.3 ± 0.5	NS
mPAP (mmHg)	**53.0 ± 3.9**	**50.8 ± 4.2**	**<0.01**	**53.3 ± 4.0**	**51.5 ± 3.8**	**<0.05**
RAP (mmHg)	5.6 ± 0.5	5.3 ± 0.4	NS	5.9 ± 0.5	5.0 ± 0.6	NS
mAoP (mmHg)	81.5 ± 2.7	86.1 ± 3.6	<0.05	84.7 ± 3.7	87.0 ± 3.2	NS
Heart rate (beats/min)	75.3 ± 3.9	75.7 ± 3.6	NS	76.2 ± 3.6	75.8 ± 3.4	NS
CI (l/min/m^2)	2.7 ± 0.2	2.7 ± 0.2	NS	2.8 ± 0.2	2.7 ± 0.1	NS
PVR (dyne/s cm^{-5})	1029 ± 191	974 ± 193	NS	1010 ± 168	999 ± 179	NS
SVR (dyne/s cm^{-5})	1626 ± 147	1735 ± 167	NS	1654 ± 146	1740 ± 147	NS
PVR/SVR	**0.61 ± 0.06**	**0.54 ± 0.05**	**<0.01**	**0.60 ± 0.06**	**0.55 ± 0.05**	**<0.05**
SvO_2 (%)	68.1 ± 1.0	68.2 ± 1.3	NS	70.4 ± 2.1	68.9 ± 1.2	NS
SaO_2 (%)	91.7 ± 1.2	92.0 ± 1.3	NS	92.7 ± 1.3	92.9 ± 1.2	NS
PaO_2 (mmHg)	68.6 ± 5.7	68.9 ± 5.6	NS	71.0 ± 5.4	70.6 ± 3.4	NS

Continuous data are shown as mean ± SE. Comparisons between "Baseline" and "NO/Fasudil" are made by use of paired *t*-test
CI, cardiac index; mAoP, mean arotic pressure; mPAP, mean pulmonary arterial pressure; NO, nitric oxide; NS, not significant; PaO_2, arterial partial pressure oxygen; PCWP, pulmonary capillary wedge pressure; PVR, pulmonary vascular resistance; RAP, right atrial pressure; SaO_2, arterial blood oxygen saturation; SvO_2, pulmonary arterial blood oxygen saturation; SVR, systemic vascular resistance

Statistical analysis

Results are expressed as mean ± SEM. All variables were analyzed by paired *t*-test or linear regression analysis, using Prism (GraphPad Software, San Diego, CA, USA). *P* values of less than 0.05 were considered to be statistically significant.

Results

Hemodynamic variables

In the 15 patients with PH, mean systolic PAP/diastolic PAP at baseline was 85/34 mmHg, mean cardiac output was 3.9 l/min, and mean PVR was 1029 dyne/s cm^{-5} (Table 2). Nitric oxide inhalation slightly but significantly decreased mean PAP (53.0 ± 3.9 to 50.8 ± 4.2 mmHg, $P < 0.01$; Table 2 and Fig. 1A) and tended to decrease PVR (1029 ± 191 to 974 ± 193 dyne/s cm^{-5}, $P = 0.07$; Table 2). Also, the inhalation of fasudil slightly but significantly reduced mean PAP (53.3 ± 4.0 to 51.5 ± 3.8 mmHg, $P < 0.05$; Table 2 and Fig. 1B) and tended to reduce PVR (1,010 ± 168 to 997 ± 179 dyne/s cm^{-5}, $P = 0.1$; Table 2). However, neither of the treatments affected cardiac index (CI). Although there was similar reduction in mean PAP (NO: –4.7% ± 1.4%, fasudil: –2.9% ± 1.3%, not significant), there was no correlation in the reduction of mean PAP between NO and fasudil (Fig. 2A). To examine the selectivity of NO and fasudil inhalation, we evaluated the ratio of PVR to SVR, which was significantly reduced both in NO and fasudil inhalation (NO: 0.61 ± 0.06 to 0.54 ± 0.05, $P < 0.01$; fasudil: 0.60 ± 0.06 to 0.55 ± 0.05 mmHg, $P < 0.05$; Table 2 and Fig. 1C,D).

Correlations between clinical features and vasodilator effects

Mean serum UA level was 5.4 mg/dl, mean plasma BNP level was 119.7 pg/dl, and mean serum level of hsCRP was 0.57 mg/dl (Table 1). The reduction in mean PAP by NO or fasudil inhalation was not significantly correlated with serum UA or plasma BNP levels (data not shown). However, the reduction in mean PAP by fasudil inhalation, but not that by NO inhalation, was significantly correlated with hsCRP levels (Fig. 2B,C). The duration of PAH did not show any significant correlation with the effects of NO or fasudil inhalation (data not shown).

Discussion

The novel finding of the present study is that the fasudil inhalation is as effective as NO inhalation in reducing mean PAP in patients with PAH. Although we have previously demonstrated that the intravenous administration of fasudil is also effective in patients with PAH, it reduced both PVR and systemic vascular resistance.[11] Thus, the inhalation of fasudil may be more favorable than its intravenous administration in avoiding any adverse effects in this chronic disorder.

Rho-kinase and PAH

We have demonstrated that Rho-kinase is a novel therapeutic target in various cardiovascular diseases, including ischemic heart disease, essential hypertension, and PAH.[4,6–8,11,12] We also have reported the acute vasodilator effects of intravenous fasudil;[11] however, it is more desirable for PAH treatment to selectively deliver the drug to the lung. It has been demonstrated that inhaled Rho-kinase inhibitor was more effective than inhaled NO as a selective pulmonary vasodilator in a rat model of hypoxia-induced PH,[10] although it was not the case with the present study, probably because the dose of Rho-kinase inhibitor used here was much lower than in the animal study.[10] The present study is the first to report that the inhalation of fasudil can reduce mean PAP in patients with PAH.

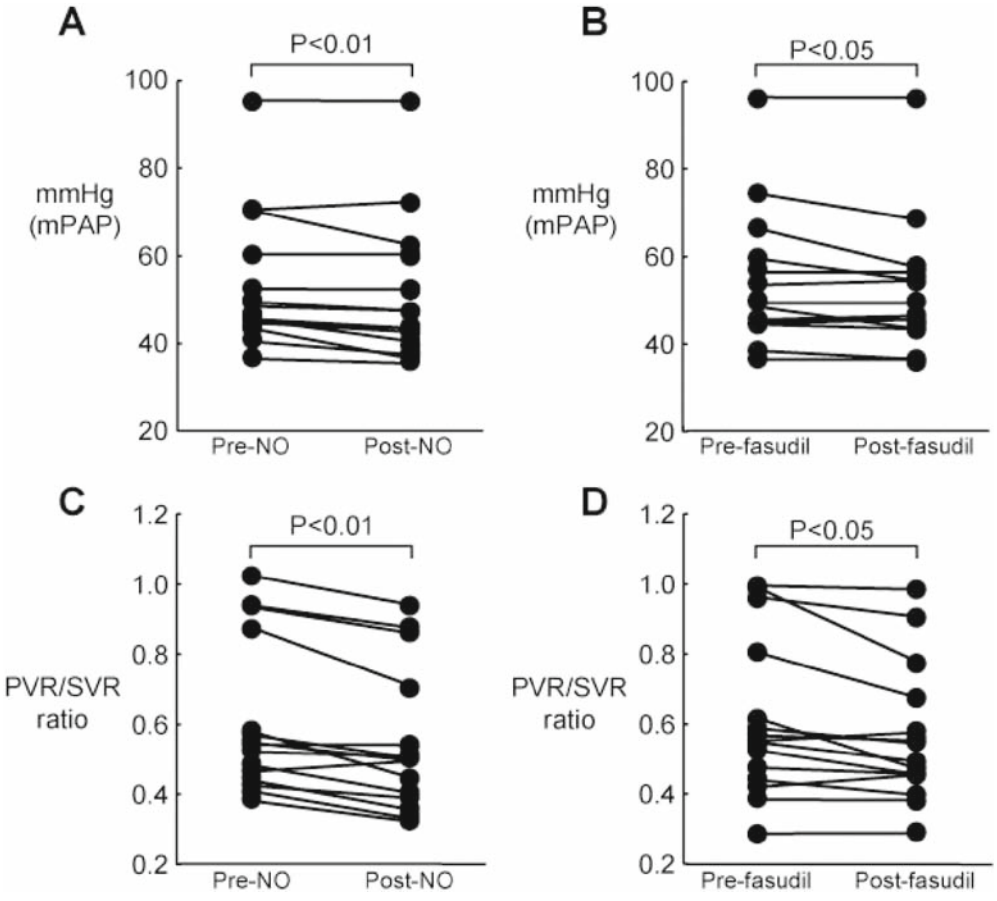

Fig. 1A–D. Acute effects of inhaled nitric oxide (*NO*) and fasudil in patients with pulmonary arterial hypertension (PAH). **A, B** Both NO and fasudil inhalation significantly reduced mean pulmonary arterial pressure (*mPAP*) in patients with PAH. **C, D** Both NO and fasudil inhalation significantly reduced the ratio of pulmonary to systemic vascular resistance (*PVR/SVR*), which is one of the selectivity markers of pulmonary circulation

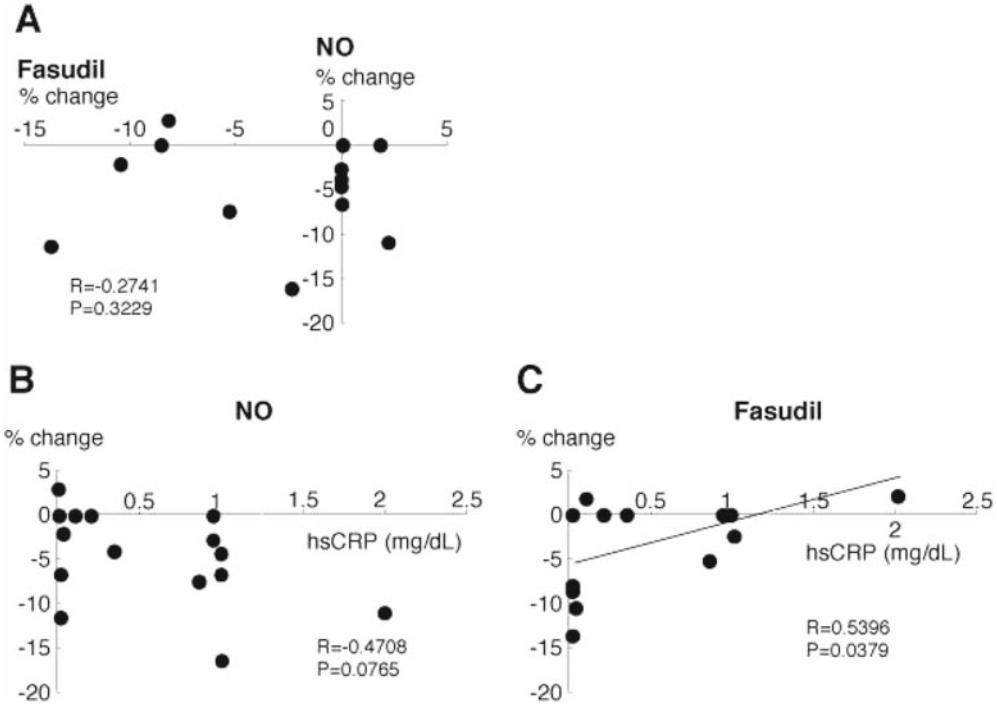

Fig. 2A–C. Correlation of vasodilator effects of nitric oxide (*NO*) and fasudil with high-sensitivity C-reactive protein (*hsCRP*) in patients with PAH. **A** There was no significant correlation on the percent reduction in mean PAP between NO and fasudil inhalation in the 15 patients with PAH. **B, C** Although there was no significant correlation between hsCRP levels and the percent reduction in mean PAP by NO inhalation, there was a significant correlation between hsCRP levels and the percent reduction in mean PAP by fasudil inhalation

Rho-kinase suppresses myosin phosphatase activity by phosphorylating the myosin-binding subunit of the enzyme, thus augmenting VSMC contraction at a given intracellular calcium concentration.[15,16] Vascular smooth muscle cell hypercontraction mediated by activated Rho-kinase plays a key role not only in coronary artery spasm[7,17,18] but also in PAH.[4,11] Rho-kinase inhibition may be preferable to calcium channel blockers because of its selective spasmolytic effect on vascular hyperconstrictive segments.[7,17]

Rho-kinase and inflammation

A number of studies have suggested that inflammation may be involved in the pathogenesis of PAH.[19,20] Some patients with idiopathic PAH have immunological disturbances (e.g., circulating autoantibodies, such as antinuclear antibodies) and elevated circulating levels of proinflammatory cytokines (e.g., interleukin-1 and -6).[20] We have demonstrated that Rho-kinase is upregulated by inflammatory stimuli.[6,21,22] We also have demonstrated that Rho-kinase inhibition increases endothelial NO synthase (eNOS) expression, and decreases inflammatory cell migration and angiotensin II-induced upregulation of monocyte chemoattractant protein-1 and plasminogen activator inhibitor-1 in vivo or in vitro,[7] in which the Rho/Rho-kinase pathway may play an important role in the development of PH. In the present study, the patients with higher levels of hsCRP showed less vasodilating response to the inhaled fasudil. Although the precise mechanism for the results remains to be elucidated, it is conceivable that the present dose of fasudil is not enough to effectively inhibit Rho-kinase activity where increased.

In the present study, there was no correlation in the vasodilator effects between inhaled NO and inhaled fasudil, and only inhaled fasudil, but not inhaled NO, showed a significant correlation with hsCRP, suggesting that the vasodilatory effects of inhaled fasudil are mediated by NO-independent mechanisms.[4]

Rho-kinase inhibitor and PAH

Fasudil is a potent and selective inhibitor of Rho-kinase,[15] with its inhibitory effect on Rho-kinase being 100 times and 1000 times more potent than on protein kinase C and myosin light chain kinase, respectively.[7] In a series of experimental studies, we have demonstrated that long-term oral treatment with fasudil markedly ameliorates monocrotaline-induced PH and pulmonary vascular lesions in rats.[8] We also have reported that intravenous administration of fasudil effectively reduces pulmonary vascular resistance in animal models and patients with PH.[11] Recently, we have started a clinical trial in which we examine the long-term effect of the oral form of fasudil in patients with PAH in Japan.

Limitations of the study

Several limitations should be mentioned for the present study. First, although we consider that the inhalation of fasudil is favorable for local delivery of the drug to the lungs to avoid systemic adverse effects, its efficacy substantially depends on the patients' skill. Obviously, more than half of our patients in the present study could not breathe deeply during the inhalation of the drug, and it is uncertain how far into the airways the fasudil was delivered. Second, fasudil used in the present study is formulated for intravenous use. Although it is not known whether fasudil is appropriate for inhalation use, no other Rho-kinase inhibitors are currently available. Third, in the present study we examined only the acute effects of inhaled fasudil alone. As mentioned above, we have just started the clinical trial to address the long-term effects of oral fasudil in patients with PAH. Fourth, we did not examine the higher dose of fasudil or its dose dependency in the present study because 30 mg of fasudil used in the present study is officially recommended for intravenous use by the Japanese government. Fifth, the extent of the reduction in mean PAP or PVR was relatively small in the present study. However, even such a small reduction in PAP or PVR should result in improvement of the fatal disorder.[1,4,11,13] Sixth, because most of the patients were already being treated by various vasodilators, such as prostaglandins, bosentan, or sildenafil, the vasodilatory effects of inhaled NO or fasudil might be masked. Seventh, we have previously shown that intravenous administration of fasudil slightly decreased mean PAP, slightly increased cardiac index, and significantly decreased PVR;[11] however, in the present study, inhalation of fasudil significantly decreased mean PAP, slightly decreased cardiac index, and slightly decreased PVR (Table 2). This discrepancy might be caused by the patients' variable characteristics or the patients' skill of inhalation of the drug. Although we usually consider that some therapy is effective if we observe a significant reduction of PVR, we can also consider that it is effective if we observe a significant reduction of mean PAP. Eighth, there were no significant differences in the hemodynamic changes among the different types of PH in the present study, probably due to the limited number of patients. Thus, further investigation needs to be performed to clarify this point.

Conclusions

The present study suggested that inhalation of fasudil is as effective as NO in patients with PAH, possibly through different mechanisms. However, future studies are required to demonstrate the chronic effects of fasudil inhalation following the development of long-acting inhalation Rho-kinase inhibitors.

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