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Double-Blind, Placebo-Controlled Clinical Trial With a Rho-Kinase Inhibitor in Pulmonary Arterial Hypertension

– A Pilot Efficacy Trial –

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Background: 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 the clinical effects of mid-term oral treatment with an extended release formulation of AT-877 (fasudil hydrochloride), a specific Rho-kinase inhibitor (AT-877ER) on PAH.

Methods and Results: 23 PAH patients were treated with either placebo (10/2 females/males, 51±16 years, idiopathic PAH (IPAH) in 6, PAH associated with connective tissue disease (CTD-PAH) in 3, PAH with congenital heart disease (CHD-PAH) in 2, and portal PAH in 1) or AT-877ER (6/5 females/males, 47±14 years, IPAH in 2, CTD-PAH in 5, and CHD-PAH in 4); 3 patients were excluded. We performed a 6-min walk test and right heart catheterization in the remaining 20 patients, before and 3 months after the treatment (placebo n=11, AT-877ER n=9). Although there were no significant differences between the 2 groups for the 6-min walk distance, pulmonary hemodynamics tended to be improved in the AT-877ER group, especially the prevalence of improved cardiac index from baseline, which was significantly higher in the AT-877ER than in the placebo group. In the AT-877ER group, serum levels of hydroxyfasudil, an active metabolite of AT-877ER tended to correlate with improvements in the cardiac index and mean pulmonary artery pressure.

Conclusions: Mid-term treatment with oral AT-877ER showed additional improvement in pulmonary hemodynamics in patients with PAH. (*Circ J* 2013; **77:** 2619–2625)

Key Words: Pulmonary arterial hypertension; Rho-kinase inhibitor; Signal transduction

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Pulmonary arterial hypertension (PAH), defined as mean pulmonary arterial pressure (PAP) ≥25 mmHg at rest during right heart catheterization,[1–3] is a fatal disease caused by small pulmonary artery obstruction from vascular proliferation and remodeling.[4] PAH is characterized by elevated PAP and increased pulmonary vascular resistance (PVR), frequently leading to right-sided heart failure and death.[4–6] The pathological changes of the pulmonary arteries in PAH include endothelial injury, proliferation and hypercontraction of vascular smooth muscle cells (VSMC), and migration of inflammatory cells.[4,6,7] Anticoagulant agents, vasodilators and lung transplantation are currently used for the treatment of PAH, but more effective treatment needs to be developed.[6,8,9]

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In mid-1990s, 2 Japanese groups and 1 Singapore group independently identified Rho-kinase/ROK/ROCK as an effecter of the small GTP-binding protein Rho,[10–12] which plays an important role in various cellular functions, including smooth muscle contraction, actin cytoskeleton organization, cell adhesion and motility, cytokinesis, and gene expressions.[13–15] We and others have demonstrated that Rho-kinase activation is substantially involved in the pathogenesis of cardiovascular diseases. First, the Rho-kinase pathway plays an important role in various cellular functions in response to various vasoactive substances.[14] Second, the so-called pleiotropic effects of statins, especially of high-doses of statins, may be mediated, at least in part, by their inhibitory effects on Rho, with a resultant inhibition of Rho-kinase.[14] Third, the effectiveness of AT877 (fasudil hydrochloride), a specific Rho-kinase inhibitor, for PAH has been demonstrated.[6,16–20]

In the present study, we examined the effects of mid-term oral treatment with an extended-release formulation of AT-877 (AT-877ER) in patients with PAH.

Methods

This study was a phase IIa clinical trial with fasudil for pulmonary arterial hypertension conducted by Asahi Kasei Pharma Corporation (Tokyo, Japan).

The ethics committees of all participating institutes approved the study protocol and all patients provided written informed consent. This report follows the recommendations of the 2010 Consolidated Standards of Reporting Trials Statement.

Study Patients

Patients with PAH were included when they had a baseline 6-min walk distance of ≥150 m with WHO functional class I–III. However, patients were excluded if they had received treatment with epoprostenol sodium, vardenafil hydrochloride hydrate, or tadalafil, or if they had changed dosages of bosentan, beraprost sodium, sildenafil citrate, warfarin potassium, calcium antagonists, cardiac glycosides or diuretics and/or doses of oxygen and nitrogen monoxide within 30 days, or had been started on any such regimens within 30 days prior to consent (for warfarin potassium, however, dosage modification as adjustment of the international normalized ratio of prothrombin was allowed). Further, patients were excluded if they had received concomitant treatment with bosentan and sildenafil citrate within 30 days prior to their informed consent. Patients with serum creatinine levels exceeding the upper limit of the study site's reference range were also excluded.

Study Design

From the viewpoint of feasibility, the sample size was planned as 30 patients in total (10 patients for WHO functional class I and 20 for functional classes II–III. The present study was designed as a 3-month, double-blind, randomized, placebo-controlled, multicenter trial in which 14 PAH centers in Japan participated. All patients were hospitalized 3–6 days before the first examination (day 1) and the last examination (week 12) (**Figure S1**). Administration of the study drug was started and ended during the hospitalization periods. Patients received either AT-877ER or placebo capsule twice daily (Asahi Kasei Pharma Corporation, Tokyo, Japan) for 12 weeks in a blind manner (**Figure S1**). The dosage of AT-877ER was increased every 3 days in a stepwise manner from 2 to 6 capsules/day (**Figure S1**). All patients were administered 2 capsules/day until day 4, when the dose was increased by the investigator's decision to 4 capsules/day. Until day 7, 4 capsules/day were given and the next doses were decided by investigators on day 7. Before increasing the study drug on days 4, 7, and 10, investigators checked the safety of the treatment in each subject and determined the subsequent treatment plan as follows. Whenever it was difficult to follow the intended regimen because of adverse effects or other reasons, the situation was required to be judged as "continuation at the dose level at the time of occurrence of the adverse drug reaction", "continuation with reduced dosage", or "discontinuation of study treatment". The treatment was randomized according to the 6-min walk distance, with drugs prescribed at baseline as stratifying factors, and used minimization with a randomized method. The 6-min walk distance was assessed before drug administration and at 4, 8 and 12 weeks of administration of the study drug. Cardiac catheterization was performed on the first and last days of the treatment protocol (**Figure S1**).

Diagnosis of Pulmonary Hypertension

PAH was defined as mean PAP ≥25 mmHg with pulmonary capillary wedge pressure (PCWP) ≤15 mmHg at rest.[1,2,6] Connective tissue disease and liver disease were diagnosed clinically and by blood tests. Congenital heart disease was diagnosed by echocardiography, and chronic thromboembolic pulmonary hypertension was diagnosed by ventilation-perfusion RI scans and computed tomography (CT).[6] Pulmonary function tests, arterial blood gases, chest X-ray and CT scan were used to diagnose lung disease and hypoxia. When the aforementioned abnormalities were ruled out, the patients were diagnosed as having idiopathic PAH (IPAH).[2,7] Heritable PAH was diagnosed as IPAH with a family history of PAH.[2,7,21]

Data Collection

Baseline demographic information (including age, sex, height and body weight), clinical diagnosis, comorbidities (connective tissue disease, liver disease, congenital heart disease, and thyroid dysfunction) and hemodynamic data from catheterization were recorded for each patient. Hemodynamic parameters examined included PCWP, PAP, right atrial pressure (RAP), cardiac output (CO), cardiac index (CI), systolic, diastolic and mean blood pressures, PVR, systolic vascular resistance, and mixed venous oxygen saturation. Blood data, including serum levels of creatinine and N-terminal pro-brain natriuretic peptide (NT-pro-BNP), plasma levels of BNP and other renal and liver functions, and urinary data were also collected.

To measure CO, both thermodilution and the Fick method were performed in 7 patients in the placebo group and in 4 in the AT-877ER group. In the present study, thermodilution data

Table 1. Baseline Characteristics of the Patients

	Placebo (n=12)	AT-877ER (n=11)
Age (years)	51.4±16.2	47.4±14.2
Female/Male (n)	10/2	6/5
Weight (kg)	51.0±7.9	62.9±12.6
WHO functional class (n)		
I	2	0
II	9	9
III	1	2
IV	0	0
Cause of PAH (n)		
Idiopathic	6	2
Connective tissue disease	3	5
Congenital heart disease	2	4
Portal hypertension	1	0
Background therapy		
Naive	2	0
Naive within 1 month of written informed consent	1	1
Beraprost alone	4	3
Bosentan	4	5
Sildenafil	1	2
Diuretics		
Without	8	5
With	4	6
Warfarin potassium		
Without	5	5
With	7	6
Oxygen therapy		
Without	5	3
With	7	8
Duration of PAH (years)		
<1	5	4
1–10	4	4
≥10	3	3
Pulmonary arterial pressure (mmHg)	47.2±14.3	40.5±17.2
Pulmonary vascular resistance (dyne·s^{-1}·cm^{-5})	865.7±476.9	687.9±550.3
Cardiac index (L·min^{-1}·m^{-2})	2.456±0.542	2.609±1.035
Arterial oxygen saturation (%)	95.56±2.58	94.63±2.30
Oxygen saturation of pulmonary artery (%)	70.18±6.36	70.30±6.18
6-min walk distance (m)	397.3±107.7	392.9±107.5
BNP (pg/ml)	119.3±140.5*	107.2±236.3
Creatinine (mg/dl)	0.65±0.11	0.77±0.18

Data are shown as mean±SD. *n=11.
BNP, B-type natriuretic peptide; PAH, pulmonary arterial hypertension.

acquired by the same technique during cardiac catheterization on the first and the last days of the treatment protocol took precedence over that acquired with the Fick method.

Blood samples were taken before oral administration of placebo or AT-877ER to measure the blood concentration of hydroxyfasudil, an active metabolite of fasudil, on the same day as cardiac catheterization was performed.[18]

Efficacy Endpoint

Efficacy was judged as a change in pulmonary hemodynamics and 6-min walk distance from baseline after 12 weeks of therapy.

Statistical Analysis

The analysis data set comprised all randomized patients who received at least 1 dose of the study medications. No estimation of missing data was performed. Demographics and baseline characteristics were summarized with descriptive statistics, including mean and standard deviation (SD) for continuous variables or counts and percentages for categorical variables. Changes from baseline for the hemodynamic parameters and 6-min walk distance were summarized with mean and SD. Student's t-test was performed for comparison of the AT-877ER and placebo treatment groups, and 2-sided 95% confidence intervals for the difference between treatment groups were calculated. Percent changes from baseline were also analyzed in a similar manner. Treatment comparison of the pro-

Table 2. Changes in Cardiac Hemodynamics and 6-min Walk Distance in the Placebo and AT-877ER Groups at Week 12

	Change from baseline		Difference between groups	
	Placebo (n=11)	AT-877ER (n=9)	Difference (95% CI)	P value*
Mean pulmonary arterial pressure (mmHg)	2.2±8.6 (11)	−0.6±2.9 (9)	−2.7 (−8.7 to 3.2)	0.3398
Pulmonary vascular resistance (dyne·s^{-1}·cm^{-5})	72.2±252.1 (11)	−31.8±137.6 (9)	−104.0 (−301.5 to 93.4)	0.2829
Cardiac index (L·min^{-1}·m^{-2})	0.09±0.397 (11)	0.368±0.496 (9)	0.278 (−0.141 to 0.697)	0.1805
Arterial oxygen saturation (%)	−0.48±3.61 (10)	−0.96±2.68 (9)	−0.48 (−3.58 to 2.63)	0.7507
Oxygen saturation of pulmonary artery (%)	−0.53±4.75 (11)	−2.10±3.48 (9)	−1.57 (−5.57 to 2.42)	0.4192
6-min walk distance (m)	31.3±47.9 (12)	18.9±32.3 (9)	−12.4 (−51.1 to 26.4)	0.5131

Data are shown as mean±SD. *t-test.
95% CI, 95% confidence interval.

Table 3. Categorical Counting for Cardiac Index

	Change from baseline >0		Difference between groups	
	Placebo (n=11) % (n/N)	AT-877ER (n=9) % (n/N)	Difference (95% CI)*	P value#
Cardiac index (L·min^{-1}·m^{-2})	45.5% (5/11)	88.9% (8/9)	43.4% (7.6 to 79.3)	0.0428

*No continuity correction; #Chi-square test.

portion of patients who showed improvement in their CI from baseline was performed using the chi-square test. No multiplicity adjustment was performed. All statistical analyses were performed using SAS (version 9.1.3 or later, SAS Institute, Cary, NC, USA).

Results

Patient Enrollment

Of the 34 patients included in this trial, 32 were enrolled for randomization of treatment with either AT-877ER or placebo (Figure S2); 2 patients failed to meet the inclusion criteria. After randomization, 23 of the 32 patients started receiving the study drug, because 5 patients in the AT-877ER group and 4 in the placebo group were excluded according to the inclusion/exclusion criteria (mean PAP <25 mmHg in 4 in the AT-877ER group and in 3 in the placebo group; PCWP >15 mmHg in 1 in the placebo group; serum creatinine level exceeding the upper limit in 1 in the AT-877ER group) (Figure S2). Of the 23 patients, 11 were randomized into the AT-877ER group and 12 as the placebo group (Figure S2), and of them, 9 patients in the AT-877ER group and 11 in the placebo group completed the treatment; 2 patients in the AT-877ER group discontinued the treatment because of the adverse events of renal impairment and heart failure death, respectively, and 1 in the placebo group because of an investigator's decision (Figure S2).

Baseline Patient Characteristics

There were 2 males and 10 females in the placebo group and 5 males and 6 females in the AT-877ER group (Table 1). Age, WHO functional class, type of PAH, combination treatment, and pulmonary hemodynamics are listed in Table 1. In the placebo group, 6 had IPAH, 3 had PAH associated with connective tissue disease (CTD-PAH), 2 had PAH with congenital heart disease (CHD-PAH), and 1 had portal hypertension PAH. In the AT-877ER group, there were 2 cases of IPAH, 5 of CTD-PAH, and 4 of CHD-PAH. There were 3 naive patients in the placebo group and 1 in the AT-877ER group (Table 1).

Tolerance of the Trial Drugs

In the placebo group, 1 patient received 2 capsules/day, 2 patients had 4 capsules/day, and 8 patients had 6 capsules/day, while in the AT-877ER group, 3 patients received 2 capsules/day, 3 patients had 4 capsules/day, and 3 patients had 6 capsules/day; 2 of these patients discontinued the treatment because of renal impairment and heart failure death, respectively, both on day 10. In the placebo group of 6 capsules/day, 1 patient discontinued the treatment on day 84 because of an investigator's decision.

Hemodynamic Parameters

Baseline mean PAP and PVR were lower and baseline CI was higher in the AT-877ER group than in the placebo group (Table 1). After the 3-month study period, mean PAP and PVR tended to be improved in the AT-877ER group compared with the placebo group (Table 2; Tables S1,S2). The incidence of a CI change from baseline was significantly improved in the AT-877ER group compared with the placebo group (Table 3).

Serum Levels of Hydroxyfasudil and Pulmonary Hemodynamics in the AT-877ER Group

In the AT-877ER group, serum levels of hydroxyfasudil, an active metabolite of fasudil, were dose-dependent of AT-877ER (Figure A). Further, serum levels of hydroxyfasudil tended to correlate with the improvements in CI (Figure B) and mean PAP (Figure C), but not of PVR (Figure D).

Safety

Adverse events occurred in all patients in the treatment and placebo groups (Table S3). The patient in the AT-877ER group who died had comorbid heart failure, and a causal relationship with the study drug was ruled out. Three patients experienced serious adverse events other than death (1 in the AT-877ER group and 2 in the placebo group). Pulmonary edema and pleural effusion occurred in 1 patient in the AT-877ER group with resultant death and a causal relationship with the study drug was not definite but possible. Idiopathic thrombocytopenic purpura and increased BNP occurred in 2 patients, respectively, in the placebo group.

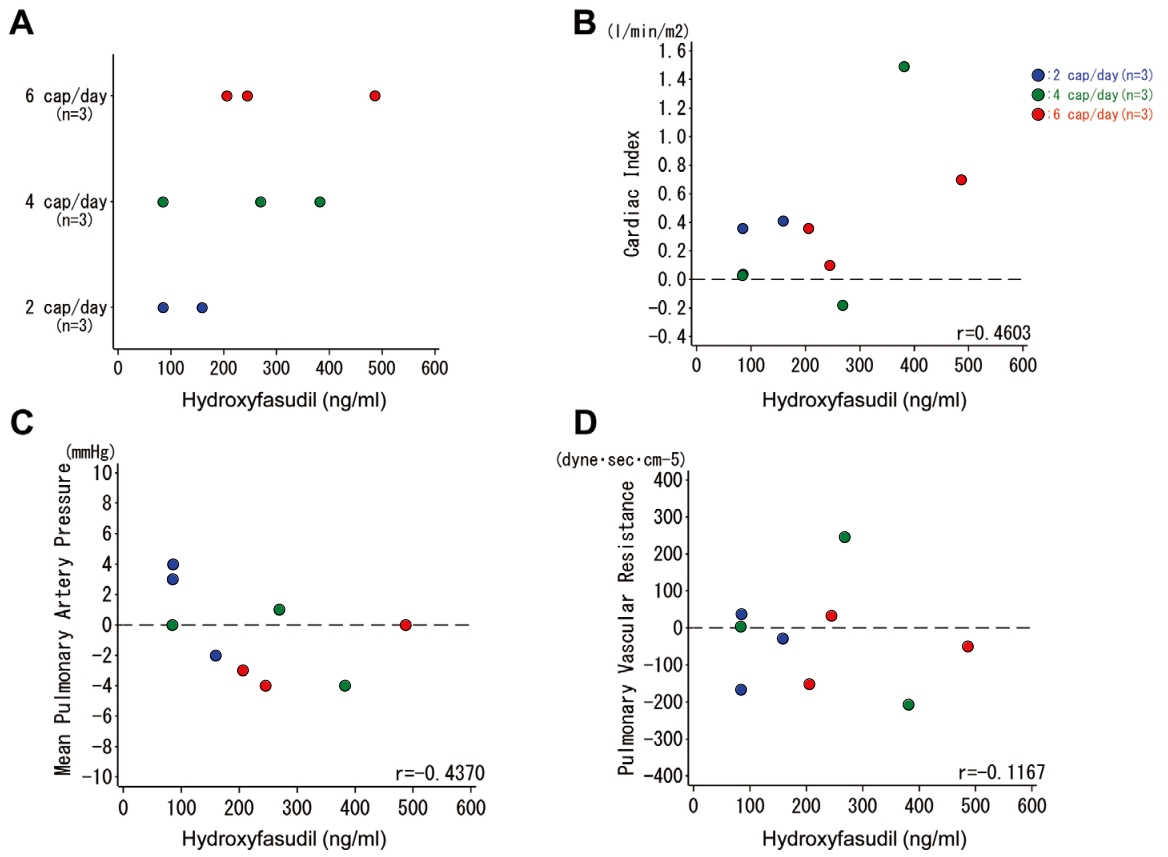

Figure. Serum levels of hydroxyfasudil and pulmonary hemodynamics. (**A**) Serum levels of hydroxyfasudil hydrochloride were dose-dependent of AT-877ER. (**B**–**D**) Serum levels of hydroxyfasudil tended to correlate with improvements in the cardiac index (**B**, r=–0.4370, P=0.2125) and mean pulmonary artery pressure (**C**, r=0.4603, P=0.2395), but not pulmonary vascular resistance (**D**, r=0.1167, P=0.7650). r, Spearman's rank correlation coefficient.

There were 3 discontinuations (2 in the AT-877ER group and 1 in the placebo group). The 2 discontinuations in the AT-877ER group included the patient who had cardiac failure, pulmonary edema and pleural effusion and eventually died, and 1 patient with renal impairment (increased BUN and creatinine levels, and positive proteinuria). The treatment was 3 capsules per dose for these patients, including the patient who discontinued the study because of renal impairment, which had recovered 13 days after discontinuation, and the patient in the placebo group who discontinued because of personal circumstances.

Discussion

The results of the present study showed that 3-month treatment with AT-877ER, a Rho-kinase inhibitor, significantly improved the CI in patients with PAH and that serum levels of AT-877ER tended to correlate with improvements in both CI and mean PAP. Importantly, all patients in the AT-877ER group had been maximally treated with pulmonary vasodilators, including 3 different vasodilators, beraprost, bosentan, and sildenafil.

Rho-Kinase and Inflammation

Inflammatory processes may be involved in the pathogenesis of PAH.[6,22] It has been demonstrated that Rho-kinase is up-regulated by inflammatory stimuli[14,23,24] and that Rho-kinase inhibition increases endothelial nitric oxide synthase (eNOS) expression and inhibits inflammatory cell migration and angiotensin II-induced upregulation of monocyte chemoattractant protein-1 and plasminogen activator inhibitor-1 in vivo or in vitro,[16] suggesting that the Rho-kinase pathway plays an important role in the pathogenesis of PAH.

Rho-Kinase Inhibitor

AT-877ER, fasudil, is a potent and selective inhibitor of Rho-kinase,[25] with its inhibitory effect on Rho-kinase being 100-fold and 1,000-fold more potent than on protein kinase C and myosin light chain kinase, respectively.[16] Several studies, including ours, have demonstrated in animal models that long-term inhibition of Rho-kinase with fasudil ameliorates monocrotaline-induced PAH and hypoxia-induced PAH.[6,17,19,26] Consistent with these findings, intravenous administration of fasudil also effectively reduces PVR in patients with PAH.[18]

Although beraprost sodium has no inhibitory effect on Rho-kinase, we have demonstrated that the combination of fasudil and beraprost is more effective than each monotherapy for ameliorating pulmonary hypertension in a rat model of monocrotaline-induced PAH.[27,28] Furthermore, it has been consistently demonstrated that IPAH patients under intravenous prostacyclin therapy show a favorable acute responses to

fasudil administration.[18,29]

Although inhibition of the ETA and ETB endothelin receptors is another effective strategy for the treatment of PAH,[30] endothelin and many other vasoactive substances (eg, serotonin, thrombin and platelet-derived growth factor) are involved in the pathogenesis of PAH, all of which could activate the Rho-kinase pathway.[14,16,24,31] Because Rho-kinase inhibitors could inhibit signal transductions initiated by all these vasoactive substances, it is highly possible that they exert more broadly beneficial effects than each single receptor antagonist.[14,16,24,31] Thus, the present clinical trial was designed to combine beraprost/sildenafil/bosentan with fasudil in order to develop additional and more beneficial treatment of PAH.

Enhanced Rho-Kinase Expression and Activity in PAH

The experimental studies using animal models have demonstrated that Rho-kinase activity in the pulmonary arteries is enhanced irrespective of etiology and that long-term treatment with Rho-kinase inhibitors ameliorates endothelial dysfunction and suppresses the hypercontraction and proliferation of VSMC and migration of inflammatory cells.[17,19,26] We and others have shown direct clinical evidence of Rho-kinase activation in patients with PAH, in whom Rho-kinase activity is enhanced in circulating neutrophils and the pulmonary arteries, resulting in hypercontraction of the pulmonary arteries[20] and thus supporting the previous findings in both animal models of PAH and patients with PAH.[14,17–19,26,29,32] Furthermore, we have demonstrated that endothelial vasodilator function is impaired and VSMC contraction is enhanced in the pulmonary arteries from patients with PAH,[20] and that inhibition of Rho-kinase abolishes hypercontraction of the VSMCs in the pulmonary arteries from IPAH patients,[20] which could explain the mechanism for the present findings.[18,29,32]

Study Limitations

Several limitations should be mentioned. First, the study group consisted of a small number of Japanese patients with PAH, demonstrating significant effects of AT-877ER on CO but not on pulmonary hemodynamics. Our calculation using the present results predicts that it would reach statistical significance for pulmonary hemodynamics if 100 patients could be recruited for each group. Thus, the present findings need to be confirmed in future studies with a large number of patients. Second, renal impairment occurred in some patients in the AT-877ER group, although a higher concentration of hydroxyfasudil seemed to be favorable for improving pulmonary hemodynamics. Thus, the appropriate dosage of AT-877ER remains to be determined in future trials. Third, the long-term effects of AT-877ER (ie, >3 months) remain to be examined in PAH patients in future clinical trials. Fourth, more male patients were enrolled in the AT-877ER group because there was no randomization by sex. The sex difference in Rho-kinase activity remains be examined in future studies with a larger number of patients. Fifth, the AT-877ER group had better pulmonary hemodynamics and more prevalence of congenital heart disease, which should be adjusted in future studies with a larger number of patients.

Conclusions

Treatment with AT-877ER, an oral form of Rho-kinase inhibitor, could be a new strategy in addition to the present medical treatment of PAH.

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YF: analysis and interpretation of data, drafting of the manuscript. NY, HM, MM, KU, AY, YK, MK, HW, YT, TA, SO, NY, TI: acquisition of data. TN: analysis and interpretation of data; HS: study conception, design, and final approval of the manuscript.

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Disclosures

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Patient Consent

Obtained.

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Supplementary Files

Supplementary File 1

Figure S1. Study design.

Figure S2. Numbers of patients enrolled in the present study who underwent screening and randomization.

Table S1. Cardiac hemodynamics changes and adverse events in the placebo and the AT-877ER groups

Table S2. %Changes in cardiac hemodynamics and 6-min walk distance in the placebo and AT-877ER groups at week 12

Table S3. Adverse events

Please find supplementary file(s);
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