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The orally active nonpeptide selective endothelin ET_A receptor antagonist YM598 prevents and reverses the development of pulmonary hypertension in monocrotaline-treated rats

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Abstract

We investigated the preventive and therapeutic effects of the selective endothelin ET_A receptor antagonist potassium(*E*)-*N*-[6-methoxy-5-(2-methoxyphenoxy)-2-(pyrimidin-2-yl)pyrimidin-4-yl]-2-phenylenthenesulfonamidate (YM598) on the development of pulmonary hypertension in monocrotaline-induced pulmonary hypertensive and hypoxemic rats. In the prevention study, oral administration of YM598 (0.1 and 1 mg/kg) or bosentan (30 mg/kg) for 4 weeks was started on the day following monocrotaline (60 mg/kg) injection. In the therapeutic study, oral administration of YM598 (0.1, 0.3 and 1 mg/kg) for 2 weeks was started 3 weeks after monocrotaline injection. In the prevention study, a marked increase in pulmonary arterial pressure and right ventricular hypertrophy, a decrease in right cardiac function and hypoxemia were observed. Histopathological examination indicated the presence of pulmonary remodeling, including medial wall thickening of the pulmonary microvasculature and alveolar disorders. YM598 suppressed the increase in pulmonary arterial pressure, right ventricular hypertrophy and systemic congestion, and improved the hypoxemia, but bosentan had only modest effects. Histopathological disorders were also ameliorated by YM598. In the therapeutic study, YM598 also ameliorated the pulmonary hypertension and hypoxemia in monocrotaline-treated rats. These results suggest that YM598 effectively prevented and reversed the development of pulmonary hypertension, and reduced the pulmonary vascular remodeling and parenchymal injury in monocrotaline-treated rats. YM598 also improved hypoxemia which accompanied with the severe pulmonary hypertension in these rats.

Keywords: Endothelin; Heart failure; Hypertension; Hypoxia

1. Introduction

Pulmonary hypertension is an elevation in pulmonary arterial pressure that can result from diverse diseases and is characterised by vascular spasm and remodeling in the resistance vessels with progressive luminal obliteration (Wagenwoort and Mooi, 1989; Yousem, 1990). In pulmonary hypertensive patients, pulmonary arterial pressure is inversely related to arterial oxygen tension, and pulmonary hypertension is typically accompanied by chronic respiratory failure with the appearance of hypoxemia (Miyamoto et al., 1983; Ferri et al., 1995). When severe, pulmonary hypertension is accompanied by cor pulmonale or right heart failure.

Endothelial cells of the pulmonary microvasculature are frequently exposed to extraneous substances such as drugs, toxins or abnormal metabolic products, and injury to these cells can result in pathological changes in the alveoli (Ogata et al., 1989). Monocrotaline is an alkaloid contained in the seeds of *Crotalaria spectalalia*, and its metabolite, monocrotaline pyrrole, injures the endothelial cells of pulmonary blood vessels, causing pulmonary hypertension and interstitial pulmonary fibrosis (Merkow and Kleinerman, 1966; Meyrick et al., 1980; Ghodsi and Will, 1981; Bruner et al., 1983). This substance also induces the proliferation of muscular intimal cells in arterioles and of fibroblasts in alveolar walls at the capillary level. Various growth factors are considered to be involved in cell proliferation following

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injury to endothelial cells. Among these, the potent vasoconstrictor peptide endothelin-1, comprising 21 amino acids, induces the proliferation and migration of smooth muscle cells and fibroblasts (Dubin et al., 1989; Takuwa et al., 1989), and its association with pulmonary hypertension has recently been indicated. Endothelin-1 was discovered in a culture medium of endothelial cells from porcine aorta (Yangisawa et al., 1988). It is known to act through two types of receptors, endothelin ET_A and endothelin ET_B receptors (Arai et al., 1990; Sakurai et al., 1990). Plasma endothelin-1 levels are reportedly high in pulmonary hypertensive patients (Cody et al., 1992; Giaid et al., 1993; Ferri et al., 1995), and the ameliorative effects of endothelin receptor antagonists in a monocrotaline-induced pulmonary hypetensive rats have been reported (Miyauchi et al., 1993; Hill et al., 1997; Prie et al., 1997; Jasmin et al., 2001). These data suggest that endogenous endothelin-1 is involved in the progression of pulmonary hypertension, and endothelin receptor antagonists are expected to prove effective in the treatment of this condition.

In these previous studies, the ameliorate effects of endothelin receptor antagonists were mainly investigated using pulmonary arterial pressure and right ventricular hypertrophy. A more comprehensive evaluation, however, would also require the assessment of effects on arterial oxygen pressure (PaO_2) and histopathological changes in the lung, these being also helpful to understanding the severity of pulmonary hypertension. In the present study, we investigated the preventive and therapeutic effects of an endothelin receptor antagonist on the progression of monocrotaline-induced pulmonary hypertension in rats by measuring not only pulmonary arterial pressure and right ventricular hypertrophy but also PaO_2 and histopathological changes, using the newly synthesized, nonpeptide, selective endothelin ET_A receptor antagonist potassium(*E*)-*N*-[6-methoxy-5-(2-methoxyphenoxy)-2-(pyrimidin-2-yl)pyrimidin-4-yl]-2-phenylenthenesulfonamidate (YM598) (Yuyama et al., 2003). We also compared the effects of YM598 in this pulmonary hypertensive model with those of bosentan (Clozel et al., 1994), which was recently launched for the treatment of primary pulmonary hypertension (Rubin et al., 2002).

2. Methods

2.1. Animals and materials

Male Wistar rats (5–7 weeks old) were obtained from SLC Japan (Shizuoka, Japan). YM598 and bosentan were synthesized at Yamanouchi Pharmaceutical (Tsukuba, Japan). YM598 and bosentan were dissolved or suspended in a 0.5% methylcellulose solution. Doses of YM598 and bosentan are represented in terms of the salt. Monocrotaline was purchased from Wako (Osaka, Japan). All other chemicals were of analytical grade.

2.2. Preparation of animal model

Monocrotaline was dissolved in 1 N HCl, and the pH was adjusted to 7.4. Pulmonary hypertensive rats were prepared by injecting the monocrotaline at a subcutaneous dose of 60 mg/kg into the dorsum of the neck.

2.3. Assessment of drug efficacy on pulmonary hypertension: prevention study

In the prevention study, 44 monocrotaline-treated and 11 sham-treated rats were used. One day after the injection of monocrotaline, the animals were randomly divided into four groups ($n = 11$ each), and given a single daily administration of either 0.5% methylcellulose solution as the vehicle-treated group, or YM598 (0.1 and 1 mg/kg) or bosentan (30 mg/kg) with a dosing cannula for 4 weeks. The sham-treated rats ($n = 11$) received vehicle. At 24 h after the final administration, hemodynamic studies were performed. The animals were anaesthetized with sodium pentobarbital (50 mg/kg, i.p.). A catheter (PE-50) was inserted into the left carotid artery to measure mean arterial blood pressure and heart rate, and a second catheter was inserted into the right ventricle (RV) to measure right ventricular systolic pressure, right ventricular end-diastolic pressure and peak positive first derivative of right ventricular pressure ($RV + dP/dt_{max}$). As right ventricular systolic pressure is nearly equal to pulmonary arterial pressure, an increase in right ventricular systolic pressure was considered as an indication of pulmonary hypertension. The position of the catheter was guided by the shape of the pressure tracing displayed on a polygraph system (RM-6000, Nihon Kohden, Tokyo, Japan). After the measurement of right ventricular systolic pressure, right ventricular end-diastolic pressure and $RV + dP/dt_{max}$, the catheter was withdrawn from the right ventricle and placed at the abdominal portion of the vena cava to measure central venous pressure as an index of systemic congestion. All hemodynamic variables were measured and recorded with a polygraph system. After measurement, the rats were exsanguinated, and cardiopulmonary tissues were excised and the wet weights of the RV, left ventricle including septum (LV + S) and lungs were measured. The RV-to-LV + S ratio [RV/(LV + S)] and RV-to-body weight (BW) ratio (RV/BW) in milligrams per gram were used as indices of right ventricular hypertrophy, while lung-to-BW ratio (Lung/BW) in milligrams per gram was used as an index of pulmonary congestion.

2.4. Blood gas analysis in arterial blood

Arterial blood gas variables were analyzed in a separate groups of rats, which had been pre-treated in the same manner as those in the hemodynamic study. Following subcutaneous administration of monocrotaline ($n = 40$), 0.5% methylcellulose solution for the vehicle-treated group ($n = 8$), YM598 (0.1 mg/kg, $n = 12$ and 1 mg/kg, $n = 8$) and

bosentan (30 mg/kg, $n=12$) were administered once daily with a dosing cannula for 4 weeks. Sham-treated rats ($n=6$) received vehicle solution. At 3 weeks of treatment, a cannula for blood sampling was inserted into the left carotid artery under ether anesthesia and secured on the back of the neck. The day after final administration, blood was sampled under conscious conditions through the catheter and subjected to analysis using an automatic blood gas analyzer (Chiron 348, Tokyo, Japan). The analysis included PaO_2 and arterial carbon dioxide pressure ($PaCO_2$). The alveolar–arterial oxygen tension gradient (A-aDO_2), an index of ventilation–perfusion inequality, was also calculated. After blood sampling, pulmonary tissues were excised from the sham, monocrotaline- and vehicle-treated, and monocrotaline- and YM598 (1 mg/kg)-treated groups. After 10% neutral-buffered formalin was injected into the trachea, the tissues were fixed by immersion in the same buffer and used for the following histopathological examinations.

2.5. Histopathological examination

Histopathological examination of tissue slides was performed at Mitsubishi Chemical Safety Institute (Ibaraki, Japan). From the left lobe of a lung, five regions, including the hilus and the two anterior and two posterior regions thereto, were cut in cross-section and stained with hematoxylin and eosin in the usual manner. Pulmonary arterioles were classified into three categories according to diameter (<100, 100–200 and >200 μm) using an image processor for analytical pathology (IPAP, Sumika Technoservice, Hyogo, Japan) by an observer blinded to the experimental group. The percent wall thickness of muscular arteries was calculated for each category using the following formula and the mean value was obtained for each animal.

$$\text{Percent wall thickness (\%)} = [(\text{cross-sectional area-luminal area})/\text{cross-sectional area}] \times 100$$

Pathological findings in the lung parenchyma were scored as follows: (−) negative, (1+) mild, (2+) moderate and (3+) severe.

2.6. Therapeutic study

In the preliminary study, a significant increase in right ventricular systolic pressure was also observed 3 weeks after monocrotaline injection, suggesting that pulmonary hypertension was already established even at this stage. This model was therefore used to study the therapeutic effects of YM598.

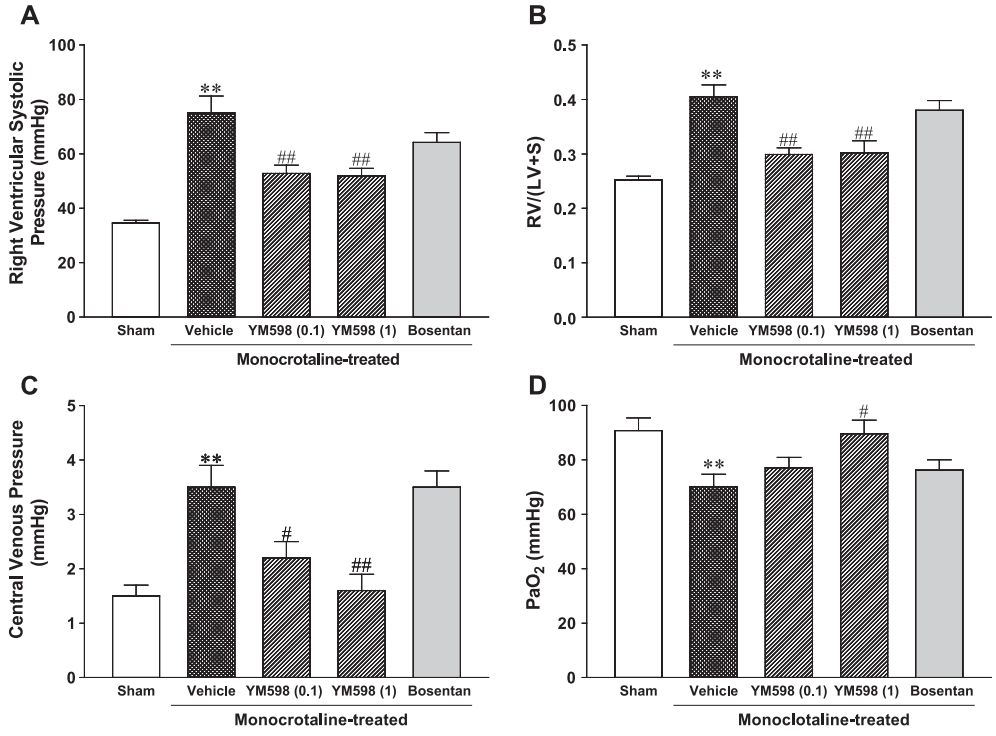

Fig. 1. Effects of YM598 and bosentan on the progression of pulmonary hypertension in monocrotaline-treated rats in the preventive study. Following subcutaneous administration of monocrotaline, drugs were administered once daily for 4 weeks. The effects on pulmonary hypertension (A), right ventricular hypertrophy (B), systemic congestion (C) and hypoxemia (D) were shown. We used right ventricular systolic pressure as an index of pulmonary hypertension, right ventricle to left ventricle including septum [RV/(LV + S)] for right ventricular hypertrophy, central venous pressure for systemic congestion, and arterial oxygen pressure (PaO_2) for hypoxemia. Each column represents the mean ± S.E.M. ($n=6–11$). The doses of YM598 were 0.1 or 1 mg/kg, and the dose of bosentan was 30 mg/kg, respectively. $^{**}P<0.01$ compared with the sham group by Student's t test. $^{\#}P<0.05$, $^{\#\#}P<0.01$ compared with the monocrotaline–vehicle group by Dunnett's multiple range test.

Table 1
Body weight and wet weight of the heart in monocrotaline-treated rats administered YM598 and bosentan in the preventive study

	Body weight (g)	RV (mg)	LV + S (mg)	Lung weight (mg)	RV/BW (mg/g)	Lung/BW (mg/g)
Sham	257 ± 3	131 ± 4	519 ± 11	923 ± 17	0.51 ± 0.01	3.59 ± 0.04
Monocrotaline-treated						
Vehicle	211 ± 5[a]	195 ± 10[a]	484 ± 19	1180 ± 40[a]	0.92 ± 0.04[a]	5.59 ± 0.14[a]
YM598 (0.1)	222 ± 3	158 ± 7[b]	530 ± 10	1145 ± 35	0.71 ± 0.03[c]	5.14 ± 0.11
YM598 (1)	218 ± 3	159 ± 10[b]	529 ± 10	1149 ± 33	0.73 ± 0.06[b]	5.29 ± 0.22
Bosentan	212 ± 6	184 ± 11	484 ± 17	1162 ± 43	0.87 ± 0.05	5.52 ± 0.25

Following subcutaneous administration of monocrotaline, drugs were administered once daily for 4 weeks. Values represent the mean ± S.E.M. ($n = 11$). RV = right ventricle; LV + S = left ventricle with septum; BW = body weight. The doses of YM598 were 0.1 or 1 mg/kg, and the dose of bosentan was 30 mg/kg.

[a] $P < 0.01$ compared with the sham group by Student's t test.

[b] $P < 0.05$ compared with the monocrotaline–vehicle group by Dunnett's multiple range test.

[c] $P < 0.01$ compared with the monocrotaline–vehicle group by Dunnett's multiple range test.

The variables measured in the therapeutic study were the same as those in the prevention study. Three weeks after the injection of monocrotaline, the animals were randomly divided into four groups, and orally given 0.5% methylcellulose solution as the vehicle ($n = 10$) or YM598 (0.1 mg/kg, $n = 9$; 0.3 mg/kg, $n = 9$; and 1 mg/kg, $n = 10$) once daily with a dosing cannula for 2 weeks. The sham-treated rats ($n = 8$) received the vehicle. One day after the final drug administration, hemodynamic variables were measured in the same way as in the prevention study.

Arterial blood gas measurements and histopathological examinations were done in separate groups of rats which had been pre-treated in the same manner as in the hemodynamic study. Three weeks after injection of monocrotaline, four groups ($n = 10$ each) were orally given the vehicle, or YM598 (0.1, 0.3 and 1 mg/kg) once daily with a dosing cannula for 2 weeks. The sham-treated group ($n = 8$) received the vehicle. During the 2nd week of administration, a cannula for blood sampling was inserted into the left carotid artery. One day after the final drug administration, blood was sampled and blood gas variables were measured. After sampling, pulmonary tissues were excised and fixed for use in histopathological examinations. Pathological observation in the lung was performed in the sham-treated, monocrotaline- and vehicle-treated, and monocrotaline- and YM598 (1 mg/kg)-treated groups.

2.7. Statistical analysis

Values are expressed as the mean ± S.E.M. Values were analyzed using SAS software (SAS Institute, NC, USA). Regarding hemodynamic and blood gas variables, the sham-treated and monocrotaline–vehicle groups were compared using Student's t test, and the monocrotaline–vehicle and YM598 groups using Dunnett's multiple range test. Thickness of the pulmonary artery in different groups was analyzed using Student's t test. Histopathological findings were analyzed using Armitage's χ^2 test. In all analyses, a P value of less than 0.05 was considered statistically significant.

2.8. Ethical considerations

The protocol for this study was approved by the Animal Ethical Committee of Yamanouchi Pharmaceutical.

3. Results

3.1. Preventive effects of YM598 in monocrotaline-treated rats: effect on hemodynamic variables, right ventricular hypertrophy and blood gas variables

Compared to sham-treated rats, monocrotaline-treated rats receiving vehicle showed a marked increase in right

Table 2
Effects of YM598 and bosentan on hemodynamic variables in monocrotaline-treated rats in the preventive study

	Heart rate (beats/min)	Mean arterial blood pressure (mm Hg)	Right ventricular end-diastolic pressure (mm Hg)	RV + dP/dt_{max} (mm Hg/s)
Sham	412 ± 14	109.7 ± 3.3	2.7 ± 0.4	3652 ± 289
Monocrotaline-treated				
Vehicle	415 ± 12	113.9 ± 4.1	6.3 ± 0.8[a]	5944 ± 434[a]
YM598 (0.1)	414 ± 13	118.9 ± 4.9	4.3 ± 0.6	5728 ± 270
YM598 (1)	404 ± 9	110.4 ± 4.3	3.3 ± 0.4[b]	6267 ± 564
Bosentan	393 ± 12	114.9 ± 3.3	5.5 ± 0.8	4840 ± 283

Following subcutaneous administration of monocrotaline, drugs were administered once daily for 4 weeks. Values represent the mean ± S.E.M. ($n = 9$–11). RV + dP/dt_{max} = peak positive first derivative of right ventricular pressure. The doses of YM598 were 0.1 or 1 mg/kg, and the dose of bosentan was 30 mg/kg.

[a] $P < 0.01$ compared with the sham group by Student's t test.

[b] $P < 0.01$ compared with the monocrotaline–vehicle group by Dunnett's multiple range test.

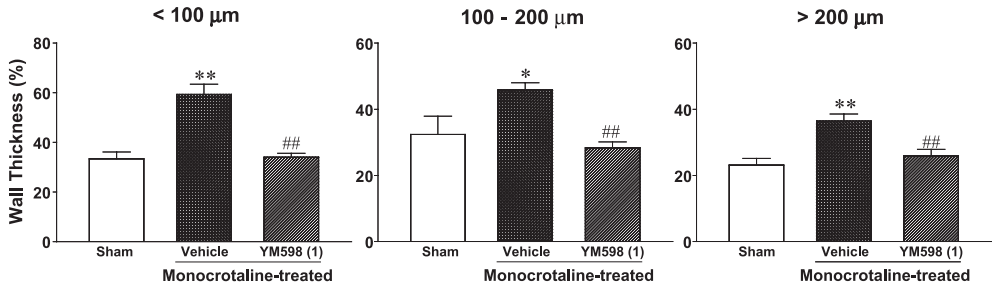

Fig. 2. Effects of YM598 on the percent wall thickness of the pulmonary arterioles in monocrotaline-treated rats in the preventive study. Following subcutaneous administration of monocrotaline, drugs were administered once daily for 4 weeks. Each column represents the mean ± S.E.M. ($n = 5–6$). The dose of YM598 was 1 mg/kg. *$P < 0.05$, **$P < 0.01$ compared with the sham group, $^{\#\#}P < 0.01$ compared with the monocrotaline–vehicle group tested by Student's t test.

ventricular systolic pressure at 4 weeks after monocrotaline injection, demonstrating the presence of pulmonary hypertension (Fig. 1A). These rats also showed increases in RV, RV/BW and RV/(LV + S) values, indicating marked right ventricular hypertrophy (Fig. 1B; Table 1). BW in the monocrotaline–vehicle group was lower than in the sham-treated group, but no differences were observed in heart rate or mean arterial blood pressure (Tables 1 and 2). A marked increase was observed in Lung weight, Lung/BW and central venous pressure values, demonstrating the presence of pulmonary congestion and systemic congestion (Table 1; Fig. 1C). A marked increase in right ventricular end-diastolic pressure suggested decreased diastolic capacity of the right ventricle (Table 2). A increase in RV + dP/dt_{max} was also observed (Table 2).

Four-week administration of YM598 (0.1 and 1 mg/kg) significantly suppressed the increases in right ventricular systolic pressure, RV, RV/BW, RV/(LV + S), central venous pressure and right ventricular end-diastolic pressure without affecting heart rate or mean arterial blood pressure (Tables 1 and 2; Fig. 1A–C). On the other hand, the non-selective endothelin receptor antagonist bosentan (30 mg/kg) had only modest effects against pulmonary hypertension and RV hypertrophy in this model (Tables 1 and 2; Fig. 1A–C).

In monocrotaline-treated rats receiving vehicle, a marked decrease in PaO_2 was observed 4 weeks after injection in comparison with sham-treated rats, demonstrating the presence of hypoxemia (Fig. 1D). Four-week administration of YM598 (0.1 and 1 mg/kg) significantly inhibited the decrease in PaO_2, indicating that an improvement in hypoxemia (Fig. 1D). Bosentan (30 mg/kg) had little effect against the decrease in PaO_2 (Fig. 1D). No difference between groups was observed in $PaCO_2$ [sham, 37.3 ± 1.2 mm Hg; monocrotaline-treated, 33.3 ± 3.4 mm Hg; monocrotaline–YM598 (0.1), 34.1 ± 1.3 mm Hg; YM598 (1), 33.8 ± 2.8 mm Hg; bosentan, 31.6 ± 2.0 mm Hg; $n = 6–11$]. Hematocrit tended to decreased in monocrotaline-treated rats (sham, 46.6 ± 2.7 vs. MCT-treated 34.9 ± 4.9, $P = 0.07$, $n = 6–7$), whereas YM598 had no effect on this change.

3.2. Preventive effects of YM598 in monocrotaline-treated rats: effect on pulmonary vascular remodeling and histopathological changes in the lung

The percent wall thickness of muscular arteries in monocrotaline-treated rats receiving vehicle was greater than that in the sham-treated rats in all diameter categories, demonstrating the progression of pulmonary vascular remodeling (Fig. 2). Administration of YM598 (1 mg/kg) significantly prevented these monocrotaline-induced increases in arterial medial thickness (Fig. 2).

Table 3
Histopathological findings in the lung and effects of YM598 in monocrotaline-treated rats in the preventive study

Findings	Group grade	Sham, $n = 5$	Control, $n = 6$	YM598 (1), $n = 5$
Emphysema	–	5	2	5
	1+	0	3	0
	2+	0	0	0
	3+	0	1	0
Interstitial pneumonia	–	5	0[a]	3[b]
	1+	0	3	2
	2+	0	2	0
	3+	0	1	0
Swelling of alveolar epithelium	–	5	0[a]	1[a]
	1+	0	6	4
	2+	0	0	0
	3+	0	0	0
Hematoidin	–	5	6	3
	1+	0	0	2
	2+	0	0	0
	3+	0	0	0
Alveolar foamy macrophages	–	5	4	1[a]
	1+	0	2	4
	2+	0	0	0
	3+	0	0	0

(–) Negative, (1+) mild, (2+) moderate, (3+) severe.
Following subcutaneous administration of monocrotaline, drugs were administered once daily for 4 weeks. Control means the monocrotaline–vehicle group, and YM598 (1) means monocrotaline–YM598 (1 mg/kg, p.o.) group.
[a] $P < 0.01$ compared with the sham group.
[b] $P < 0.05$ compared with the control group by Armitage's χ^2 test.

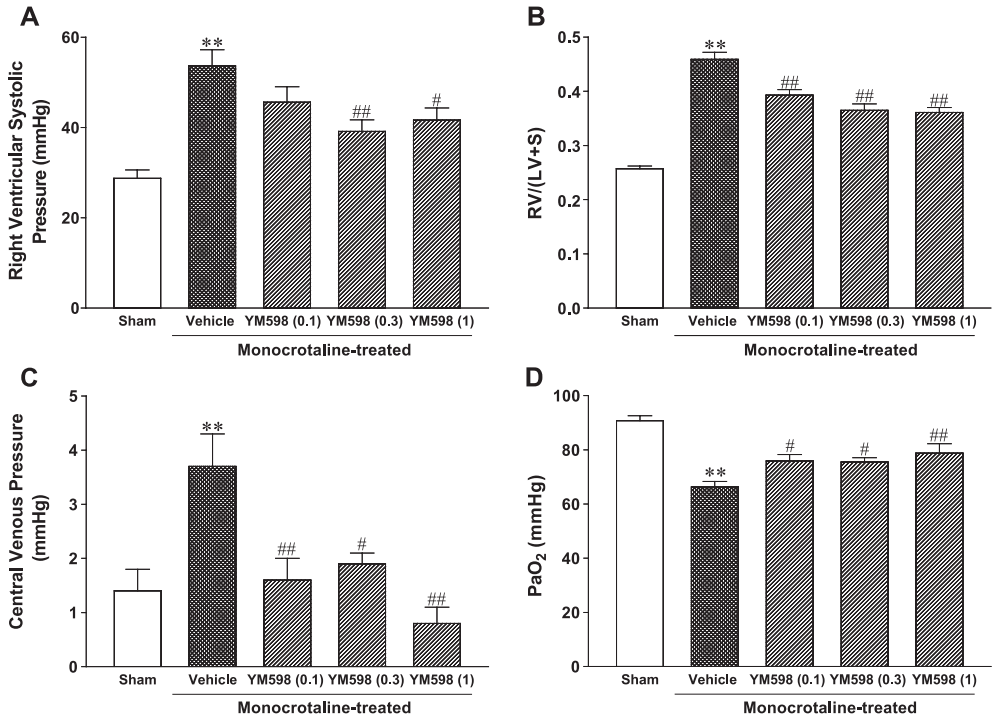

Fig. 3. Effects of YM598 on the progression of pulmonary hypertension in monocrotaline-treated rats in the therapeutic study. Three weeks after subcutaneous administration of monocrotaline, drugs were administered once daily for 2 weeks. The effects on pulmonary hypertension (A), right ventricle hypertrophy (B), systemic congestion (C) and hypoxemia (D) were shown. We used right ventricular systolic pressure as an index of pulmonary hypertension, right ventricle to left ventricle including septum [RV/(LV + S)] for right ventricular hypertrophy, central venous pressure for systemic congestion, and arterial oxygen pressure (PaO_2) for hypoxemia. Each column represents the mean ± S.E.M. ($n = 7–10$). $^{**}P < 0.01$ compared with the sham group by Student's t test. $^{\#}P < 0.05$, $^{\#\#}P < 0.01$ compared with the monocrotaline–vehicle group by Dunnett's multiple range test.

On histopathological examination, emphysema was observed in four of six monocrotalien-treated rats receiving vehicle, whereas no such findings were noted in the sham- or monocrotaline-treated rats receiving YM598 (1 mg/kg) (Table 3). Interstitial pneumonia, characterized by thickening of the alveolar septum and inflammatory cell infiltration into the thickened area, was observed more frequently in monocrotaline-treated rats receiving vehicle than in the sham-treated rats (Table 3). The incidence of interstitial pneumonia in the monocrotaline-treated rats receiving YM598 was significantly lower than in those receiving vehicle (Table 3). In addition, swelling of the alveolar epithelium, characterized by karyomegaly and basophilic cytoplasm and aggregation of alveolar foamy macrophages, was also observed in monocrotaline-treated rats. Hematoidin deposition was noted in the alveoli of the monocrotaline-treated rats receiving YM598 (Table 3).

3.3. Therapeutic effects of YM598 in monocrotaline-treated rats: effect on hemodynamic variables, right ventricular hypertrophy, and blood gas variables

Right ventricular systolic pressure was also observed in monocrotaline-treated rats receiving vehicle 3 weeks after

Table 4
Body weight and wet weight of the heart in monocrotaline-treated rats administered YM598 in the therapeutic study

	Body weight (g)	RV (mg)	LV + S (mg)	Lung weight (mg)	RV/BW (mg/g)	Lung/BW (mg/g)
Sham	255 ± 4	136 ± 3	528 ± 9	927 ± 21	0.53 ± 0.01	3.64 ± 0.06
Monocrotaline-treated						
Vehicle	203 ± 5[a]	217 ± 6[a]	475 ± 11[a]	1121 ± 40[a]	1.07 ± 0.02[a]	5.50 ± 0.08[a]
YM598 (0.1)	204 ± 5	193 ± 5[b]	492 ± 12	1059 ± 27	0.95 ± 0.02[c]	5.20 ± 0.05[b]
YM598 (0.3)	201 ± 5	180 ± 8[c]	493 ± 10	1023 ± 29	0.90 ± 0.02[c]	5.11 ± 0.09[c]
YM598 (1)	210 ± 4	182 ± 5[c]	504 ± 8	1038 ± 22	0.87 ± 0.02[c]	4.95 ± 0.08[c]

Three weeks after subcutaneous administration of monocrotaline, drugs were administered once daily for 4 weeks. Values represent the mean ± S.E.M. ($n = 8–10$). RV = right ventricle; LV + S = left ventricle with septum; BW = body weight. The doses of YM598 were 0.1, 0.3 or 1 mg/kg.

[a] $P < 0.01$ compared with the sham group by Student's t test.

[b] $P < 0.05$ compared with the monocrotaline–vehicle group by Dunnett's multiple range test.

[c] $P < 0.01$ compared with the monocrotaline–vehicle group by Dunnett's multiple range test.

injection, in comparison with sham-treated rats (sham-treated: 31 ± 1.3 mm Hg, monocrotaline-treated: 57.8 ± 6.7 mm Hg, $n = 7$), confirming the development of pulmonary hypertension 3 weeks after the injection of monocrotaline. The increase in right ventricular systolic pressure was still present at 5 weeks after monocrotaline injection, indicating persistent pulmonary hypertension (Fig. 3A). Increases in RV, RV/BW, RV/(LV + S), Lung/BW, central venous pressure and right ventricular end-diastolic pressure were observed in rats 5 weeks after monocrotaline injection, as were a decrease in BW and no change in heart rate or mean arterial blood pressure (Fig. 3B–C; Tables 4 and 5). Further, a slight increase in RV + dP/dt_{max} was observed ($P = 0.053$, Table 5). These changes were closely similar to those observed in rats 4 weeks after monocrotaline injection in the prevention study.

Two-week administration of YM598 (0.1–1 mg/kg) suppressed the increases in right ventricular systolic pressure, RV, RV/BW RV/(LV + S), central venous pressure, Lung/BW and right ventricular end-diastolic pressure without affecting heart rate and mean arterial blood pressure (Fig. 3A–C; Tables 4 and 5).

A decrease in PaO_2 was observed in the monocrotaline-treated rats receiving vehicle in comparison with the sham-treated rats (Fig. 3D). A increase in A-aDO_2 indicated the development of ventilation–perfusion inequality (sham: 14.2 ± 0.7 mm Hg, monocrotaline-treated: 41.9 ± 2.3 mm Hg, $P < 0.01$, $n = 7$ and 9). Two-week administration of YM598 (0.1–1 mg/kg) significantly improved the decrease of PaO_2, indicating the amelioration of hypoxemia (Fig. 3D). The increase in A-aDO_2 was also suppressed [monocrotaline-treated, 41.9 ± 2.3 mm Hg; monocrotaline–YM598 (0.1), 30.8 ± 2.4 mm Hg; YM598 (0.3), 33.9 ± 1.4 mm Hg; YM598 (1), 28.3 ± 2.3 mm Hg; $P < 0.05$, $n = 7–9$]. No difference between groups was observed in $PaCO_2$ [sham, 36.3 ± 1.5 mm Hg; monocrotaline-treated, 33.6 ± 1.0 mm Hg; monocrotaline–YM598 (0.1), 34.9 ± 0.9 mm Hg; YM598 (0.3), 32.7 ± 0.9 mm Hg, YM598 (1), 34.5 ± 1.3 mm Hg; $n = 6–11$]. Hematocrit was decreased in monocrotaline-treated rats (sham, 40.7 ± 1.3 vs. monocrotaline-treated 31.8 ± 1.6, $P < 0.01$, $n = 7–9$), whereas YM598 had no effect on this change.

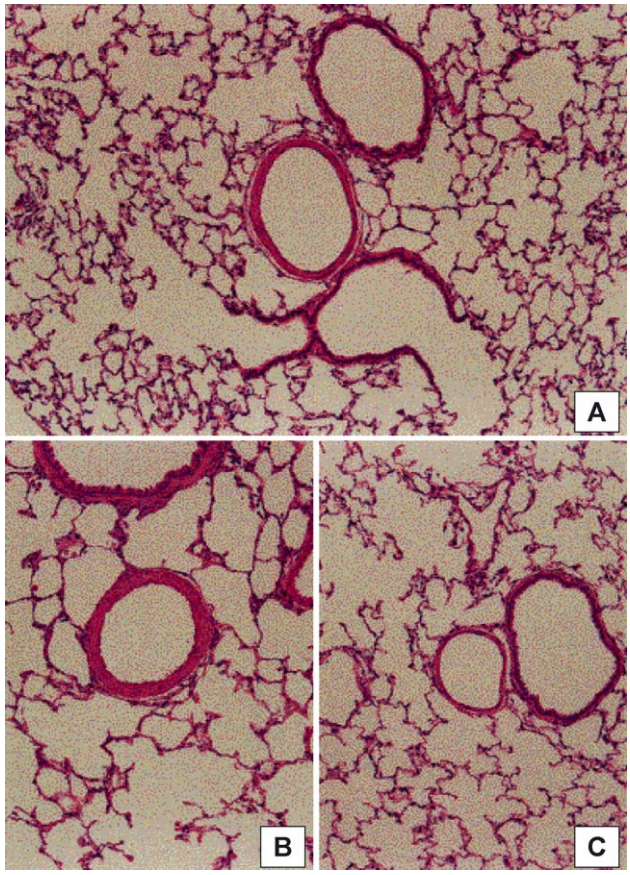

Fig. 4. Effects of YM598 on lung vascular morphology in monocrotaline-treated rats. Three weeks after subcutaneous administration of monocrotaline, drugs were administered once daily for 2 weeks. Lung and pulmonary arterioles were stained with hematoxylin and eosin (HE × 50). (A) Sham rats given vehicle, (B) monocrotaline-control rats given vehicle, (C) monocrotaline–YM rats given YM598 (1 mg/kg) for 2 weeks.

Table 5
Effects of YM598 on the hemodynamic variables in monocrotaline-treated rats in the therapeutic study

	Heart rate (beats/min)	Mean arterial blood pressure (mm Hg)	Right ventricular end-diastolic pressure (mm Hg)	RV + dP/dt_{max} (mm Hg/s)
Sham	391 ± 18	113.1 ± 6.5	1.4 ± 0.5	2197 ± 217
Monocrotaline-treated				
Vehicle	394 ± 11	109.7 ± 11.9	5.4 ± 0.4[a]	2972 ± 283
YM598 (0.1)	407 ± 4	114.2 ± 8.7	3.8 ± 0.7	2600 ± 322
YM598 (0.3)	409 ± 10	126.5 ± 9.8	3.3 ± 0.3[b]	2450 ± 301
YM598 (1)	404 ± 12	103.7 ± 8.7	2.7 ± 0.3[c]	2438 ± 303

Three weeks after subcutaneous administration of monocrotaline, drugs were administered once daily for 4 weeks. Values represent the mean ± S.E.M. ($n = 6–9$). RV + dP/dt_{max} = peak positive first derivative of right ventricular pressure. The doses of YM598 were 0.1, 0.3 or 1 mg/kg. The number of parentheses indicates the number of rats in respective experiments.

[a] $P < 0.01$ compared with the sham group by Student's t test.

[b] $P < 0.05$ compared with the monocrotaline–vehicle group by Dunnett's multiple range test.

[c] $P < 0.01$ compared with the monocrotaline–vehicle group by Dunnett's multiple range test.

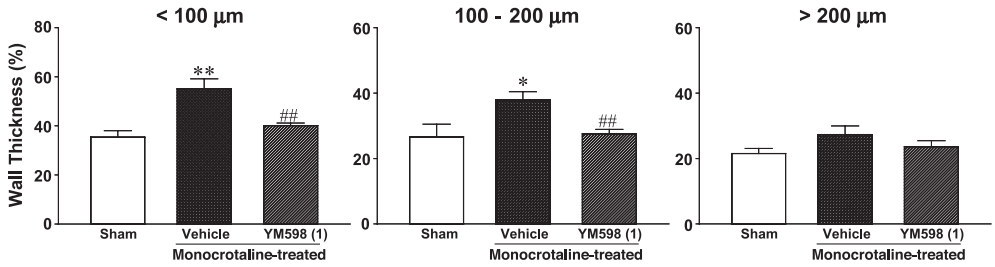

Fig. 5. Effects of YM598 on the percent wall thickness of the pulmonary arterioles in monocrotaline-treated rats in the therapeutic study. Three weeks after subcutaneous administration of monocrotaline, drugs were administered once daily for 2 weeks. Each column represents the mean ± S.E.M. ($n = 7–8$). The dose of YM598 was 1 mg/kg. $^{*}P < 0.05$, $^{**}P < 0.01$ compared with the sham group, $^{\#\#}P < 0.01$ compared with the monocrotaline–vehicle group by Student's t test.

3.4. Therapeutic effects of YM598 in monocrotaline-treated rats: effect on pulmonary vascular remodeling and histopathological changes in the lung

The typical lung morphology was shown in Fig. 4. The percent wall thickness of pulmonary arterioles in the monocrotaline-treated rats was significantly greater than in the sham-treated rats at the < 100 and 100–200 μm levels, indicating the progression of pulmonary vascular remodeling (Fig. 5). Administration of YM598 (1 mg/kg) significantly reduced the arterial medial thickening (Fig. 5).

On histopathological examination, emphysema was observed in two of eight rats in the monocrotaline-treated rats receiving vehicle, whereas no such findings were noted in the sham- or monocrotaline-treated rats receiving YM598 (1 mg/kg) (Table 6). Interstitial pneumonia was present in two of eight monocrotaline-treated rats. These changes were not observed in the sham-treated rats, while findings in the monocrotaline-treated rats receiving YM598 resembled those in the monocrotaline-treated rats (Table 6). Swelling of alveolar epithelia and the presence of alveolar foamy macrophages were also detected in the monocrotaline-treated rats. These findings were absent in the sham-treated group (Table 6).

Table 6
Histopathological findings in the lung and effects of YM598 in monocrotaline-treated rats in the therapeutic study

Findings	Group grade	Sham $n = 5$	Control $n = 6$	YM598 (1) $n = 5$
Emphysema	–	7	6	7
	1+	0	2	0
	2+	0	0	0
	3+	0	0	0
Interstitial pneumonia	–	7	6	5
	1+	0	2	1
	2+	0	0	1
	3+	0	0	0
Swelling of alveolar epithelium	–	5	0[a]	0[a]
	1+	0	8	7
	2+	0	0	0
	3+	0	0	0
Hematoidin	–	7	5	5
	1+	0	3	2
	2+	0	0	0
	3+	0	0	0
Alveolar foamy macrophages	–	7	1[a]	2[a]
	1+	0	7	4
	2+	0	0	0
	3+	0	0	0

(–) Negative, (1+) mild, (2+) moderate, (3+) severe.
Three weeks after subcutaneous administration of monocrotaline, drugs were administered once daily for 4 weeks. Control means the monocrotaline–vehicle group, and YM598 (1) means monocrotaline–YM598 (1 mg/kg, p.o.) group.
[a] $P < 0.01$ compared with the sham group by Armitage's χ^2 test.

4. Discussion

In the present study, we investigated in detail the preventive and therapeutic effects of endothelin receptor antagonist on the progression of pulmonary hypertension and cor pulmonale, using the novel selective endothelin ET_A receptor antagonist YM598. A marked increase in pulmonary arterial pressure, right ventricular hypertrophy, pulmonary congestion, decreased right cardiac function and systemic congestion were observed in rats 4 weeks after treatment of monocrotaline, and hypoxemia also developed. These conditions closely resemble the clinical characteristics of pulmonary hypertension and cor pulmonale. Histopathological examination indicated the existences of arterial medial thickening in the pulmonary microvasculature and alveolar disorders such as emphysema or interstitial pneumonia, suggesting that, in addition to disorders in pulmonary circulation, respiratory function was also suppressed in this model. In the prevention study, the increases in pulmonary arterial pressure, right ventricular hypertrophy and systemic congestion were significantly suppressed in the YM598-treated rats, and hypoxemia was also improved. Arterial medial thickening in the pulmonary microvasculature and alveolar disorders were also significantly reduced. Further, in addition to these preventive effects, the same improvements were also seen in the therapeutic study. These findings indicate that YM598 prevented and reversed the development of pulmonary hypertension in monocrotaline-induced pulmonary hypertensive rats, and strongly suggest that YM598 may ameliorate the condition of patients with pulmonary hypertension and cor pulmonale in clinical use.

In our preliminary study, the rate of death of rats increased 6 weeks after treatment with monocrotaline (60 mg/kg, s.c.). On this basis, we set both the prevention and therapeutic studies to finish within 5 weeks in the present study (prevention study, 4 weeks treatment; therapeutic study, 2 weeks treatment from 3 weeks after monocrotaline injection).

Other selective endothelin ET_A receptor antagonists, namely Cyclo(-D-Trp-D-Asp-Pro-D-Val-Leu-) (BQ-123) and (+)-(*S*)-2-(4,6-dimethoxy-pyrimidin-2-yloxy)-3-methoxy-3,3-diphenyl-propionic acid (LU 135252), have also been shown to be effective in reducing pulmonary hypertension and right ventricular hypertrophy in monocrotaline-induced pulmonary hypertensive rats (Miyauchi et al., 1993; Prie et al., 1997). Together, these and the present results indicate that the effectiveness of selective endothelin ET_A receptor antagonists is unequivocal in pulmonary hypertension in monocrotaline-treated rats. Since the effectiveness of non-selective endothelin receptor antagonists has also been demonstrated (Hill et al., 1997; Jasmin et al., 2001), it remains uncertain whether pulmonary hypertension is better treated with non-selective or selective ET_A receptor antagonists. Jasmin et al. (2001) reported that treatment with a non-selective endothelin receptor antagonist was more effective than that with a selective endothelin ET_A receptor antagonist on pulmonary hypertension in monocrotaline-treated rats. In addition, contraction via endothelin ET_B receptors has also been reported in rat pulmonary vascular and broncho smooth muscle (LaDouceur et al., 1993; Adner et al., 1996). On the other hand, the existence of endothelin ET_B receptor-mediated dilatation in the pulmonary vasculature has been shown from the finding that selective endothelin ET_B receptor antagonists increased pulmonary arterial pressure, suggesting that selective endothelin ET_A receptor antagonists are more favorable as they do not suppress this dilatation of pulmonary vasculature via endothelin ET_B receptors (Okada et al., 1995; Sakai et al., 2000). Furthermore, endothelin ET_B receptors are reported to be clearance receptors of endothelin-1 (Dupuis et al., 1994), and both selective endothelin ET_B and non-selective endothelin receptor antagonists have been reported to delay the clearance of endothelin-1 in the lungs and thereby increase plasma endothelin-1 levels (Dupuis et al., 1996; Hemsen et al., 1996). In the present study, treatment with YM598 at only 1 mg/kg/day prevented and reversed not only pulmonary hypertension and right ventricular hypertrophy but also histopathological changes. Further, bosentan was found to be ineffective at 30 mg/kg. Hill et al. (1997) used bosentan at more than 100 mg/kg to confirm its ameliorating effects on pulmonary hypertension in monocrotaline-treated rats. The dose of bosentan we used was selected to be equipotent to 1 mg/kg of YM598 in terms of in vivo endothelin ET_A receptor antagonistic activity (Yuyama et al., 2003). Because of the suppression of vasodilatation or endothelin-1 clearance via endothelin ET_B receptors, it is possible that higher doses of bosentan might be needed to improve pulmonary hypertension in the face of co-existing endothelin ET_B receptor antagonistic activity. Although further investigation is necessary to determine whether non-selective or selective endothelin ET_A receptor antagonists are better in the treatment of pulmonary hypertension, YM598 may be at least 30-fold more potent than bosentan in pulmonary hypertensive patients. Bosentan has already shown its clinical benefit in patients with pulmonary hypertension at a dose of 125 mg twice daily, and was recently launched for the treatment of primary pulmonary hypertension (Channick et al., 2001; Rubin et al., 2002). In heart failure patients, however, the effective dose of bosentan has been shown to cause liver toxicity or fluid retention in clinical study (Mylona and Cleland, 1999; Kalra et al., 2002). Although it is uncertain whether these adverse effects relate to endothelin receptor antagonistic activities or the chemical structure of bosentan, YM598, which showed curative and palliative effects pulmonary hypertension at quite low doses, may be useful in the treatment of patients with severe pulmonary hypertension without risk of adverse events than bosentan.

Gillespie et al. (1985) described pulmonary function in monocrotaline-treated rats. They demonstrated decreases in tidal volume, lung compliance and respiratory frequency and an increase in lung resistance. They also demonstrated decreased diffusion capacity of the lung. These changes indicate the impairment of gas exchange in the lung and suggest the existence of hypoxemia in monocrotaline-induced pulmonary hypertensive rats. Lai et al. (1996) directly demonstrated the existence of hypoxemia, as in pulmonary hypertension in humans. In the present study, we demonstrated the ameliorative effects of YM598 on hypoxemia without the induction of hyperventilation in monocrotaline-treated rats. As far as we are aware, this is the first report to demonstrate the effectiveness of agents, especially endothelin receptor antagonists, on hypoxemia in monocrotaline-treated rats. On histological examination, emphysema, interstitial pneumonia and swelling of alveolar epithelium were observed in the monocrotaline–vehicle group, but not in the sham-treated group. These changes were regarded as having resulted from monocrotaline injection. As these changes were reduced in the YM598-treated group, this compound is considered to exert preventive effects against emphysema, interstitial pneumonia and swelling of alveolar epithelium. These histopathological results suggested that YM598's improvement of hypoxemia in these monocrotaline-treated rats was partly due to the recovery of respiratory function through histopathological repair in the lung. Most patients with pulmonary hypertension and cor pulmonale have accompanying chronic respiratory failure, resulting in hypoxemia (Miyamoto et al., 1983). Defects in gas exchange during respiratory failure are classified according to $PaCO_2$. At a high $PaCO_2$ tension, alveolar hypoventilation predominates, whereas at a normal $PaCO_2$ tension, ventilation–perfusion inequality typically occurs. Increases in A-aDO_2 are an index of ventilation–perfusion inequality.

Because YM598 inhibited increases in this variable significantly, in addition to the reduction in organic pulmonary injury, correction of the ventilation–perfusion inequality might be a second mechanisms by which YM598 improves hypoxemia.

In conclusion, we have demonstrated that YM598 effectively prevented and reversed the development of pulmonary hypertension, and reduced the pulmonary vascular remodeling and parenchymal injury in monocrotaline-treated rats. YM598 also improved the hypoxemia which accompanied the severe pulmonary hypertension in these rats. These data strongly suggest that YM598 may be clinically useful in patients with both primary and secondary pulmonary hypertension.

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