INTRODUCTION

Pulmonary hypertension (PH) is a poor prognostic and drug-resistant disease characterized by the progressive elevation of pulmonary arterial pressure and pulmonary vascular resistance, ultimately producing right ventricular (RV) failure and leading to death (1). Previously, a variety of drugs have been tried for treating PH patients, such as anticoagulant and vasodilator agents (2, 3). Anticoagulant agents are believed to reduce *in situ* thrombosis in the pulmonary circulation, thus the progression of this disease may be slowed down (2). As to the vasodilator agents, calcium-channel blockers have been used to reduce the vasoconstriction of pulmonary vasculature (3). Furthermore, other treatments such as inotropic agents, diuretics, and oxygen supplement have been used for patients with PH. All of these conventional therapies are partially effective in some patients; however, none of them has resulted in improved survival of these patients (4).

Prostacyclin (PGI_2) is a potent, short-acting vasodilator and inhibitor of platelet aggregation that is endogenously produced from the vascular endothelium (5). Vascular tone is maintained by the balance of vasodilative and vasoconstrictive prostanoids released by platelets and vascular endothelium to a considerable extent (6). An imbalanced production of PGI_2, a vasodilator, and thromboxane A_2 (TXA_2), a vasoconstrictor, in pulmonary circulation exists in patients with PH, and the disorder of this balance seems to cause the progression of PH (6, 7). It has been reported that intravenous PGI_2 infusion significantly reduces pulmonary arterial pressure and pulmonary vascular resistance in patients with PH (5, 8). Recently, continuous intravenous infusion of PGI_2 was reported to improve exercise capacity (4, 9) and long-term survival in patients with PH (4, 10, 11) in addition to lowering pulmonary vascular resistance (12). However, it must be given continuously through a central intravenous catheter infusion system; thus, several complications are serious hazards including recurrent intravenous route infections, blood clotting, and severe systemic hypotension (1, 4, 13).

Beraprost sodium (BPS) is a chemically stable oral PGI_2 analogue, whose pharmacological profile is similar to that of PGI_2 (14-16). It was reported that BPS has a protective effect on the development of PH in animal models (14) and that BPS is effectual for primary and secondary PH

in patients (15, 17). However, the effectiveness of BPS is limited and BPS is not sufficient to treat all of patients with PH.

Endothelin (ET)-1, a potent vasoconstrictor peptide derived from endothelial cells (18, 19), induces the growth of vascular smooth muscle cells (19, 20) and myocardial cell hypertrophy (19-21). Plasma ET-1 level is reported to be increased in patients with PH, and ET-1 is thought to play an important role in the progression of PH (22). We have reported that the expression of ET-1 in the lungs of rats with PH due to congestive heart failure was markedly increased (23) and that the high plasma ET-1 concentration in patients with PH due to congenital heart disease was normalized by successful surgical repair accompanied with great improvement in the hemodynamic state of the pulmonary circulation (24). These observations suggest that endogenous ET-1 may contribute to an increase in pulmonary vascular tone in patients with PH. Furthermore, we have shown that an ET-A receptor antagonist inhibited the progression of PH and ameliorated the vascular structure, RV hypertrophy, and poor survival in rats with PH induced by monocrotaline (MCT) (25, 26). We also have reported that an ET-A receptor antagonist improved PH associated with congestive heart failure (23). Other groups also have reported that an ET receptor antagonist ameliorated PH induced by hypoxia in rats (27, 28). Recently, it was reported that acute ET-A receptor blockade caused selective pulmonary vasodilation in human patients with PH due to chronic heart failure (29). These findings suggest that an ET receptor antagonists are another candidate for the treatment of PH as well as an oral PGI_2 analogue.

We hypothesized that the combination of an oral ET-A receptor antagonist and an oral PGI_2 analogue would be more effective than the single use of each drug alone for ameliorating PH. Because there is no report, in which both drugs were simultaneously administered to PH, we investigated the effectiveness of these drugs against the progression of PH in an animal model with or without combination of the two drugs. The rat treated with MCT is established as an animal model of PH (25, 30-32). A single subcutaneous injection of MCT, a pyrrolizidine alkaloid, causes pulmonary vascular endothelial cell damage and medial wall thickening of muscular pulmonary arteries, which is involved in etiology of PH by MCT, in the lungs (25, 30, 33, 34). Therefore, we used MCT-treated rats as a PH model in this study.