

Endothelin Receptor Antagonists in a Beagle Model of Pulmonary Hypertension: Contribution to Possible Potential Therapy?

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Objectives. This study investigated the pharmacologic effect of endothelin receptor antagonists on cardiopulmonary hemodynamic variables in a beagle model of pulmonary hypertension.

Background. We recently developed a beagle model of pulmonary hypertension that allows accurate determination of cardiopulmonary hemodynamic variables and is associated with elevated plasma endothelin-1 concentrations similar to those in pulmonary hypertension in humans.

Methods. Twelve beagles (pulmonary hypertension, $n = 6$; control group, $n = 6$) were studied during baseline conditions and during right atrial infusion of FR139317 (an ETA receptor antagonist), RES-701-1 (an ETB receptor antagonist), nitroglycerin and prostaglandin E_1 . Pulmonary hypertension was induced in experimental beagles 8 weeks after injection with 3 mg/kg body weight of dehydromonocrotaline.

Results. FR139317 lowered pulmonary artery and systemic arterial pressures in both pulmonary hypertensive and control beagles, with a significantly greater effect on pulmonary artery pressure in pulmonary hypertensive dogs. RES-701-1 tended to increase pulmonary artery pressure only in pulmonary hypertensive beagles. Nitroglycerin depressed pulmonary artery and systemic arterial tone equally well in control and pulmonary hypertensive animals. Prostaglandin E_1 produced a greater decrease in systemic arterial pressure in pulmonary hypertensive than in normal beagles despite having the same effect on pulmonary artery pressure in both.

Conclusions. ETA receptor antagonists decrease pulmonary artery pressure in a beagle model and may therefore be clinically useful for treatment of pulmonary hypertension.

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Clinical use of vasodilator therapy for pulmonary hypertension has yielded inconsistent results (1,2), largely because of such adverse effects as systemic hypotension, right ventricular ischemia, arrhythmias, arterial oxygen desaturation and exacerbation of pulmonary hypertension (3-5). The ideal vasodilator agent for pulmonary hypertension would specifically lower pulmonary artery pressure and minimize the risk of systemic complications. Clinical studies have shown considerable variation in the response to vasodilator agents in small numbers of pulmonary hypertensive patients. This variability may reflect differences in pulmonary vascular pathophysiology as well as in the dose, timing and route of administration of therapeutic agents. Nonrandomized studies using vasodilator therapy (2,6-8) have been performed as a result of the concept that pulmonary hypertension was caused by pulmonary vasoconstriction in response to an external stimulus (9,10). Unfortunately, such therapy has not been demonstrated to improve survival (8). Because thorough comparative studies in humans

are impossible, we developed an animal model of pulmonary hypertension in a relatively large animal to evaluate cardiopulmonary hemodynamic variables accurately. Pulmonary hypertension can be induced in beagles by injection of 3 mg/kg body weight of dehydromonocrotaline (11).

Recent studies have examined the role of abnormal pulmonary endothelial cell function in pulmonary hypertension (12,13) and established that elevated plasma concentrations of endothelin-1 are associated with pulmonary hypertension (14-17). The finding that our model showed elevated plasma endothelin-1 concentrations similar to clinical pulmonary hypertension prompted us to consider whether endothelin receptor antagonists might be efficacious for the treatment of pulmonary hypertension. This study was therefore designed to investigate the hemodynamic response of the cardiopulmonary circulation to the endothelin ETA receptor antagonist FR139317 and the ETB receptor antagonist RES-701-1 in dehydromonocrotaline-induced pulmonary hypertensive beagles. Physiologic effects were compared with those of the vasoactive agents nitroglycerin and prostaglandin E_1 .

Methods

Study protocol. Twelve purebred beagles (mean age 3 months, mean [\pm SD] weight 5.7 ± 1.3 kg) were studied. Before initiation of an experiment, each beagle was anesthetized with sodium pentobarbital (25 mg/kg intravenously) and

From the Department of Surgery, Division II, Kobe University School of Medicine, Kobe, Japan. Fujisawa Pharmaceutical Co., Ltd., Tsukuba, Japan, supplied the FR139317, and Kyowa Hakko Kogyo Co., Ltd., Tokyo, Japan, supplied the RES-701-1.

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permitted to breathe spontaneously. At a venous infusion port, a 5F flow-directed pulmonary artery thermodilution catheter (Baxter Healthcare Corporation) was introduced, and cardiac output was determined by taking the mean of at least three measurements recorded by a cardiac output computer. Another catheter was placed in the femoral artery for monitoring systemic arterial pressure. Arterial and intracardiac pressures were measured by a polygraph (363, NEC San-ei Instruments Ltd.) and recorded (8M14, NEC San-ei Instruments Ltd.). We injected 3 mg/kg of dehydromonocrotaline, which was prepared from monocrotaline (18), into the right atrium of the beagles and investigated cardiopulmonary hemodynamic variables at 8 weeks after injection. A blood sample was collected from the pulmonary artery for determining the plasma endothelin-1 concentration. Each blood sample was placed in a chilled tube containing ethylenediaminetetraacetic acid (EDTA) and aprotinin, and after centrifugation, the plasma was stored at -30°C until used. After endothelin-1 had been extracted through C_{18} (Waters Associates), the concentration was measured by radioimmunoassay with the use of an anti-endothelin-1 antibody (Peninsula Lab. Inc.) and iodine-125-labeled endothelin-1 (Amersham Japan Co.).

FR139317 (Fujisawa Pharmaceutical Co., Ltd.) and RES-701-1 (Kyowa Hakkō Kogyō Co., Ltd.) were infused at doses of 40 or 200 and 20 or 100 $\mu\text{g}/\text{kg}$ per min, respectively. We determined the infusion dose of nitroglycerin and prostaglandin E_1 that reduced pulmonary arterial pressures by $\sim 20\%$ in our pulmonary hypertension model. The appropriate doses were found to be 10 and 0.4 $\mu\text{g}/\text{kg}$ per min for nitroglycerin and prostaglandin E_1 , respectively. FR139317 was dissolved in 1 N sodium hydroxide, and RES-701-1 was dissolved in dimethylsulfoxide. Nitroglycerin and prostaglandin E_1 were dissolved in sterile water. All solutions were prepared on the day of the study and kept on ice until administered. All drugs were delivered through the pulmonary artery by an infusion pump. Under sterile conditions, baseline values were obtained with infusion of vehicle for 30 min, followed by infusion of the drug, each given for 30 min.

Hemodynamic variables. Hemodynamic variables were measured during the last 5 min of each infusion. There was a pause of 24 h between infusion of different drugs. Therefore, we believed that there were no interactions between the treatments with different drugs administered to the same beagles. The experiments were performed in the same order of drug administration in every beagle at baseline and at 8 weeks after dehydromonocrotaline. Systemic vascular resistance was calculated by subtracting mean right atrial pressure (mm Hg) from mean systemic arterial pressure (mm Hg) and dividing by cardiac output (liters/min). Pulmonary vascular resistance was calculated by subtracting mean wedge pressure (mm Hg) from mean pulmonary artery pressure (mm Hg) and dividing by cardiac output. Both values were expressed as $\text{dynes}\cdot\text{s}\cdot\text{cm}^{-5}$ by multiplying the values obtained by a factor of 80. The percent change in the variables was calculated as follows: (Postinjection value - Preinjection value)/Preinjection value. All beagles were kept in clean cages, with regular food and sterile water as

desired, and received humane care in compliance with the "Principles of Laboratory Animal Care" formulated by the Institute of Laboratory Animal Resources and the "Guide for the Care and Use of Laboratory Animals" published by the National Institutes of Health.

Statistical analysis. All data are given as the mean value \pm SD. Data and variables were compared using one-way repeated-measures analysis of variance to determine the effect of dehydromonocrotaline and to compare the efficacies of different drugs. When the one-way repeated-measures analysis of variance was significant, the differences were tested by the Sheffé F test; $p < 0.05$ was considered significant.

Results

Development of pulmonary hypertension in dehydromonocrotaline-treated beagles. A single injection of dehydromonocrotaline produced a significant elevation in pulmonary artery pressure compared with the preinjection value. During the 8 weeks after injection, systolic pulmonary artery pressure, mean pulmonary artery pressure and pulmonary vascular resistance increased significantly from 21.1 ± 2.4 to 55.9 ± 8.3 mm Hg, from 11.8 ± 1.8 to 33.2 ± 5.9 mm Hg and from 196 ± 33 to $1,237 \pm 441$ $\text{dynes}\cdot\text{s}\cdot\text{cm}^{-5}$, respectively. Cardiac output decreased significantly from 2.0 ± 0.2 to 1.7 ± 0.3 liters/min. Heart rate, systemic arterial pressure, right atrial pressure, pulmonary capillary wedge pressure and systemic vascular resistance remained relatively stable and revealed no significant differences. Plasma endothelin-1 levels increased significantly from 1.25 ± 0.23 to 3.12 ± 1.06 pg/ml.

Hemodynamic effects of drug infusion in normal beagles. The physiologic responses to the four drugs in the six normal beagles are shown in Table 1. FR139317 at doses of 40 or 200 $\mu\text{g}/\text{kg}$ per min or RES-701-1 at 20 or 100 $\mu\text{g}/\text{kg}$ per min caused no significant changes. Nitroglycerin at 10 $\mu\text{g}/\text{kg}$ per min significantly decreased mean systemic arterial pressure (from 115 ± 6 to 94 ± 6 mm Hg), pulmonary artery pressure (from 11.8 ± 1.8 to 9.7 ± 2.0 mm Hg), systemic vascular resistance (from $4,511 \pm 458$ to $3,618 \pm 383$ $\text{dynes}\cdot\text{s}\cdot\text{cm}^{-5}$) and pulmonary vascular resistance (from 196 ± 33 to 141 ± 32 $\text{dynes}\cdot\text{s}\cdot\text{cm}^{-5}$). Prostaglandin E_1 at 0.4 $\mu\text{g}/\text{kg}$ per min significantly decreased mean pulmonary artery pressure (from 11.8 ± 1.8 to 9.8 ± 0.8 mm Hg) and pulmonary vascular resistance (from 196 ± 33 to 135 ± 40 $\text{dynes}\cdot\text{s}\cdot\text{cm}^{-5}$) but did not produce significant effects in the systemic circulation. Heart rate and cardiac output were not altered by any of the drugs tested.

Hemodynamic effects of drug infusion in pulmonary hypertensive beagles. The physiologic responses to the four drugs in the six beagles with pulmonary hypertension are shown in Table 2. FR139317 at 200 $\mu\text{g}/\text{kg}$ per min significantly decreased mean pulmonary artery pressure (from 33.2 ± 5.9 to 26.8 ± 3.7 mm Hg) and pulmonary vascular resistance (from $1,237 \pm 441$ to 867 ± 164 $\text{dynes}\cdot\text{s}\cdot\text{cm}^{-5}$). FR139317 also decreased mean systemic arterial pressure and systemic vascular resistance ($p = \text{NS}$). RES-701-1 at 100 $\mu\text{g}/\text{kg}$ per min increased mean pulmonary artery pressure (from 33.2 ± 5.9 to

Table 1. Hemodynamic Values for Normal Beagles at Baseline and During Drug Infusion

	Baseline	FR139317		RES-701-1		Nitroglycerin (10 µg/kg per min)	Prostaglandin E ₁ (0.4 µg/kg per min)
		40 µg/kg per min	200 µg/kg per min	20 µg/kg per min	100 µg/kg per min		
HR (beats/min)	124 ± 13	123 ± 14	125 ± 16	123 ± 13	123 ± 12	127 ± 16	130 ± 14
MSAP (mm Hg)	115 ± 6	115 ± 8	109 ± 9	116 ± 9	116 ± 10	94 ± 6*	110 ± 11
MPAP (mm Hg)	11.8 ± 1.8	11.7 ± 2.1	11.3 ± 2.5	12.0 ± 1.9	11.7 ± 2.3	9.7 ± 2.0*	9.8 ± 0.8*
CO (liters/min)	2.0 ± 0.2	1.9 ± 0.2	1.9 ± 0.3	1.9 ± 0.2	1.9 ± 0.2	1.9 ± 0.2	2.1 ± 0.2
SVR (dynes·cm ⁻⁵)	4,511 ± 458	4,595 ± 651	4,426 ± 663	4,651 ± 568	4,601 ± 563	3,618 ± 383*	4,084 ± 586
PVR (dynes·cm ⁻⁵)	196 ± 33	211 ± 82	195 ± 52	198 ± 69	211 ± 76	141 ± 32*	135 ± 40*

*p < 0.05, baseline versus drug infusions. Data presented are mean value ± SD. CO = cardiac output; HR = heart rate; MPAP = mean pulmonary artery pressure; MSAP = mean systemic arterial pressure; PVR (SVR) = pulmonary (systemic) vascular resistance.

36.2 ± 6.4 mm Hg) but did not affect pulmonary vascular resistance, mean systemic arterial pressure or systemic vascular resistance. Nitroglycerin at 10 µg/kg per min significantly decreased mean systemic arterial pressure (from 106 ± 5 to 87 ± 6 mm Hg), pulmonary artery pressure (from 33.2 ± 5.9 to 27.0 ± 5.1 mm Hg), systemic vascular resistance (from 4,685 ± 439 to 3,767 ± 229 dynes·cm⁻⁵) and pulmonary vascular resistance (from 1,237 ± 441 to 856 ± 207 dynes·cm⁻⁵). Prostaglandin E₁ at 0.4 µg/kg per min significantly decreased mean systemic arterial pressure (from 106 ± 5 to 93 ± 7 mm Hg), pulmonary artery pressure (from 33.2 ± 5.9 to 27.8 ± 4.1 mm Hg), systemic vascular resistance (from 4,685 ± 439 to 3,718 ± 120 dynes·cm⁻⁵) and pulmonary vascular resistance (from 1,237 ± 441 to 870 ± 243 dynes·cm⁻⁵). Both nitroglycerin at 10 µg/kg per min and prostaglandin E₁ at 0.4 µg/kg per min increased heart rate but did not produce significant differences.

Vasodepressor effects of drug infusion on systemic arterial and pulmonary artery pressures. The decreases in mean systemic arterial and pulmonary artery pressures are summarized in Figure 1. FR139317 at 200 µg/kg per min produced selectively reduced pulmonary artery pressure in pulmonary hypertensive beagles, with little effect on systemic arterial pressure in pulmonary hypertensive or normal beagles (pulmonary artery and systemic arterial pressures, 14.6% and 5.2%, respectively, in pulmonary hypertensive beagles and 4.2% and 4.8%, respectively in normal beagles). Nitroglycerin at 10 µg/kg per min produced a decrease in systemic arterial and pulmonary artery pressures. However, there were no significant differences in response between normal and pulmonary hypertensive beagles (systemic arterial and pulmonary artery

pressures 17.8% and 18.3%, respectively, in normal beagles and 18.2% and 18.6%, respectively, in pulmonary hypertensive beagles). Prostaglandin E₁ at 0.4 µg/kg per min caused a similar decrease in pulmonary artery pressure in normal and pulmonary hypertensive beagles but produced a greater decrease in systemic arterial pressure in pulmonary hypertensive than in normal beagles (pulmonary artery pressure 15.5% and 16.1%, respectively, in normal and pulmonary hypertensive beagles; systemic arterial pressure 11.0% and 4.1%, respectively, in normal and pulmonary hypertensive beagles).

Discussion

Reports that patients with either primary or secondary pulmonary hypertension had increased plasma endothelin-1 concentrations (14-17) and injured vascular endothelial cells in the pulmonary circulation (19) inspired the series of experiments described in the present study. A single subcutaneous injection of monocrotaline causes pulmonary hypertension in rats (20-24) and a progressive increase in plasma endothelin-1 concentration (25). To examine the pharmacologic effects on the pulmonary vasculature, we sought a larger animal model of pulmonary hypertension in which cardiopulmonary hemodynamic variables could be accurately measured. We therefore established a beagle model of pulmonary hypertension induced by right atrial injection of 3 mg/kg of dehydromonocrotaline. This experimental model has promising similarities to pulmonary hypertension in humans, which also develops long after induction by a variety of factors. Indeed, our pulmonary hypertension model showed an elevation in plasma endothelin-1 concentration similar to that in clinical pulmonary hypertension. This observa-

Table 2. Hemodynamic Values for Pulmonary Hypertensive Beagles at Baseline and During Drug Infusion

	Baseline	FR139317		RES-701-1		Nitroglycerin (10 µg/kg per min)	Prostaglandin E ₁ (0.4 µg/kg per min)
		40 µg/kg per min	200 µg/kg per min	20 µg/kg per min	100 µg/kg per min		
HR (beats/min)	135 ± 13	135 ± 10	136 ± 9	134 ± 9	133 ± 12	146 ± 18	146 ± 10
MSAP (mm Hg)	106 ± 5	105 ± 6	101 ± 7	105 ± 5	109 ± 6	87 ± 6*	93 ± 7*
MPAP (mm Hg)	33.2 ± 5.9	31.5 ± 7.9	26.8 ± 3.7*	34.5 ± 6.0	36.2 ± 6.4	27.0 ± 5.1*	27.8 ± 4.1*
CO (liters/min)	1.7 ± 0.3	1.7 ± 0.3	1.6 ± 0.3	1.7 ± 0.4	1.7 ± 0.3	1.6 ± 0.3	1.8 ± 0.2
SVR (dynes·cm ⁻⁵)	4,685 ± 439	4,597 ± 535	4,581 ± 608	4,621 ± 693	4,771 ± 597	3,767 ± 229*	3,718 ± 120*
PVR (dynes·cm ⁻⁵)	1,237 ± 441	1,124 ± 349	867 ± 164*	1,272 ± 351	1,319 ± 299	856 ± 207*	870 ± 243*

*p < 0.05, baseline versus drug infusions. Data presented are mean value ± SD. Abbreviations as in Table 1.

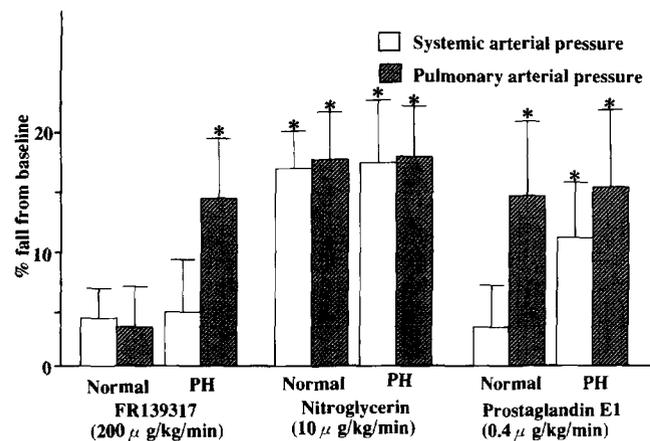


Figure 1. Bar graph showing percent decrease in gradient (mm Hg) from baseline in normal and pulmonary hypertensive (PH) beagles. Differences among FR139317, nitroglycerin and prostaglandin E₁ are indicated. Data shown are mean value \pm SD. * $p < 0.05$ versus baseline value.

tion formed the basis of our hypothesis that endothelin receptor antagonists might be effective in pulmonary hypertension. Because drug therapy resulted in a decrease in both pulmonary artery pressure and pulmonary vascular resistance, some degree of reversible pulmonary vasoconstriction was present. This finding supported the validity of our model for comparison of the pulmonary vasodilator effect of the drugs under study.

We considered the ideal pulmonary vasodilator response to be a decrease in both pulmonary artery pressure and pulmonary vascular resistance that exceeded the decrease in systemic arterial pressure and systemic vascular resistance. The effects of nitroglycerin have generally been attributed to decreased systemic venous tone, with a subsequent shift of blood from the heart and lungs to the systemic veins (26,27). In the present study, nitroglycerin was an effective vasodilator, but it lacked pulmonary vascular selectivity. Therefore, nitroglycerin had the least favorable hemodynamic profile.

Prostaglandin E₁ has generated interest because prostaglandins may contribute to the physiologic regulation of pulmonary blood flow and because a deficiency of endogenous prostaglandins has been associated with primary pulmonary hypertension (28,29). Prostaglandin E₁ is presently one of the most readily available prostaglandins for clinical use. Our data indicated that prostaglandin E₁ produced a greater decrease in systemic arterial pressure in pulmonary hypertensive than normal beagles despite a similar decrease in pulmonary artery pressure in both groups. Progression of pulmonary hypertension appears to prevent its selectivity as a pulmonary vasodilator. It has been proposed that the normally rapid pulmonary clearance of prostaglandin E₁ by 15-hydroxyprostaglandin dehydrogenase in the lungs (30,31) does not occur in pulmonary hypertension and that the active drug concentration in the systemic circulation therefore becomes elevated.

Endothelin, which may be isolated from the supernatant of cultured porcine endothelial cells, has potent vasoactivity (32)

and may play an important role in the regulation of pulmonary vascular tone (33). At least two endothelin receptor subtypes, termed *ETA* and *ETB*, have been described (34,35). *ETA* receptors are located primarily on smooth muscle cells and mediate vasoconstriction, whereas activation of *ETB* receptors on endothelial or smooth muscle cells can cause vasodilation or vasoconstriction (36,37). Both *ETA* and *ETB* receptors coexist in the pulmonary circulation (38). The distribution of receptor subtypes, which varies with the vascular bed, may therefore explain differences in regional hemodynamic responses to endothelin.

There is some evidence that endothelin-1 has a potential role in pulmonary hypertension and is associated with altered vasoreactivity (39,40). Elevated plasma endothelin-1 concentrations precede the development of pulmonary hypertension, suggesting that endothelin-1 may be involved in the pathogenesis of pulmonary hypertension in monocrotaline-treated rats (25). Endothelin-1 activates *ETA* receptors, thereby causing vasoconstriction by intracellular signal transduction (41). If endothelin contributes to the progression of pulmonary hypertension, and endothelin receptors mediate vasoconstriction, then endothelin receptor antagonists might be clinically useful in the management of pulmonary hypertension. Although Miyauchi et al. (25) reported that infusion of BQ-123, a selective *ETA* receptor antagonist, concurrently with an injection of monocrotaline, inhibited the progression of monocrotaline-induced pulmonary hypertension in rats, they studied the effect of BQ-123 before the establishment of pulmonary hypertension. We found that reduction in pulmonary artery pressure by FR139317 was minor under basal conditions but that the vasodilator response was greatly enhanced when vasoconstrictor tone was elevated. This observation is consistent with excessive stimulation of *ETA* receptors in the pulmonary circulation by endogenous endothelin-1 in pulmonary hypertension and suggests a possible role of FR139317 for the blockade of endothelin-1-induced vasoconstriction. Our experiments showed that RES-701-1 increased pulmonary artery pressure only in pulmonary hypertensive beagles, although not significantly. This observation suggests that *ETB* receptors in our pulmonary hypertension model mediate pulmonary vasodilation but not vasoconstriction and that pulmonary hypertension might be exacerbated by *ETB* receptor antagonists.

Although the specific mechanism of action of endothelin receptor antagonists remains unclear, the present results have important therapeutic implications in the development of therapy for pulmonary hypertension.

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