Effectiveness of a Nonselective ET<sub>AB</sub> and a Selective ET<sub>A</sub> Antagonist in Rats With Monocrotaline-Induced Pulmonary Hypertension

Jean-François Jasmin, BSc; Martin Lucas, MSc; Peter Cernacek, MD; Jocelyn Dupuis, MD, PhD

Background—Both nonselective ET<sub>AB</sub> receptor and selective ET<sub>A</sub> receptor antagonists can reduce pulmonary hypertension (PH) and right ventricular hypertrophy (RVH) in various animal models. Depending on their net effects after blockade of endothelial and smooth muscle ET<sub>B</sub> receptors, nonselective ET<sub>AB</sub> antagonists could be more or less effective than selective ET<sub>A</sub> antagonists.

Methods and Results—Two weeks after injection of saline or 60 mg/kg monocrotaline (MCT), rats received 50 mg · kg<sup>–1</sup> · d<sup>–1</sup> of a selective (LU135252) or nonselective (BSF420627) antagonist for 3 weeks. This resulted in 4 groups: control (n=15), MCT (n=60), MCT+ET<sub>A</sub> (n=39), and MCT+ET<sub>AB</sub> (n=40). Five-week survival was 35% in the MCT group; this was increased to 56% in the MCT+ET<sub>A</sub> group (P=0.10) and to 67% in the MCT+ET<sub>AB</sub> group (P=0.0015). Drug administration was stopped 48 hours before hemodynamic measurements to evaluate the chronic effects of therapy: PH in the MCT group (RV systolic pressure 87±1 mm Hg) was improved similarly in both MCT+ET<sub>A</sub> and MCT+ET<sub>AB</sub> groups (72±3 and 70±3 mm Hg, respectively, P<0.05). Severe RVH in the MCT group (RV/left ventricle+septum weight ratio 73±1%) was not affected by the selective antagonist (70±2%) but was reduced to 54±2% in the MCT+ET<sub>AB</sub> group (P<0.01). Pulmonary resistive properties, assessed from isolated lung pressure-flow relationships, were improved similarly in survivors from both treated groups.

Conclusions—Both the nonselective ET<sub>AB</sub> antagonist BSF420627 and the selective ET<sub>A</sub> antagonist LU135252 are effective in this model of PH. Similar direct comparative studies in other models of PH and with various dosage regimens are warranted to define the optimal pharmacological approach of PH when ET receptor antagonists are used. (Circulation. 2001;103:314-318.)

Key Words: receptors ■ hypertension, pulmonary ■ pulmonary heart disease ■ endothelium-derived factors

The endothelin (ET) system is activated in human pulmonary hypertension (PH) of various pathogeneses. ET-1 could contribute to the development of human PH through its strong vasoconstrictive and promitogenic properties. The effectiveness of ET receptor antagonists has been convincingly demonstrated in various animal models of PH. The interpretation of these studies, however, is complicated by the pharmacology of the ET system, consisting of 2 receptor subtypes demonstrating opposite actions.

The ET<sub>A</sub> receptors are located on smooth muscle cells, where they mediate vasoconstrictive and proliferative effects. The ET<sub>B</sub> receptor is the only subtype found predominantly on the vascular endothelium, where it promotes vasodilation through the release of nitric oxide and prostacyclin. There is also evidence that the ET<sub>B</sub> receptor indirectly modulates ET-1 synthesis through negative feedback under the action of nitric oxide. The endothelial ET<sub>B</sub> receptor is also responsible for the clearance of circulating ET-1. In humans, the pulmonary circulation clears 50% of circulating ET in a single pulmonary transit time. Acute selective ET<sub>B</sub> receptor blockade causes adverse hemodynamic effects in animals with PH, suggesting that this receptor attenuates the severity of PH. The ET system is further complicated by the presence of smooth muscle ET<sub>B</sub> receptors, which, like the ET<sub>A</sub> receptors, cause vasoconstriction on stimulation. The ET<sub>B</sub> receptor is therefore present both on the endothelium and on the smooth muscle, where it mediates opposite effects.

The endothelin receptor antagonists demonstrate various affinities and selectivities for the ET<sub>A</sub> and the ET<sub>B</sub> receptors. Both selective ET<sub>A</sub> and nonselective ET<sub>AB</sub> receptor antagonists have been shown to be effective in the therapy of PH in animal models. However, no direct comparison of a selective versus a nonselective antagonist has been made. The net effect of nonselective ET<sub>AB</sub> antagonists could be to provide greater benefit by blocking all of the ET-1–induced vasoconstriction or less benefit by removing the potentially protective...
role of the endothelial ET_{B} receptor. Because ET antagonists are currently under clinical development for conditions associated with PH, it becomes important to resolve this issue. This study was designed to directly compare the effectiveness of a selective ET_{A} (LU135252) versus a nonselective ET_{A/B} (BSF420627) receptor antagonist in the treatment of rats with established PH. To facilitate comparison, we used the monocrotaline (MCT) model of PH because it causes severe PH with high reproducibility.

Methods
Male Sprague Dawley rats (Charles River, St-Constant, Quebec, Canada) weighing between 150 and 200 g received an intraperitoneal (IP) injection of either 0.5 mL 0.9% saline or 0.5 mL 60 mg/kg MCT. Two weeks later, they were randomly assigned to receive oral therapy with either the selective ET_{A} antagonist LU135252 (50 mg \* kg^{-1} \* d^{-1}) or the nonselective ET_{A/B} antagonist BSF420627 (50 mg \* kg^{-1} \* d^{-1}) for a 3-week period. The antagonists were mixed with rat chow. This resulted in the following 4 groups: Control (n=15), MCT (n=60), MCT+ET_{A} (n=39), and MCT+ET_{A/B} (n=40).

Experimental Protocol
Forty-eight hours after active treatment was stopped, surviving rats were anesthetized with xylazine (10 mg/kg)/ketamine (50 mg/kg), followed by 2000 U heparin (Sigma Chemical Co). Study drugs were stopped 48 hours before hemodynamic measurements to obtain an evaluation of the chronic effect of therapy with no active medications in plasma. After stable anesthesia was obtained, the right jugular vein and the right carotid artery were isolated and incised, and polyethylene catheters (PE 50; 0.97 mm OD, 0.58 mm ID) were advanced into the right and left ventricles (RV and LV) for hemodynamic measurements. The RV and LV pressures were measured and recorded with a polygraph (Gould TA 4000). Venous blood samples were collected for circulating ET level determination, as previously described in detail.13

The trachea was then cannulated with tubing connected to a rodent ventilator (Harvard Apparatus) and ventilated with room air at a tidal volume of 1 mL and 2 cm H_{2}O positive end-expiratory pressure. After a midline sternotomy, the heart and lungs were exposed. The pulmonary artery was cannulated through an incision in the upper portion of the RV. The lungs were initially perfused with Krebs' solution supplemented with 100 U/mL heparin (Sigma Chemical Co). Two minutes before the perfusion, the Krebs solution had the following composition (mmol/L): NaCl 120, NaHCO_{3} 25, KCl 4.7, KH_{2}PO_{3} 1.18, MgSO_{4} 1.18, CaCl_{2} 2.5, and glucose 5.5. This solution was bubbled with a mixture of 95% O_{2} and 5% CO_{2}, and pH was adjusted to 7.4. The lungs were rapidly removed and suspended in a water-jacketed chamber maintained at 37°C. Lungs were perfused with Krebs' solution supplemented with 3% albumin. After a 10-minute equilibration period, the relationship between perfusion pressure and flow rate was assessed by increasing the flow rate in the range of 5 to 25 mL/min and recording the corresponding perfusion pressure (P-Q curves). After the in vitro experiments had been completed, the heart was dissected and weighed to determine RV hypertrophy [RV/(LV+septum) weight]. To evaluate the presence of pulmonary edema, the right lower lobe was gravity-drained and weighed to determine its wet weight and then set aside for later determination of dry weight.

Drugs
The MCT was purchased from Sigma Chemical Co. The MCT was dissolved in 1.0N HCl, and the pH was adjusted to 7.4 with 1.0N NaOH. The endothelin receptor antagonists LU135252 and BSF420627 were kindly provided by Dr M. Kirchengast (Knoll AG, BASF Pharma, Ludwigshafen, Germany). The K_{i} for LU135252 is 1.4 nmoL/L for the ET_{A} receptor and 184 nmoL/L for the ET_{B} receptor. The K_{i} for BSF420627 is 2.2 nmoL/L for the ET_{A} receptor and 5.8 nmoL/L for the ET_{B} receptor.

Statistical Analysis
All values were expressed as mean±SEM. Differences in Kaplan-Meier survival curves between groups were evaluated by the log-rank test. Differences between all other parameters for the 4 groups were evaluated by ANOVA followed by multiple-group comparisons with the Bonferroni correction. Pressure-flow relationships were compared by repeated-measures ANOVA. The individual P-Q relationships for each group were fitted by linear regression to determine their slope and intercept. Statistical significance was assumed at a value of P<0.05.

Results
Effect of ET Receptor Antagonists on Survival
There were no deaths in the control rats (Figure 1). Five-week survival was only 35% in the MCT group, with 21 of 60 rats alive. Chronic ET receptor antagonist therapy begun 2 weeks after MCT injury nonsignificantly increased survival to 56.4% in the MCT+ET_{A} group (n=22/39, P=0.10) but further and significantly improved survival to 66.7% in the MCT+ET_{A/B} group (n=27/40, P=0.0015).

Chronic Hemodynamic and Morphological Effects of ET Receptor Antagonists
The MCT group developed severe PH, with an RV systolic pressure (RVSP) of 86.5±6.0 mm Hg, compared with 24.7±0.5 mm Hg in the control group (P<0.01, Figure 2). Both the MCT+ET_{A} and MCT+ET_{A/B} groups demonstrated a significant and similar improvement in RVSP, with 72.1±2.5 and 69.7±2.4 mm Hg, respectively (P<0.01). This translated into higher RV end-diastolic pressure in the MCT group (8.5±0.1 mm Hg) than in the control group (1.9±0.2 mm Hg, P<0.01), which was also significantly and similarly reduced to 5.9±0.3 mm Hg in the MCT+ET_{A} group and to 5.5±0.5 mm Hg in the MCT+ET_{A/B} group (P<0.01, Table). Central venous pressure and indices of RV contractility (dP/dt) behaved similarly, with an increase in the MCT group, which was similarly reduced in both groups receiving the ET antagonists (Table).

The MCT group developed severe RV hypertrophy, manifested by an RV/(LV+septum) ratio of 73±1%, compared with 24±1% in the control group (P<0.01, Figure 3). This
marked RV hypertrophy was not modified by the selective ET\textsubscript{A} antagonist (70±2\%) but was reduced significantly, to 54±2\%, in the MCT+ET\textsubscript{A} group (P<0.01 versus MCT and MCT+ET\textsubscript{A} groups).

Systemic hemodynamic parameters are also presented in the Table. Heart rate did not differ between the 4 groups. Mean arterial pressure was lowered to 66±4 mm Hg in the MCT group, compared with 120±4 mm Hg in the control group (P<0.01). The mean arterial pressure increased non-significantly to 77±6 mm Hg in the MCT+ET\textsubscript{A} group but was significantly higher, at 88±6 mm Hg in the MCT+ET\textsubscript{AB} group (P<0.01 versus Control, **P<0.01 vs MCT). The mean arterial pressure increased non-significantly to 77±6 mm Hg in the MCT+ET\textsubscript{A} group but was significantly higher, at 88±6 mm Hg, in the MCT+ET\textsubscript{AB} group (P<0.05 versus the MCT group). Indices of LV contractility and relaxation were significantly depressed only in the MCT group. There was no evidence of pulmonary edema as assessed by the ratio of dry to wet lung weight in any of the groups.

Immunoreactive ET-1 levels measured in a subset of the animals were nonsignificantly elevated in the MCT group as well as in both ET antagonist groups (Table).

**Effects of ET Receptor Antagonists on Vascular Resistive Properties of Isolated Lungs**

A common source of difficulty in the evaluation of pulmonary vascular resistance by use of single time points resides in the passive variation induced by modifications in pulmonary blood flow and pressure. Analysis of numerous points with construction of the P-Q relationships provides greater insight into pulmonary vascular resistive properties. The P-Q relationship of isolated lungs from the MCT group was shifted upward (Figure 4), with an increase in both the slope (2.34±0.13 versus 0.44±0.13 mm Hg·mL\textsuperscript{-1}·s\textsuperscript{-1} in controls, P<0.01) and the intercept (6.28±0.62 versus −0.10±0.60 mm Hg, P<0.01). In the animals treated with the ET antagonists, there was a similar improvement in the P-Q relationship with lowering of the slope to 1.69±0.13 mm Hg·mL\textsuperscript{-1}·s\textsuperscript{-1} in the ET\textsubscript{A} group and 1.59±0.13 mm Hg·mL\textsuperscript{-1}·s\textsuperscript{-1} in the ET\textsubscript{AB} group (P<0.05 versus MCT). In these 2 groups, the intercept was similar to that in the MCT group.

**Discussion**

We evaluated the efficacy of a selective ET\textsubscript{A} (LU135252) and nonselective ET\textsubscript{AB} (BSF420627) antagonist in the treatment of MCT-induced PH in rats. Our results demonstrate, for the first time, the efficacy of both treatment strategies in a side-by-side comparison.

Although both selective ET\textsubscript{A} and nonselective ET\textsubscript{AB} receptor antagonists have been successfully used in the treatment of PH in various animal models, no direct comparison of the 2 strategies had been performed. Because of its dichotomous role, blockade of the ET\textsubscript{B} receptor could theoretically provide more or less benefit in addition to ET\textsubscript{A} receptor blockade. Studies supporting a protective role demonstrate that acute selective ET\textsubscript{B} blockade increases pulmonary pressures in dogs with dehydromonocrotaline-induced PH\textsuperscript{14} and with tachycardia-induced heart failure.\textsuperscript{15} Another concern with chronic ET\textsubscript{B} antagonist therapy is the inhibition of ET-1 clearance, which is mediated by the endothelial ET\textsubscript{B} receptor,\textsuperscript{12} because the ultimate physiological significance and impact of this function is currently unknown. There is also evidence that the ET\textsubscript{B} receptor indirectly modulates ET-1 synthesis through negative feedback under the action of nitric oxide.\textsuperscript{11} We have previously shown that chronic ET\textsubscript{B} blockade with LU135252 in the MCT model not only improved PH but also improved endothelium-dependent pulmonary vasodilation to acetylcholine.\textsuperscript{18} This suggests additional potentially important interactions between the ET-1 and the nitric oxide systems to which the endothelial ET\textsubscript{B} receptor may contribute.

Conversely, other studies have shown that blockade of both ET\textsubscript{A} and ET\textsubscript{B} receptors is necessary to achieve optimal inhibition of ET-1–induced vasoconstriction in both systemic\textsuperscript{19,20} and pulmonary\textsuperscript{16,21} vascular beds. Because these studies used acute administration of the ET antagonists, they fail to provide accurate insight into the role of the ET\textsubscript{B} receptor in the development of PH, which is usually a slow and long-term process. From previous studies using long-term administration of either selective or nonselective ET antagonists, it is evident that both strategies are effective in various models of PH. In the MCT model, the selective ET\textsubscript{A} antagonists LU135252 and BQ123,\textsuperscript{5,22} as well as the nonselective ET\textsubscript{AB} antagonist bosentan,\textsuperscript{23} have shown their efficacy. The net effect after ET\textsubscript{B} receptor blockade therefore seems favorable,
but the points raised above suggest that combined blockade could provide more or less benefit than selective ET_α blockade. The present study was designed to try to resolve this issue.

In certain aspects, our results demonstrate differences in the effect of a nonselective ET_{AB} versus a selective ET_α antagonist in the treatment of MCT-induced PH. The nonselective antagonist doubled survival compared with the untreated animals and increased it by 10% compared with the animals receiving the selective antagonist. There was also a reduction of RV hypertrophy only in the animals receiving the nonselective antagonist. These 2 findings, however, were not accompanied by a greater reduction of RV systolic pressure measured in vivo in the surviving animals. In addition, the pressure-flow curves, which are a very accurate way of evaluating the resistive properties of the lungs, were similarly improved in both treated groups. We therefore cannot conclude that one agent is clearly superior to the other.

The MCT model of PH is associated with initial severe damage to the pulmonary vascular endothelium. Thus, it could be argued that blockade of the remaining and possibly nonfunctional endothelial ET_α would have very little pharmacological effect. This, however, is not supported by studies in dogs with dehydromonocrotaline PH, because endogenous ET attenuates the increase in pulmonary pressure through the ET_α receptor. Recent data from our laboratory also support functionality of the endothelial ET_α receptor after monocrotaline injury, because pulmonary ET-1 clearance is slightly reduced but maintained. We have also recently demonstrated preservation of endothelium-dependent pulmonary vasodilation to acetylcholine 3 weeks after MCT injury in rats. These findings therefore suggest at least partial preservation of some endothelial functions after MCT injury, including that of the ET_α receptor.

Many studies suggest an important role of the ET_β receptor in ET-1–induced pulmonary vasconstriction. A combination of the selective ET_α antagonist BQ123 and the selective ET_β antagonist BQ788 inhibits ET-1–induced pulmonary vasconstriction more effectively than BQ123 alone. The selective ET_β agonist IRL-1620 caused pulmonary vasconstriction and edema, both of which were completely inhibited by BQ788. ET-1 is a potent thromboxane-dependent pulmonary constritor in the guinea pig lung, with an important role of the ET_β receptor in thromboxane A2 release in normal guinea pigs and in endotoxin-treated rats.

Limitations of This Study
The MCT model of PH has no human equivalent. Activation of the ET system, however, is present in all forms of human PH and in all animal models, including the MCT model. We chose the MCT model because of the severe and reproducible PH obtained, which more easily allows comparison of 2 treatment regimens.

The ET_α-selective antagonist LU135252 (K_i ET_α 1.4 mol/L, K_i ET_β 184 mol/L) has a bioavailability of 86%, with a plasma half-life of 10 hours (Knoll Investigator’s Brochure). The nonselective ET_{AB} antagonist BSF420627 (K_i ET_α 1.22 mol/L, K_i ET_β 5.8 mol/L) has a 60% bioavailability.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Control (n=15)</th>
<th>MCT (n=20)</th>
<th>MCT+ET_α (n=22)</th>
<th>MCT+ET_{AB} (n=27)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CVP, mm Hg</td>
<td>1.5 ± 0.9</td>
<td>7.8 ± 0.4†</td>
<td>5.5 ± 0.5†</td>
<td>5.2 ± 0.4§</td>
</tr>
<tr>
<td>RVEDP, mm Hg</td>
<td>1.9 ± 0.2</td>
<td>8.5 ± 0.5†</td>
<td>5.9 ± 0.3†</td>
<td>5.5 ± 0.4§</td>
</tr>
<tr>
<td>RV +dP/dt, mm Hg/s</td>
<td>1460 ± 29</td>
<td>3688 ± 112†</td>
<td>2938 ± 99†</td>
<td>2815 ± 108§</td>
</tr>
<tr>
<td>RV –dP/dt, mm Hg/s</td>
<td>906 ± 25</td>
<td>2732 ± 72†</td>
<td>2305 ± 78§</td>
<td>2190 ± 86§</td>
</tr>
<tr>
<td>Heart rate, bpm</td>
<td>262.9 ± 4.6</td>
<td>275.7 ± 3.4</td>
<td>266.2 ± 4.3</td>
<td>273.0 ± 7.6</td>
</tr>
<tr>
<td>MAP, mm Hg</td>
<td>120 ± 4</td>
<td>66 ± 4†</td>
<td>77 ± 7†</td>
<td>88 ± 7†</td>
</tr>
<tr>
<td>LVEDP, mm Hg</td>
<td>10 ± 2</td>
<td>8 ± 1</td>
<td>7 ± 1</td>
<td>6 ± 1</td>
</tr>
<tr>
<td>LV +dP/dt, mm Hg/s</td>
<td>7415 ± 337</td>
<td>5656 ± 253‡</td>
<td>6211 ± 549</td>
<td>6156 ± 435</td>
</tr>
<tr>
<td>LV –dP/dt, mm Hg/s</td>
<td>4801 ± 322</td>
<td>3375 ± 212‡</td>
<td>3438 ± 374</td>
<td>4594 ± 475</td>
</tr>
<tr>
<td>5-week BW, g</td>
<td>447 ± 9</td>
<td>319 ± 10†</td>
<td>323 ± 9†</td>
<td>330 ± 13†</td>
</tr>
<tr>
<td>Lung dry/wet weight</td>
<td>0.201 ± 0.002</td>
<td>0.203 ± 0.003</td>
<td>0.202 ± 0.002</td>
<td>0.211 ± 0.006</td>
</tr>
<tr>
<td>ET-1, pg/mL</td>
<td>1.6 ± 0.3 (n=7)</td>
<td>3.4 ± 1.2 (n=9)</td>
<td>4.1 ± 0.7 (n=9)</td>
<td>3.1 ± 0.6 (n=9)</td>
</tr>
</tbody>
</table>

CVP indicates central venous pressure; RVEDP, RV end-diastolic pressure; MAP, mean arterial pressure; LVEDP, LV end-diastolic pressure; and BW, body weight. For ET-1, the number of samples analyzed is indicated for each group.

*P<0.05 vs MCT, †P<0.01 vs Control, ‡P<0.05 vs Control, §P<0.01 vs MCT.

**Figure 4.** Relationship between perfusion pressure and flow rate in isolated lungs of control rats and MCT pulmonary hypertensive rats treated with a selective ET_α (LU135252) or nonselective ET_{AB} (BSF420627) antagonist. †P<0.01 vs Control, *P<0.05 vs MCT.
with a 2-hour half-life (Knoll Investigator’s Brochure). The lower bioavailability and half-life of BSF420627 may have resulted in lower plasma concentrations of the nonselective antagonist. Our results, therefore, cannot be generalized to other endothelin antagonists or to other models of PH.

Conclusions
Chronic administration of the nonselective ET₁/ET₂ antagonist BSF420627 or the selective antagonist LU135252 is effective in the treatment of MCT-induced PH. Similar direct comparative studies in other models of PH and with various dosage regimens are warranted to define the optimal pharmacological approach of PH when using ET receptor antagonists.

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