Nonspecific endothelin-receptor antagonist blunts monocrotaline-induced pulmonary hypertension in rats

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Hill, Nicholas S., Rod R. Warburton, Linda Pietras, and James R. Klinger. Nonspecific endothelin-receptor antagonist blunts monocrotaline-induced pulmonary hypertension in rats. J. Appl. Physiol. 83(4): 1209–1215, 1997.—Endothelin-1 (ET-1), a potent vasoactive and mitogenic peptide, has been implicated in the pathogenesis of several forms of pulmonary hypertension. We hypothesized that nonspecific blockade of ET receptors would blunt the development of monocrotaline (MCT)-induced pulmonary hypertension in rats. A single dose of the nonspecific ET blocker bosentan (100 mg/kg) given to intact rats by gavage completely blocked the pulmonary vasoconstrictor actions of Big ET-1 and partially blunted hypoxic pulmonary vasconstriction. After 3 wk, MCT-injected (105 mg/kg sc) rats gavaged once daily with bosentan (200 mg/kg) had lower right ventricular (RV) systolic pressure (RVSP), RV-to-body weight (RV/BW) and RV-to-left ventricular (LV) plus septal (S) weight [RV/(LV+S)] ratios and less percent medial thickness of small pulmonary arteries than control MCT-injected rats. Lower dose bosentan (100 mg/kg) had no effect on these parameters after MCT or saline injection. Bosentan raised plasma ET-1 levels but had no effect on lung ET-1 levels. Bosentan (200 mg/kg) also had no effect on wet-to-dry lung weight ratios 6 days after MCT injection. When given during the last 10 days, but not the first 11 days of a 3-wk period after MCT injection, bosentan reduced RV/(LV+S) compared with MCT-injected controls. We conclude that ET-1 contributes to the pathogenesis of MCT-induced pulmonary hypertension and acts mainly during the later inflammatory phase rather than the acute injury phase after injection.

Endothelin (ET) is a potent vasoactive and mitogenic peptide that is synthesized and released by endothelial cells (30). It exists in three isoforms, ET-1, ET-2, and ET-3, with ET-1 appearing to be the most important in vascular regulation (16). ET-1 constricts pulmonary arteries under resting conditions but may have vasodilator actions when administered to preconstricted arteries (9). These opposing effects have been ascribed to the different actions of the ET receptors, of which two have been described: the endothelin A (ET\textsubscript{A}) receptor, which mediates vasoconstrictor and mitogenic actions (15), and the endothelin B (ET\textsubscript{B}) receptor. The ET\textsubscript{B} receptor has two subtypes: ET\textsubscript{B1}, which mediates vasodilation, at least partially via release of nitric oxide and prostacyclin (5), and ET\textsubscript{B2}, which mediates vasoconstrictor actions (5, 29).

These properties have led investigators to posit that ET-1 contributes to the pathogenesis of experimental and clinical forms of pulmonary hypertension. In chronic hypoxic pulmonary hypertension in rats, lung homogenate concentrations and steady-state mRNA levels for ET-1 increase (6). In addition, blockade of ET activity by using either the specific ET\textsubscript{A}-receptor blocker cyclo(-Trp-D-Asp-Pro-d-Val-Leu) (BQ-123) or the nonspecific ET-1-receptor blocker bosentan prevents the development of both acute (2, 23) and chronic forms of hypoxic pulmonary hypertension (2). ET-1 has also been implicated in the Fawn-hooded rat model of idiopathic pulmonary hypertension (26). These rats have elevated lung tissue ET-1 levels, and vascular smooth muscle cells cultured from their pulmonary arteries have enhanced proliferative responses to ET-1 (26). Furthermore, humans with primary pulmonary hypertension have increased plasma ET-1 levels (27) as well as increased gene expression and immunoreactivity for ET-1 in perivascular lung tissue (7), suggesting a possible pathogenic role.

Monocrotaline (MCT) is a pyrrole alkaloid that consistently causes pulmonary hypertension in rats (1). The mechanism of this form of pulmonary hypertension is unknown, but an initial acute injury phase characterized by morphological and functional evidence of endothelial cell damage occurs during the first week after administration (19). This is followed by an inflammatory phase characterized by the gradual development of pulmonary hypertension. Structural changes occur, including proliferation of medial smooth muscle in small pulmonary arteries (19), and pulmonary function abnormalities develop, including restriction and mild hypoxemia (8).

Miyauchi et al. (20) have recently implicated ET-1 in the pathogenesis of MCT-induced pulmonary hypertension. They demonstrated an increase in circulating ET-1 levels after MCT injection, although lung concentrations of both peptide and steady-state mRNA for ET-1 decreased. In addition, they showed that BQ-123 blunts MCT-induced pulmonary hypertension and pulmonary arterial medial thickening. However, responses to a specific ET\textsubscript{A} blocker could be misleading if ET-1 acting on the ET\textsubscript{B} receptor caused vasodilation, thereby blunting the development of pulmonary hypertension. In addition, the possible contribution of reduced cardiac output to the blunting of pulmonary hypertension during BQ-123 administration was not evaluated.

In the present study, we reasoned that blunting of MCT-induced pulmonary hypertension by simultaneous blockade of both ET\textsubscript{A} and ET\textsubscript{B} receptors would strengthen the evidence that ET-1 is involved in pathogenesis. We hypothesized that the nonspecific ET-receptor blocker bosentan (3, 4) would reduce pulmonary hypertension, pulmonary vascular remodeling, and right ventricular (RV) hypertrophy in MCT-injected rats. We also sought to determine whether bosentan inhibits pulmonary hypertension without af-
fecting cardiac output. Finally, we examined the effect of administering bosentan early as opposed to later after MCT administration to assess relative contributions of ET-1 to the acute and later inflammatory phases. Because bosentan is an oral agent suitable for once-a-day administration, demonstrated efficacy in experimental models of pulmonary hypertension may have important clinical implications.

METHODS

Animals and materials. Male Sprague-Dawley rats (250–300 g) were obtained from Harlan Sprague Dawley Laboratories (Madison, WI). Bosentan [Ro 47–0203; 4-tert-butyl-N-[6-(2-hydroxy-ethoxy)-5-(2-methoxy-phenoxy)-2,2'-bipyrimidin-4-yl]-benzene sulfonamide] was a gift of M. Clozel, Hoffmann-LaRoche (Basel, Switzerland). MCT was obtained from Transworld Chemical (Rockville, MD). Big ET-1 was purchased from Peptides International (Louisville, KY). All other reagents were obtained from Sigma Chemical (St. Louis, MO) unless otherwise indicated.

Experimental design. A preliminary series of experiments determined the efficacy of bosentan in blocking the constrictor actions of big ET-1 and acute hypoxic pulmonary vasconstriction in intact rats. Bosentan was dispersed in 5% gum arabic (25 mg/ml) and administered by gavage (100 mg/kg or an equal volume of vehicle in controls). Two hours later, after placement of a right-heart catheter and an aortic thermistor (see Hemodynamic analysis and heart weights), rats were exposed to hypoxia (10% inspired O2 fraction), normoxia, or increasing doses of Big ET-1 (or saline in controls) with subsequent monitoring of RV pressures and cardiac output.

To test the hypothesis that bosentan blunts MCT-induced pulmonary hypertension, four groups of rats were initially studied. The first two groups consisted of saline-injected controls, gavaged with vehicle alone or bosentan (100 mg/kg), to test for independent effects of the inhibitor. Another two groups of rats received a single injection of MCT (105 mg/kg), followed by gavage with bosentan (100 mg·kg⁻¹·day⁻¹), or vehicle alone. Because the initial series of experiments showed no anti hypertensive effect of bosentan, a fifth group of MCT-injected rats that received bosentan (200 mg·kg⁻¹·day⁻¹) was compared with appropriate controls in a second series of experiments. At the end of 3 wk, all groups were anesthetized for measurement of hemodynamics and heart weights (see below).

To assess the effects of ET blockade on the acute lung injury induced by MCT, rats were euthanized 6 days after MCT injection, after receiving bosentan (200 mg·kg⁻¹·day⁻¹) or vehicle alone. This time point was based on prior observations demonstrating increased lung lavage protein concentrations and wet-to-dry lung weight ratios 5–7 days after MCT injection (28). The left main stem bronchus was ligated, and the trachea was cannulated with a blunt 19-gauge needle. The right lung was lavaged twice with 2.5 ml saline (0.9%), and the aspirate was centrifuged at 4,000 revolutions/min × 15 min to remove cells and at 18,000 revolutions/min × 15 min to remove lipids (28). Total protein concentrations were assayed by using the Bradford method. The left lungs were weighed wet, placed in an 80°C oven for 48 h, and weighed dry, and the wet-to-dry weight ratio was calculated.

An additional experiment was performed to examine the effects of bosentan on the time course of development of MCT-induced pulmonary hypertension. Six groups of rats were studied. The first two groups were euthanized 11 days after MCT injection after receiving bosentan (200 mg·kg⁻¹·day⁻¹) or vehicle alone. The second two groups consisted of MCT-injected rats gavaged with vehicle alone for the first 11 days and then received bosentan (200 mg·kg⁻¹·day⁻¹) for the next 10 days, or continued receiving vehicle alone. The last two groups were gavaged with bosentan (200 mg·kg⁻¹·day⁻¹) for the first 11 days after MCT injection, and then continued receiving bosentan, or were switched to vehicle alone. These latter four groups were then studied at 21 days.

Hemodynamic analysis and heart weights. Rats were anesthetized with ketamine (60 mg/kg im) and pentobarbital (20 mg/kg ip). A V3 catheter (Biplab Products, Lake Havasu City, AZ) was inserted into the right external jugular vein for removal of 2 ml of blood for hemocrit and ET-1 measurements. After replacement of the blood with an equal volume of saline, the catheter was advanced into the right ventricle. RV systolic pressure (RVSP) was measured as the mean systolic pressure over 30 s. For experiments involving repeated RVSP measurements, the catheter was left in place. A V3 catheter was then inserted into the right internal carotid artery for measurement of mean systemic blood pressure over 30 s. This catheter was removed and a 3-Fr thermistor was positioned in the aorta 2–3 mm above the aortic valve for measurement of cardiac output by thermodilution (Columbus Instruments, Columbus, OH). The mean of three 0.2 ml saline injections into the RV catheter was used for determination of cardiac index [cardiac output/kg body wt (BW)]. Animals were then exsanguinated, and the hearts and lungs were dissected and weighed. The RV-to-BW ratio (RV/BW) in milligrams per gram and the RV-to-left ventricular (LV) plus septal (S) weight ratio [RV/(LV+S)] were used as indexes of RV hypertrophy.

In some experiments, cannulas were inserted into the trachea and main pulmonary artery. The left pulmonary artery was ligated, and buffered formalin (10%) was infused at 23 cmH2O pressure via the trachea and 75 cmH2O pressure via the pulmonary artery for fixation of the right lung before histological analysis (see Morphological analysis). Left lungs were homogenized for 15 s in RNAzol (Biotek Laboratories, Houston, TX) with a Brinkmann homogenizer (speed setting 6; Westbury, NY) and stored at −80°C for later measurement of ET-1 concentration.

ET-1 assays. Venous blood was collected in chilled vials containing EDTA. Plasma was separated by centrifugation and stored at −80°C until assay (within 1 mo). ET-1 was measured by enzyme immunoassay (Cayman Chemical, Ann Arbor, MI). Plasma samples were extracted with C₁₈ extraction cartridges (Fisher Scientific, Pittsburgh, PA) activated with methanol. ET-1 was eluted by using methanol-water-0.1% trifluoroacetic acid, with a recovery rate of 80%. Tissue extracts were diluted fivefold in assay buffer. Samples were read by using an unextracted standard curve, with an interassay variability of 12%.

Morphological analysis. Transverse sections, 5 µm in thickness, were cut from the right mid- and lower zones of fixed, paraffin-mounted lungs. Sections were stained with elastic tissue stain. A computerized visual imaging system was used to photograph areas of interest by using a Hitachi KP 160 camera (Tokyo, Japan), with the image digitized by using Biovision Software (Perseptics, Knoxville, TN). Measurements of medial thickness (MT) and vessel diameter were made digitally on all appropriately sized vessels in each photographic field by an observer blinded to experimental group. Vessels were grouped according to diameter: 25–50, 50–125, and 125–250 µm. MT was calculated as MT (%) = 2 × MT/Vessel diameter × 100.

Statistical analysis. Differences between time-matched pairs of mean values were assessed by using unpaired t-tests.
Multiple-group comparisons were performed by using two-way analysis of variance. When F-ratios indicated statistical significance, post hoc analysis was done by using the Student-Newman-Keuls test. Differences were considered significant when P < 0.05. Values are presented as means ± SE.

RESULTS

Acute effects of bosentan. Two hours after gavage with bosentan (100 mg/kg), the acute pulmonary hypertensive effect of Big ET-1 in intact rats was completely blocked (Fig. 1). Bosentan had a similar blocking action on systemic hypertension induced by Big ET-1 (data not shown). The single dose of bosentan (100 mg/kg) blunted but did not completely abolish acute hypoxic vasoconstriction in intact rats (Fig. 2). Cardiac output was unchanged by acute hypoxia (data not shown).

Effects of bosentan on MCT-induced pulmonary hypertension. Saline-injected rats gavaged with bosentan (100 mg·kg⁻¹·day⁻¹) had slightly greater BWs than those given vehicle alone (Table 1), but otherwise there were no differences in heart weight, cardiac index, or hematocrit between the two groups. Among MCT-injected rats, BWs were lower than in saline-injected controls but were unaffected by administration of bosentan. As anticipated, MCT-injected rats had significantly elevated RV/BW and RV/(LV+S) values as well as RVSPs (Table 1, Fig. 3), indicating the development of RV hypertrophy and pulmonary hypertension after 21 days. Bosentan at a daily dose of 200 mg/kg, but not 100 mg/kg, significantly reduced the severity of both RV hypertrophy and pulmonary hypertension, as demonstrated by reductions in RV/(LV+S) and RVSP (Fig. 3). Neither MCT nor bosentan had any significant effect on LV mass, hematocrit, systemic blood pressure, or cardiac index (Table 1).

Rats injected with MCT had increased MTs of pulmonary vessels with diameters between 25–50 and 50–125 µm, but not 125–250 µm, compared with saline controls (Fig. 4). Bosentan (200 mg·kg⁻¹·day⁻¹) prevented the MCT-induced increase in MT of vessels with diameters between 25–50 and 50–125 µm. Bosentan (100 mg·kg⁻¹·day⁻¹) had no effect on MT of smaller pulmonary vessels (diameters of 25–50 and 50–125 µm) of saline-injected rats, but among 125- to 250-µm vessels, saline-injected rats that received bosentan had reduced MTs.

Effects of MCT and bosentan on ET-1 levels. Plasma levels of ET-1 were unchanged in rats 21 days after injection of MCT (Fig. 5A). Bosentan increased circulating ET-1 levels in both saline- and MCT-injected rats. ET-1 levels in lung homogenates from rats 21 days after MCT injection tended to be lower than those of saline-injected rats, but the difference was not statistically significant. Bosentan had no effect on lung homogenate ET-1 levels in either group (Fig. 5B).

Effects of bosentan on acute MCT-induced lung injury. Lung lavage protein concentration was 195 ± 39 µg/ml and wet-to-dry lung weight ratio was 4.80 ± 0.04 6 days after saline injection. These values increased to 351 ± 160 µg/ml and 4.96 ± 0.13, respectively, 6 days after MCT injection in rats gavaged with vehicle alone (P < 0.05 compared with saline). Daily gavage with 200 mg/kg bosentan had no effect on lung lavage protein concentration (366 ± 156 µg/ml) or wet-to-dry lung weight ratio (5.00 ± 0.08) 6 days after MCT injection (P = not significant compared with MCT-injected rats gavaged with vehicle alone).

Effects of bosentan on development of pulmonary hypertension. Eleven days after MCT injection, bosentan (200 mg·kg⁻¹·day⁻¹) prevented the increase in RVSP that occurred in MCT-injected rats, but no effect was seen on RV/(LV+S) (Fig. 6, A and B). At 21 days, RV/(LV+S) and RVSP were similar in bosentan-treated rats that were switched to vehicle alone 11 days after MCT injection, and in those treated with vehicle alone for the entire 3-wk period (Fig. 6, A and B). On the other hand, bosentan administered for only the last 10
of the 21 days after MCT injection was as effective as 21 days of bosentan in reducing RV/(LV + S). There was a trend toward a lower RVSP and RV/(LV + S) in the group receiving bosentan for only the last 10 days compared with that receiving it for only the first 11 days of the 3-wk period after MCT injection, but the difference was not statistically significant \( P \leq 0.23 \) for RVSP; \( P \leq 0.15 \) for RV/(LV + S).

### DISCUSSION

Our study is the first to show a statistically significant inhibition of MCT-induced pulmonary hypertension, pulmonary vascular thickening, and RV hypertrophy by a nonspecific ET blocker, thus strengthening the case that ET-1 plays a role in pathogenesis. Previous studies have used specific ETA blockade to inhibit pulmonary hypertension (20), leaving open the possibility that vasodilation caused by unopposed stimulation of the ETB1 receptor is responsible for the inhibition and that ET-1 is actually not involved in pathogenesis. On the assumption that bosentan effectively blocked both receptors in the present study, our findings eliminate this possibility from consideration. In addition, we also found no alterations in cardiac output attributable to bosentan administration, eliminating reduced blood flow as a possible contributor to the blunting of pulmonary hypertension.

In a prior study that posited a role for ET-1 in the pathogenesis of MCT-induced pulmonary hypertension, Miyauchi et al. (20) observed increases in plasma ET-1 levels preceding increases in pulmonary arterial pressure in MCT-injected rats. They also demonstrated

### Table 1. Body and heart weights, hematocrit, and systemic hemodynamics

<table>
<thead>
<tr>
<th>Protocol</th>
<th>n</th>
<th>Body Wt, g</th>
<th>RV/BW</th>
<th>(LV + S)/BW</th>
<th>Hct, %</th>
<th>MSBP, mmHg</th>
<th>Cl, ml·kg⁻¹·min⁻¹</th>
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<tr>
<td>Saline</td>
<td>15</td>
<td>378±11</td>
<td>0.56±0.01</td>
<td>2.01±0.03</td>
<td>44±1</td>
<td>119±6</td>
<td>216±12</td>
</tr>
<tr>
<td>Saline/bosentan (100 mg·kg⁻¹·day⁻¹)</td>
<td>13</td>
<td>416±16*</td>
<td>0.53±0.02</td>
<td>2.01±0.04</td>
<td>42±1</td>
<td>114±9</td>
<td>221±15</td>
</tr>
<tr>
<td>MCT</td>
<td>20</td>
<td>358±9*</td>
<td>0.69±0.02*</td>
<td>2.01±0.03</td>
<td>43±1</td>
<td>103±5</td>
<td>215±14</td>
</tr>
<tr>
<td>MCT/bosentan (100 mg·kg⁻¹·day⁻¹)</td>
<td>7</td>
<td>350±12*</td>
<td>0.67±0.05*</td>
<td>1.99±0.07</td>
<td>41±1</td>
<td>118±10</td>
<td>216±48</td>
</tr>
<tr>
<td>MCT/bosentan (200 mg·kg⁻¹·day⁻¹)</td>
<td>17</td>
<td>362±8</td>
<td>0.63±0.02*</td>
<td>2.01±0.04</td>
<td>43±1</td>
<td>97±4</td>
<td>205±12</td>
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</table>

Values are means ± SE; n, no. of animals. RV/BW, right ventricular-to-body weight ratio; (LV + S)/BW, left ventricular plus septal-to-body weight ratio; Hct, hematocrit; MSBP, mean systolic blood pressure; Cl, cardiac index; MCT, monocrotaline. *P < 0.05 compared with saline-to-body-treated animals. †P < 0.05 compared with MCT-treated animals.

![Fig. 3. RVSP (A) and right ventricular-to-left ventricular plus septal weight ratios (RV/(LV + S)) (B) are shown in 2 saline-injected groups (left) and 3 monocrotaline (MCT)-injected groups (right). Values are means ± SE; n = measurements made on 52–130 vessels/group from 4 lungs. Measurements were made on lung sections from saline-injected control rats gavaged with vehicle or bosentan (100 mg·kg⁻¹·day⁻¹) and MCT-injected rats gavaged with vehicle or bosentan (200 mg·kg⁻¹·day⁻¹). *P < 0.05 compared with saline control group. †P < 0.05 compared with MCT control group.](http://jap.physiology.org/)

![Fig. 4. Percent medial thickness \((2 \times \text{medial thickness/vessel diameter} 	imes 100)\) for pulmonary vessels with diameters ranging from 25 to 50, 50 to 125, and 125 to 250 µm. Values are means ± SE; n = measurements made on 52–130 vessels/group from 4 lungs. Measurements were made on lung sections from saline-injected control rats gavaged with vehicle or bosentan (100 mg·kg⁻¹·day⁻¹) and MCT-injected rats gavaged with vehicle or bosentan (200 mg·kg⁻¹·day⁻¹). *P < 0.05 compared with saline control group. †P < 0.05 compared with MCT control group.](http://jap.physiology.org/)
that the specific ET_A blocker BQ-123 inhibits MCT-induced pulmonary hypertension, RV hypertrophy, and pulmonary arterial medial wall thickening. Although lung homogenate and steady-state mRNA concentrations for ET-1 in the above-mentioned study dropped significantly from control levels 14 days after MCT injection, mRNA levels for ET-1 were elevated in the kidney. The authors speculated that the increase in circulating ET-1 levels derived from increased renal production and contributed to the pulmonary hypertension.

A recent report on telemetry in rats as a method for monitoring pulmonary arterial pressure observed trends for decreased pulmonary arterial pressures and RV hypertrophy in rats given bosentan (100 mg·kg^{-1}·day^{-1} in saline and 200 mg·kg^{-1}·day^{-1} in MCT-injected) or vehicle daily for 21 days. Values are means ± SE: n = 15–25 for plasma and n = 5–6 for lung homogenate levels. *P < 0.05 compared with saline control group. **P < 0.05 compared with MCT control group.

Fig. 5. Plasma endothelin-1 (A) and lung homogenate endothelin-1 levels (B) in saline and MCT groups gavaged with bosentan (100 mg·kg^{-1}·day^{-1} in saline and 200 mg·kg^{-1}·day^{-1} in MCT-injected) or vehicle daily for 21 days. Values are means ± SE: n = 15–25 for plasma and n = 5–6 for lung homogenate levels. *P < 0.05 compared with saline control group. **P < 0.05 compared with MCT control group.

The specific mechanism by which ET-1 contributes to MCT-induced pulmonary hypertension remains unclear. Unlike Miyauchi et al. (20), we failed to detect any elevation of plasma ET-1 in MCT-injected controls, even 21 days after injection. However, like Miyauchi et al. (20), we observed a decrease in lung homogenate ET-1 levels, although the decrease was not quite statistically significant in our study. In addition, Miyauchi et al. (20) found a significant decrease in steady-state lung mRNA levels for ET-1. The question must be raised, then, How does ET-1 contribute to MCT-induced pulmonary hypertension if lung levels decrease and circulating levels remain steady? Regional variations in lung ET-1 production that decrease whole lung homogenate levels by 10.220.33.4

Fig. 6. RVSP (A) and RV/(LV+S) (B) at 0, 11, and 21 days after MCT injection. Values are means ± SE; n = 9–20 animals. *P < 0.05 compared with MCT-injected rats gavaged with vehicle alone.
concentrations but increase local concentrations, such as those adjacent to vascular smooth muscle, could offer an explanation. Alternatively, pulmonary vascular responsiveness to ET-1 could be upregulated, perhaps by alterations in the sensitivity or abundance of ET\(_{A}\) receptors. Previous studies in isolated rat lungs have shown potentiation of pulmonary pressor responses to serotonin both 7 and 14 days after MCT pyrrole injection (14). Also, steady-state mRNA levels for the ET\(_{A}\) receptor are increased in lung homogenates from rats with hypoxic pulmonary hypertension (17), although these have not been assessed in MCT-injected rats. In addition, Miyauchi et al. (20) observed an increase in oscillations induced by ET-1 in pulmonary arteries isolated from rats 14 days after MCT injection and speculated that they might reflect increased vascular responsiveness. By 25 days after MCT injection, however, the isolated pulmonary arteries had reduced contractility to ET-1 (20), suggesting that, if enhanced vascular responsiveness plays a role, it occurs transiently during the early development of pulmonary hypertension.

Regardless of the specific mechanism, blunting of MCT-induced pulmonary hypertension by bosentan is consistent with inhibition of vascular remodeling and possibly vasoconstriction. The dose of bosentan that was used completely blocked pulmonary vasoconstriction induced by Big ET-1 and partially blocked acute hypoxic vasoconstriction. This, combined with our observation that bosentan prevents the elevation of pulmonary arterial pressures seen 11 days after MCT injection before RV hypertrophy develops, is consistent with an inhibition of ET-1-induced vasoconstriction. In addition, our finding that bosentan inhibits MCT-induced pulmonary vascular medial thickening is consistent with blockade of the proliferative effect of ET-1 on vascular smooth muscle cells (15). This inhibition was most pronounced in smaller, peripheral vessels, where progenitor cells are thought to undergo transformation to smooth muscle cells, giving rise to neomuscularization (25).

The pathogenesis of MCT-induced pulmonary hypertension remains poorly understood. A liver-derived pyrrole metabolite of MCT is thought to acutely injure endothelial cells (1), causing diffuse endothelial vacuolization and swelling (24), metabolic dysfunction (21), and in situ thrombosis (19). Vascular permeability temporarily increases (28), but pulmonary hypertension and RV hypertrophy do not occur until 10–14 days after injection or ingestion, when diffuse inflammatory changes are apparent (19). This inflammatory process is accompanied by vascular and parenchymal histological changes (19), including vessel wall thickening, inflammatory interstitial changes, and the development of pulmonary function abnormalities typical of those seen with interstitial lung diseases (8). Multiple agents have been demonstrated to partially inhibit the severity of pulmonary hypertension and RV hypertrophy after MCT injection, including vasodilators (13, 22), corticosteroids (13), antiplatelet serum (12), and mild hyperoxia (11). This has led investigators to speculate that the pathogenesis of MCT-induced pulmonary hypertension is multifactorial, consisting of vasoreactive, inflammatory, cytokine, and hypoxic components.

Our findings indicate that ET-1 acts during the later inflammatory phase after MCT injection. We found that bosentan had no effect on the severity of the acute lung injury after injection, as assessed by lung lavage protein concentrations and wet-to-dry lung weight ratios. Furthermore, we found that bosentan inhibition of MCT-induced pulmonary hypertension tended to be greater during the latter half than the first half of the 3-wk period after injection. This suggests that the inhibitory effect of bosentan is unrelated to any early alteration of MCT metabolism by the liver that might have blunted the initial injury or delayed the development of pulmonary hypertension. This also suggests that ET-1 is involved mainly in the augmentation of pulmonary vascular reactivity and cellular proliferation that are thought to contribute to the development of pulmonary hypertension after the first week after MCT injection. However, the high dose of bosentan required and the relatively minor degree of inhibition achieved imply that, although ET-1 may contribute to MCT-induced pulmonary hypertension, other important agents and mediators are likely to be involved. Finally, even though MCT-induced pulmonary hypertension has not been established as a suitable model for human idiopathic pulmonary hypertension, the inhibition of pulmonary hypertension by an oral once-a-day ET blocker raises the possibility that such agents may prove useful in the therapy of pulmonary hypertension in humans.

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