Nonselective Endothelin-Receptor Antagonism Attenuates Hemodynamic Changes After Massive Pulmonary Air Embolism in Dogs*

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Study objectives: To evaluate the effects of nonselective endothelin (ET)-receptor antagonism on the hemodynamic changes and serum thromboxane (TX)-A2 levels after a massive pulmonary air embolism (PAE) in dogs.

Design: Prospective trial.

Setting: University laboratory.

Interventions: Anesthetized mongrel dogs (ET-receptor antagonist group; n = 6) received a bolus injection of 1 mg of the nonselective ET-A/ET-B-receptor antagonist PD 145065 (Sigma Chemical; St. Louis, MO), and dogs in the control group (n = 6) received saline solution. Hemodynamic data were recorded 5 min after the administration of antagonist or saline solution. Subsequently, each dog received 2.5-mL air/kg via the right femoral vein (the PAE), and the hemodynamic data were recorded for up to 60 min thereafter. Arterial blood samples were drawn at baseline and 15 min after PAE for the determination of plasma TX-A2, measured by enzyme-linked immunosorbent assay as TX-B2 (the stable metabolite of TX-A2).

Results: PD 145065 alone produced no hemodynamic effects. However, dogs pretreated with PD 145065 had significantly lower increases in mean pulmonary arterial pressure and in pulmonary vascular resistance after the PAE (116% and 165%, respectively) compared to the control dogs (187% and 367%, respectively). The mean arterial pressure (MAP), cardiac index (CI), and plasma TX-B2 levels were unaltered after PAE in the presence of ET-receptor antagonist, whereas CI and MAP decreased 5 to 10 min after PAE, and TX-B2 concentrations increased 15 min after PAE in control dogs (p < 0.05 in all cases).

Conclusions: Nonselective antagonism of ET receptors attenuates the pulmonary hypertension and blunts the TX-A2 release caused by massive PAE in dogs.

Key words: embolism; endothelin; endothelium; lung; pathophysiology; pulmonary hypertension; thromboxane

Abbreviations: BL = baseline; CI = cardiac index; CVP = central venous pressure; ET = endothelin; HR = heart rate; MAP = mean arterial pressure; MPAP = mean pulmonary artery pressure; PAE = pulmonary air embolism; PVRI = pulmonary vascular resistance index; PWP = pulmonary capillary wedge pressure; SVRI = systemic vascular resistance index; TX = thromboxane

Massive pulmonary air embolism (PAE) is a serious complication that may occur in many therapeutic interventions. Some studies have focused on the effectiveness of monitoring for the occurrence of PAE. Although an early diagnosis is essential for the prompt treatment of this complication, little progress has been made in understanding the pathophysiologic mechanisms involved in the cardiovascular consequences of PAE and its therapy. Despite the fact that the selective pulmonary vasodilation caused by inhaled nitric oxide attenuates the hemodynamic changes after a massive PAE7 and

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Jose E. Tanus-Santos and Wladimir M. Gordo are supported by FAPESP-SP-Brazil. This work was supported by FAPESP-SP-Brazil.

Manuscript received July 19, 1999; revision accepted January 5, 2000.

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during a continuous venous air embolism in dogs, no further progress has been reported on this issue.

The pathophysiologic changes seen in a PAE may differ significantly from those seen in a pulmonary thromboembolism. However, the obstruction of the pulmonary artery blood flow and the release of vasoactive mediators such as serotonin, histamine, thromboxane (TX)-A₂, and endothelin (ET)-1, occur and increase pulmonary vascular resistance in both conditions. Thus, the pharmacologic blockade of the receptors for the potent pulmonary vasoconstrictor ET-1, a member of the ET family, may have beneficial hemodynamic effects during pulmonary embolism. This suggestion is supported by the observation that abnormal ET-1 metabolism leads to higher serum levels of this peptide after pulmonary thromboembolism in patients. In addition, the vasoconstrictor and cardiodepressive effects of ET-1 during pulmonary embolism suggest that this peptide has a role in the hemodynamic changes after pulmonary embolism. However, conflicting results have been reported for the role of ET-1 in the pulmonary hypertension after PAE in rabbit isolated perfused lungs.

To determine the importance of ET-1 in the cardiovascular responses to PAE, we examined the effects of nonselective ET-receptor antagonism on the hemodynamic changes induced by a massive PAE in dogs. Since it is possible that ET-1 released after PAE stimulates the formation of TX-A₂, we also measured the plasma concentrations of TX-B₂ (the stable breakdown product of TX-A₂) to assess whether nonselective ET-receptor antagonism affects the in vivo production of TX-A₂, a known pulmonary vasoconstrictor released after air embolism.

**Materials and Methods**

All procedures were approved by our animal care committee. The dogs were handled according to the guideline principles published by the National Institutes of Health and the Council of the American Physiology Society. Twelve mongrel dogs (weight, 10.9 ± 0.6 kg) of either sex were anesthetized with sodium pentobarbital, 40 mg/kg IV; paralyzed with pancuronium bromide, 0.1 mg/kg IV; and tracheally intubated; and their lungs were given mechanical ventilation with room air using a volume-cycled respirator (Dual Phase Control Respirator; Harvard Apparatus; Boston, MA). The tidal volume was 15 mL/kg, and the respiratory rate was adjusted to maintain a baseline (BL) physiologic PaO₂ (as revealed by end-tidal CO₂ monitoring). Anesthesia was maintained with an infusion of sodium pentobarbital, 6 to 10 mg/kg/h. Fluid-filled catheters were placed in the right femoral artery and right femoral vein for mean arterial pressure (MAP) monitoring via a pressure transducer and fluid administration, respectively. A 7F balloon-tipped Swan-Ganz thermodilution catheter was placed into the pulmonary artery via the right external jugular vein, and its correct location was confirmed by detection of the typical pressure wave of this artery. The catheter was connected to a pressure transducer to allow the monitoring of mean pulmonary artery pressure (MPAP), central venous pressure (CVP), and pulmonary capillary wedge pressure (PCWP). The transducers were zeroed at the level of the right heart and recalibrated before each set of measurements. Cardiac output was determined in triplicate by injecting 5 mL of saline solution, and the results were recorded on a computerized system (Datex-Engstrom; Helsinki, Finland). The heart rate (HR) was measured using a surface ECG (lead I). Before initiating the experiments, blood samples were drawn from the femoral artery for PaO₂, arterial oxygen saturation, PaCO₂, arterial blood pH, and hemoglobin determination using a blood gas analyzer (Stat Profile 5; Nova Biomedical; Waltham, MA). These respiratory variables were within the physiologic limits in all of the dogs.

After at least 20 min of stabilization, a BL time point hemodynamic evaluation was performed, and the dogs were randomly assigned to one of two experimental groups: the dogs in ET-receptor antagonist group (n = 6) received a bolus injection of 1 mg of the mixed ET-A/ET-B-receptor antagonist PD 145065 (Sigma Chemical; St. Louis, MO), and dogs in the control group (n = 6) received the same volume (3 mL) of saline solution. Hemodynamic data were recorded 5 min (antagonist time point) after the administration of antagonist (or saline solution), and each dog subsequently received 2.5-mL air/kg body weight (the same dose of previous studies) at a rate of 5 mL/s via the right femoral vein, followed by a 10-mL saline solution flush. The hemodynamic data were recorded 5, 10, 15, 30, and 60 min after the PAE.

The dose of PD 145065 we selected in the present study was based on a previous study demonstrating that equimolar doses (1 mol) of specific ET-A- and ET-B-receptor antagonists produced no significant hemodynamic effects in dogs. Interestingly, this study showed that the specific ET-B-receptor antagonist completely abolished the removal of ET-1 during a single pulmonary transit time.

The cardiac index (CI), systemic vascular resistance index (SVRI), and pulmonary vascular resistance index (PVRI) were calculated by using standard formulas.

Blood samples were drawn from the femoral artery at BL and 15-min time points for the determination of plasma TX-A₂ levels, measured as TX-B₂, using a commercial enzyme immunoassay (Cayman Chemical; Ann Arbor, MI).

The results are presented as mean ± SEM. One-way analysis of variance for repeated measures was used to determine the changes in the hemodynamic parameters and serum TX-B₂ concentrations in each group (SigmaStat for Windows; Jandel Scientific; San Rafael, CA). When the one-way analysis of variance for repeated measures was significant, the differences were tested by the Dunnett multiple comparisons test. The between-groups comparisons for single measurements were assessed using Student’s unpaired t test. A probability value < 0.05 was considered significant.

**Results**

There were no significant differences in the BL hemodynamic variables between groups, and the administration of the ET-receptor antagonist alone produced no important hemodynamic effects (Fig 1 and Table 1).

The CI and MAP were decreased and CVP was increased for 5 to 10 min after PAE in the control group, while pretreatment with the ET-receptor antagonist resulted in unchanged CI, MAP, and CVP...
MPAP and PVRI were higher in the control group for > 10 min after PAE, when compared with the ET-receptor antagonist group. These variables returned to baseline 15 to 60 min after PAE in both groups (Fig 1 and Table 1). There were no significant changes in HR in both groups, and PWP was increased in both groups for 5 to 10 min after PAE (Table 1).

In pilot experiments, a group of four dogs received the same dose of PD 145065 without air emboli, and were observed for 60 min. In addition, hemodynamic data were registered for 60 min in another group of four sham-operated-on animals. There were no significant changes in the hemodynamic variables in both groups of animals during this period of 60 min (data not shown).

TX-B₂ concentrations increased 15 min after the PAE in control dogs, but not in dogs pretreated with the ET-receptor antagonist (Fig 2). Since the major hemodynamic changes occurred 5 min after the PAE, we determined TX-B₂ levels at this time point in two animals. Dogs pretreated with the ET-receptor antagonist tended to show lower TX-B₂ concentrations as compared to control dogs (590 ± 90 vs 1115 ± 140 pg/mL).

**Discussion**

Little is known of the mechanisms underlying pulmonary embolism-induced vascular injury. The main finding of this study was that nonselective antagonism of ET receptors attenuated the pulmonary hypertension and blunted TX-A₂ formation after a massive PAE in dogs.

The hemodynamic responses observed after PAE in the control group were similar to those previously reported by others. The lack of increase in HR may be the result of cardiovascular reflexes involving the activation of vagal afferents in the atria or ventricles by volume or pressure loading. The increase in MPAP and PVRI after pulmonary embolism results from the interaction of three components, namely, massive mechanical obstruction of the right ventricle and pulmonary vessels, general arteriolar neurogenic constriction, and the release of humoral mediators. With regard to the latter component, a previous study using a selective ET-A-receptor antagonist in isolated perfused lungs suggested that the acute hemodynamic responses to PAE were mainly mediated by the potent pulmonary vasoconstrictor ET-1 acting via ET-A receptors. However, conflicting results have been reported on this issue, and it is probable that isolated perfused lungs may not possess the pathophysiologic features found in intact animals. It is important to note that in...
isolated perfused lungs studies, the absence of blood cells and plasma proteins in the perfusate may lead to significantly different conclusions when compared to intact animal studies. Our results showed lower increases in MPAP and PVRI after PAE in dogs pretreated with the nonselective ET-receptor antagonist (116% and 165%, respectively), compared to the control dogs (187% and 367%, respectively), giving further support to the hypothesis that ET-1 is an important vasoactive mediator released after PAE.19 In contrast with the control dogs, the MAP and CI did not change significantly after PAE in the ET-receptor antagonist group, probably reflecting a maintained pulmonary blood flow in dogs pretreated with the ET-receptor antagonist.

Increases in circulating ET-1 levels have been observed in patients with both primary and secondary pulmonary hypertension.20 However, the relevance of the increased ET-1 levels after pulmonary embolism in patients14 or laboratory animals11,12,19 and the use of ET antagonists in this setting remains to be elucidated. Interestingly, ET-B receptors on pulmonary endothelial cells are responsible for pulmonary clearance of ET-1 in dogs,18 and the blockade of these receptors by nonselective ET antagonists may adversely increase the circulating levels of ET-1 causing pulmonary vasoconstriction via ET-A receptors.18

The increased levels of TX-B2 15 min after PAE in the control group may reflect the increase in TX-A2 formation from 0 to 15 min after PAE. These findings were similar to those previously reported,1,9,12,19 and strengthen the idea that pulmonary vasoconstrictors are released after pulmonary embolism, thereby causing further deterioration of pulmonary hemodynamics. This idea is supported by previous studies showing that ET-1 activates the cyclooxygenase pathway, resulting in enhanced TX-A2 formation.16,21 We did not measure the levels of ET-1 in the present study; however, the attenuation of hemodynamic changes and the lower levels of TX-B2 after PAE in the ET-receptor antagonist group suggest that ET-1 is released after PAE and stimulates TX-A2 formation. These results are in accordance with previous studies suggesting that the release of ET-1 may result in increased TX-A2 formation after PAE.12 In conclusion, the antagonism of ET receptors attenuated the hemodynamic effects of ET-1 on the pulmonary circulation.

**Table 1—Hemodynamic Variables**

<table>
<thead>
<tr>
<th>Variables</th>
<th>BL</th>
<th>ANTAG</th>
</tr>
</thead>
<tbody>
<tr>
<td>HR, beats/min</td>
<td>136 ± 9</td>
<td>134 ± 8</td>
</tr>
<tr>
<td>MAP, mm Hg</td>
<td>107 ± 6</td>
<td>107 ± 6</td>
</tr>
<tr>
<td>MPAP, mm Hg</td>
<td>13 ± 1</td>
<td>14 ± 2</td>
</tr>
<tr>
<td>CI, L/min/m²</td>
<td>4.2 ± 0.4</td>
<td>4.3 ± 0.4</td>
</tr>
<tr>
<td>PWP, mm Hg</td>
<td>5 ± 1</td>
<td>5 ± 1</td>
</tr>
<tr>
<td>CVP, mm Hg</td>
<td>2.1 ± 1.0</td>
<td>2.3 ± 1.0</td>
</tr>
<tr>
<td>PVRI, dynecm⁻⁵/m²</td>
<td>150 ± 16</td>
<td>150 ± 17</td>
</tr>
<tr>
<td>SVRI, dynecm⁻⁵/m²</td>
<td>2,184 ± 328</td>
<td>2,138 ± 299</td>
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</tbody>
</table>

<table>
<thead>
<tr>
<th>Time After PAE, min</th>
<th>5</th>
<th>10</th>
<th>15</th>
<th>30</th>
<th>60</th>
</tr>
</thead>
<tbody>
<tr>
<td>HR beats/min</td>
<td>152 ± 9</td>
<td>152 ± 8</td>
<td>145 ± 9</td>
<td>146 ± 9</td>
<td>144 ± 11</td>
</tr>
<tr>
<td>MAP, mm Hg</td>
<td>119 ± 7</td>
<td>118 ± 9</td>
<td>108 ± 17</td>
<td>109 ± 13</td>
<td>107 ± 12</td>
</tr>
<tr>
<td>MPAP, mm Hg</td>
<td>12 ± 2</td>
<td>12 ± 2</td>
<td>26 ± 21</td>
<td>19 ± 21</td>
<td>17 ± 2</td>
</tr>
<tr>
<td>CI, L/min/m²</td>
<td>4.9 ± 0.5</td>
<td>4.8 ± 0.5</td>
<td>4.3 ± 0.4</td>
<td>4.3 ± 0.2</td>
<td>4.5 ± 0.4</td>
</tr>
<tr>
<td>PWP, mm Hg</td>
<td>5 ± 1</td>
<td>5 ± 1</td>
<td>9 ± 11</td>
<td>7 ± 1</td>
<td>6 ± 1</td>
</tr>
<tr>
<td>CVP, mm Hg</td>
<td>2.8 ± 0.8</td>
<td>2.7 ± 0.9</td>
<td>3.0 ± 1.0</td>
<td>2.9 ± 1.0</td>
<td>2.0 ± 0.9</td>
</tr>
<tr>
<td>PVRI, dynecm⁻⁵/m²</td>
<td>127 ± 14</td>
<td>119 ± 13</td>
<td>336 ± 56</td>
<td>261 ± 25</td>
<td>203 ± 34</td>
</tr>
<tr>
<td>SVRI, dynecm⁻⁵/m²</td>
<td>2,029 ± 331</td>
<td>1,963 ± 350</td>
<td>1,891 ± 273</td>
<td>2,026 ± 265</td>
<td>1,916 ± 234</td>
</tr>
</tbody>
</table>

*Data are presented as mean ± SEM of six dogs per group. ANTAG = 5 min after ET-receptor antagonist administration.

†p < 0.05 ET-receptor antagonist group vs control group.

†p < 0.05 vs BL.

**Figure 2.** Serum TX-B₂ concentrations at BL and 15 min after PAE. Values are mean ± SEM; * = p < 0.05 vs BL; # = p < 0.05 ET-receptor antagonist group vs control group.
changes caused by massive PAE in intact animals. This occurred because the ET blocker may have attenuated the vasoconstriction caused by ET-1 and prevented the formation of TX-A2.

ACKNOWLEDGMENT: The authors thank the Center of Experimental Medicine and Surgery (UNICAMP) and Dr. Stephen Hyslop for reviewing the manuscript.

REFERENCES
1. Glenski JA, Cucchiara RF, Michenfelder JD. Transesophageal echocardiography and transcutaneous O2 and CO2 monitoring for the detection of venous air embolism. Anesthesiology 1986; 64:541–545
4. Pearl R, Larson P. Hemodynamic effects of positive end-expiratory pressure during continuous venous air embolism in the dog. Anesthesiology 1986; 64:724–729

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