Pulmonary Responses to Selective Phosphodiesterase-5 and Phosphodiesterase-3 Inhibitors*

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Objective: To compare the direct pulmonary vasodilating activity and specificity of phosphodiesterase-5 (zaprinast) and phosphodiesterase-3 (milrinone) inhibitors on the pulmonary vascular (PV) bed of the spontaneously breathing cat with an intact chest.

Design: Prospective, randomized animal study.

Setting: Laboratory of university hospital.

Subjects: Experiments were performed in vivo in intact-chest, spontaneously breathing cats with controlled pulmonary blood flow and constant left atrial pressure.

Interventions: The responses to intralobar injections of zaprinast and milrinone were investigated at low PV tone. PV tone was then increased by intralobar arterial infusion of a thromboxane A_2 mimic, U46619. Animals received intralobar bolus injections of zaprinast or milrinone, followed by continuous IV infusion of the drug, which was administered in incremental doses titrated to produce a 20% reduction in mean systemic arterial pressure.

Measurements and main results: At low PV tone, zaprinast, but not milrinone, decreased lobar arterial pressure (LoAP). At elevated PV tone, both drugs caused dose-dependent decreases in LoAP; however, milrinone caused significantly less pulmonary vasodilation. Dose-related decreases in mean systemic arterial pressure were observed with milrinone, but not with zaprinast. When the continuous IV infusion was titrated to produce a 20% reduction in mean systemic arterial pressure, the decreases in lobar arterial pressure with zaprinast infusion were significantly greater than those produced by milrinone.

Conclusions: These data show that zaprinast and milrinone exert a direct in vivo vasodilator effect on the PV bed at low (zaprinast) and elevated (zaprinast and milrinone) PV tone; however, at elevated PV tone, the pulmonary vasodilator effect was greater with zaprinast than with milrinone. This suggests that phosphodiesterase-5 inhibitors may potentially offer a therapeutic alternative in the management of acute pulmonary hypertension. (CHEST 2004; 125:644–651)

Key words: milrinone; phosphodiesterase inhibitor; pulmonary hypertension; thromboxane A_2; vasodilation; zaprinast

Abbreviations: cAMP = cyclic adenosine monophosphate; cGMP = cyclic guanosine monophosphate; LoAP = lobar arterial pressure; PDE = phosphodiesterase; PGE_1 = prostaglandin E_1; PV = pulmonary vascular

The cyclic nucleotides, cyclic guanosine monophosphate (cGMP) and cyclic adenosine monophosphate (cAMP), have been implicated as mediators of vasodilation.1,2 Because nitric oxide and prostacyclin cause pulmonary vasodilation by increasing these cyclic nucleotides levels, it follows that inhibiting enzymatic inactivation of cGMP and cAMP would likewise result in pulmonary vasodilatation.3,4 Phosphodiesterase (PDE) inhibitors prevent the breakdown of cGMP and cAMP; therefore, these agents are potentially useful in clinical situations where active pulmonary vasoconstriction is one of the pathophysiologic features.6,7

Previous data suggest that the predominant PDE isoenzymes in pulmonary arteries are PDE-5 (cGMP specific) and PDE-3 (cAMP specific, cGMP inhibitable),5 suggesting that these enzymes are potential pharmacologic targets for pulmonary vasodilation. In previous studies8–24 that used various models of pulmonary hypertension, both zaprinast (PDE-5 in-
hhibitor) and milrinone (PDE-3 inhibitor) exhibited pulmonary vasodilating activity; however, for these drugs to be useful in the treatment of pulmonary hypertension, they should lower pulmonary artery pressure while minimizing the risk of systemic hypotension. The pulmonary selectivity of zaprinast and milrinone is unclear; therefore, the aim of this study was to compare the pulmonary vasodilating effects of PDE-5 and PDE-3 inhibitors, and especially their selectivity for the pulmonary vasculature. This was performed in spontaneously breathing cats with intact chests, under conditions of controlled pulmonary blood flow and increased pulmonary vascular (PV) tone.

Materials and Methods

Animal Preparation

With the approval of the Institutional Animal Care and Use Committee, adult cats weighing 2.8 to 4.5 kg were anesthetized with sodium pentobarbital, 20 mg/kg IV, and were secured in the supine position on a fluoroscopic table. Additional sodium pentobarbital was administered hourly. The cats spontaneously breathed room air through an endotracheal tube. Polyethylene catheters were inserted into both femoral veins and femoral arteries to measure mean arterial pressure, to obtain arterial blood samples, to perfuse the left lower lung lobe, and for drug administration.

To selectively perfuse the left lower lobe, a specially designed 6F, triple-lumen, balloon perfusion catheter was advanced under fluoroscopic guidance from the external jugular vein into the arterial branch of the left lower lobe. After heparinization (1,000 U/kg), the lobe was perfused at a constant rate with blood withdrawn from the aorta through a port immediately distal to the balloon cuff of the perfusion catheter. After initiation of left lower lobe perfusion, the lobar artery was isolated by inflation of the balloon cuff on the catheter. Perfusion pressure in the lobar artery was measured through a third lumen port that was located distal to the perfusion port. The lobe was perfused using a peristaltic pump (model 1210; Harvard Apparatus; South Natick, MA) at a flow rate of 38 to 40 mL/min. The flow rate was measured before every experiment and did not change during the experiment. Left atrial pressure was measured with a 5F, double-lumen, Teflon catheter passed transseptally under fluoroscopic guidance into the vein draining the left lower lung lobe. Because flow to the lobe was constant, changes in lobar arterial pressure reflect changes in vascular resistance. These procedures have been described previously.25 All vascular pressures were measured with Sorenson disposable transducers (Abbott Laboratories; North Chicago, IL) that were set to zero at the right atrial level, and mean pressures obtained by electronic integration were continuously recorded on a Gould Recorder (Gould, Cleveland, OH). After completion of catheterization procedures, the animals were allowed to stabilize for 30 min.

Experimental Protocol

Low-Tone Experiments: At low baseline PV tone (mean lobar arterial pressure [LoAP] < 15 mm Hg), boluses of either milrinone (n = 5; 1 μg, 10 μg, 100 μg, 1,000 μg) or zaprinast (n = 5; 1 μg, 10 μg, 100 μg, 1,000 μg) were injected into the lobar perfusion circuit in random order (ie, order of bolus injections was determined by table of random numbers).

Elevated-Tone Experiments: In another set of experiments, PV tone was raised to a mean LoAP of 36 to 40 mm Hg by infusing the thromboxane analog U46619 (9,11-dideoxy-9α,11e-methanoepoxy-prostaglandin F2α; Upjohn Laboratories; Kalamazoo, MI) (dissolved in ethanol and diluted with 0.9% sodium chloride to a concentration of 1 mg/mL). The infusion rate was maintained constant for the duration of the experiment. Cats were then assigned to one of two vasodilator (zaprinast or milrinone) groups (seven cats per group).

Bolus Injections

Initially, the acute responses to bolus injections of the drug were assessed. Animals received either intralobar injections (order of bolus injections was randomized) of zaprinast (Sigma Chemicals; St. Louis, MO) [1 μg, 10 μg, 100 μg, 1,000 μg; n = 7] or milrinone (Sanofi Pharmaceuticals; New York, NY) [1 μg, 10 μg, 100 μg, 1,000 μg; n = 7]. All agents were injected in small volumes as a rapid bolus directly into the perfused lobar artery. Injections were made only after lobar arterial and systemic pressures had returned to preinjection levels, and when a minimum of 15 min between injections had elapsed.

Continuous IV Infusion

After the last dose of zaprinast or milrinone was administered and once the pulmonary pressures had returned to baseline elevated-tone conditions (36 to 40 mm Hg), either milrinone or zaprinast were administered as a continuous drug infusion in incremental doses titrated to produce a 20% reduction in mean systemic arterial pressure. The infusions were started at a low dose (100 μg/kg/min for zaprinast, 2 μg/kg/min for milrinone) and increased in a stepwise fashion (infusion of zaprinast was titrated up by 40 μg/kg/min, and infusion of milrinone was titrated up by 0.5 μg/kg/min). Each dose was infused until lobar and systemic arterial pressure stabilized. After recovery from the infusion, carrier solutions used for each dose were administered at the same volume (bolus injections) and the same infusion rate (continuous infusion). Administration of carrier solutions had no significant effect on lobar or systemic arterial pressure. Arterial blood gases and pH (178PH; Corning; Medfield, MA) were measured before bolus injections of the drug, before drug infusion, and after establishing a stable infusion rate and a 20% reduction in mean systemic arterial pressure.

Statistical Analysis

A power analysis for a difference of 8 to 10 mm Hg between the two groups (when the maximal bolus dose was injected or during continuous IV infusion), with estimated SD of the change of 7, showed that the sample size needed to detect this difference with a statistical power of 80 to 88% (depending on the difference) was 7. All hemodynamic data are expressed in absolute units and presented as mean ± SE. Responses represent peak changes from baseline. Paired t tests and unpaired t tests were performed for intragroup and intergroup comparison, respectively. To test for intragroup differences among doses, comparisons were made using one-way analysis of variance with the Bonferroni correction for multiple comparisons between groups. Analysis was performed using Statistical Analysis System software (version 6.12; SAS Institute; Cary, NC); p < 0.05 was considered significant.

Results

Responses at Low PV Tone

Resting mean LoAP was not significantly different between the groups. Under low-tone conditions,
intralobar injections of zaprinast (1 to 1,000 μg) produced dose-dependent decreases in mean LoAP (Fig 1, top, a). Milrinone had no significant effect on mean LoAP (Fig 1, bottom, b).

Responses at Elevated PV Tone, Bolus Injection

Intralobar infusion of U46619 caused a rapid increase in mean LoAP without affecting mean systemic arterial pressure or left atrial pressure (Table 1). This effect remained constant throughout the infusion and was similar during all subsequent experiments.

The effects of intralobar injections of zaprinast, an inhibitor of type 5 (cGMP specific) PDE, on the PV bed of the cat are illustrated in Figures 2, 3. Intralobar administration of zaprinast (1 to 1,000 μg)
into the perfused left lower lobe caused dose-related decreases in LoAP. Left atrial pressure was unchanged and mean systemic arterial pressure decreased significantly only at the highest dose (Fig 4). Inasmuch as lobar blood flow was held constant and left atrial pressure was unchanged, the fall in LoAP reflected decreased lobar vascular resistance. The responses were rapid in onset, and pressures returned toward control values over a 2- to 8-min period, depending on the dose injected.

Intralobar injections of milrinone significantly decreased mean LoAP at the two highest doses (100 µg and 1,000 µg) [Fig 3]; however, mean systemic arterial pressure decreased in a dose-related manner (Fig 4). The pulmonary vasodilating response with milrinone was significantly less than with zaprinast (p < 0.05), whereas the decreases in mean systemic arterial pressure were significantly greater (p < 0.05) [Figs 3, 4]. The efficacy and the pulmonary vasoselectivity of milrinone and zaprinast were compared by dividing the changes in mean LoAP by the changes in mean systemic arterial pressure at the different doses. For milrinone at 1 µg, 10 µg, 100 µg, and 1,000 µg, the results were 0.12 ± 0.03, 0.08 ± 0.01, 0.25 ± 0.02, and 0.28 ± 0.04, respectively. The corresponding results for zaprinast were 1 ± 0.2, 2.3 ± 0.3, 3 ± 0.3, and 1.9 ± 0.2 (p < 0.05 compared to milrinone at the corresponding doses).

Continuous IV Infusion

Lobar vascular responses to continuous infusion of zaprinast and milrinone were also investigated during increased PV tone. The two drugs produced equivalent reductions in systemic arterial pressure (approximately 20%) [Table 2]. Drug infusion rates were 757 ± 101 µg/kg/min for zaprinast, and 8 ± 1 µg/kg/min for milrinone. Both drugs decreased mean LoAP without changing left atrial pressure. The decreases in mean LoAP with zaprinast infusion

<table>
<thead>
<tr>
<th>Drugs</th>
<th>LoAP, mm Hg</th>
<th>Systemic Arterial Pressure, mm Hg</th>
<th>Left Atrial Pressure, mm Hg</th>
</tr>
</thead>
<tbody>
<tr>
<td>Zaprinast</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Baseline</td>
<td>8.2 (0.5)</td>
<td>132 (3)</td>
<td>4.7 (1.0)</td>
</tr>
<tr>
<td>U46619†</td>
<td>37.5 (0.5)</td>
<td>131 (5)</td>
<td>4.3 (1.1)</td>
</tr>
<tr>
<td>Milrinone</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Baseline</td>
<td>7.3 (0.6)</td>
<td>132 (5)</td>
<td>3.8 (0.9)</td>
</tr>
<tr>
<td>U46619†</td>
<td>37.2 (0.4)</td>
<td>136 (5)</td>
<td>4.5 (1.2)</td>
</tr>
</tbody>
</table>

*Values are mean ± SE of seven animals.
†Values recorded after production of elevated PV tone with U46619. Mean vascular pressures were comparable in both groups before and after the administration of U46619. The administration of U46619 did not cause any significant changes (vs baseline) in mean systemic or left atrial pressures (paired t test).
were significantly greater than with milrinone: 39 ± 1% with zaprinast, vs 11 ± 1% with milrinone (p < 0.0001; 95% confidence interval, 25 to 32%). There were no significant changes in arterial oxygen tension during drug therapy (Table 2).

**Discussion**

In treating pulmonary hypertension, a drug with preferential pulmonary vasodilator effect is desirable. We found that both zaprinast and milrinone
exerted direct vasodilating effect on the pulmonary vascular bed. Zaprinast demonstrated the greatest pulmonary selectivity and caused the greatest decrease in lobar arterial pressure.

There are at least five well-described isoforms of PDE with varying specificity for the hydrolysis of cyclic nucleotides. Selective inhibitors for some of the isoforms have been synthesized. Rabe et al showed that selective inhibition of PDE-3 and PDE-5 relaxed pulmonary artery rings. These results were in agreement with their finding of high tissue PDE-5 relaxed pulmonary artery rings. These results showed that selective inhibition of PDE-3 and pulmonary selectivity and caused the greatest de-

 vascular bed. Zaprinast demonstrated the greatest pulmonary hypertension and different modes of drug ad-

 Table 2—Effect of IV Infusion of Zaprinast and Milrinone When Lobar Vascular Pressures Were Increased by U46619 (n = 7)*

<table>
<thead>
<tr>
<th>Variables</th>
<th>Baseline Value†</th>
<th>Value at the Time of Therapeutic Response‡</th>
</tr>
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<tbody>
<tr>
<td>Mean systemic arterial</td>
<td></td>
<td></td>
</tr>
<tr>
<td>pressure, mm Hg</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Zaprinast</td>
<td>129 (6)</td>
<td>104 (5)</td>
</tr>
<tr>
<td>Milrinone</td>
<td>132 (5)</td>
<td>106 (5)</td>
</tr>
<tr>
<td>Pa02, torr</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Zaprinast</td>
<td>103 (5)</td>
<td>99 (6)</td>
</tr>
<tr>
<td>Milrinone</td>
<td>98 (6)</td>
<td>102 (5)</td>
</tr>
</tbody>
</table>

*Data are presented as mean ± SE.
†Before drug infusion started.
‡When 20% reduction in mean systemic arterial pressure was reached. There were no significant differences between groups in mean systemic arterial pressure at baseline and when 20% reduction in mean systemic arterial pressure was reached (unpaired t test). Pa02 did not significantly change (vs baseline) when IV administra-

tion of zaprinast or milrinone caused 20% reduction in mean systemic arterial pressure (paired t test).

U46619-induced pulmonary vasoconstriction, the two PDE inhibitors decrease LoAP in a dose-dependent manner. Since blood flow to the lobe and left atrial pressure were maintained constant, the decreases in LoAP reflect decreases in lobar vascular resistance. U46619-induced pulmonary vasoconstric-

tion was more completely reversed with zaprinast compared to milrinone. Both zaprinast and mili-

rnone decreased systemic arterial pressure; however, the effect of zaprinast was noted only at the highest dose. Nitroglycerin and PGE1 have previously been reported to exert favorable vasodilating effects on the pulmonary circulation in similar experimental preparations. In the present study, intralobar injections of nitroglycerin and PGE1 (0.1 to 10 μg, five animals per group) produced comparable dose-related decreases in mean lobar and systemic arterial pressures that were significantly greater than the decreases produced by zaprinast and milrinone. Since cardiac output and central venous pressure were not measured, the relative changes in pulmonary and systemic vascular resistances could not be compared in the present study.

The relative pulmonary vasoselectivity of zaprinast were also demonstrated when zaprinast and milri-

none were titrated (continuous IV administration) to produce equivalent reductions in mean systemic arterial pressure; with zaprinast, mean LoAP decreased by 39%, compared to 11% with milrinone. Using the same experimental conditions, PGE1 and nitroglycerin decreased LoAP by 28% and 24%, respectively. The observed minimal effect of milri-

none in dilating the PV bed suggests that the benefi-
cial effects of milrinone in the clinical setting (for example, patients with pulmonary hypertension awaiting heart transplantation) is mainly attributed to its inotropic and systemic vasodilator actions. In addition, the smaller dilating responses with the PDE-3 inhibitor may simply reflect a lower level of this isozyme in the pulmonary arteries of the cat. Alternatively, our findings may support the hypoth-

esis that it is the activity of guanylate cyclase which plays a major role in the regulation of PV tone.15,17,27 In the present study, the PDE inhibitors were administered IV. Ichinose et al 11 reported that aerosolized zaprinast selectively dilated the pulmonary circulation in a lamb model of acute pulmonary hypertension. Inhalation of zaprinast may be preferable to IV or oral administration since it selectively delivers high concentrations of the drug to ventilated regions of the lung and this may produce less systemic vasodilation.

U46619, a stable prostaglandin endoperoxide anal-

og, is a thromboxane A2 mimetic. In the present study, it was infused directly into the left lower lobe to increase pulmonary vascular tone. U46619 admin-
istration produced sustained pulmonary hypertension without increasing systemic arterial pressure. The increase in pulmonary arterial pressure dissipated within a few minutes after stopping the U46619 infusion. The administration of vasodilator agents reduced pulmonary arterial pressure, indicating that reversible pulmonary vasoconstriction was present. Moreover, the use of a thromboxane mimic most likely simulates what occurs in the pulmonary vascular bed after lung injury since many pathologic states with pulmonary vasoconstriction are in part mediated by thromboxane A$_2$. Finally, this model has previously been reported to be effective for examining the effects of various pulmonary vasodilators used in the clinical arena. These properties of U46619 permit comparisons of the pulmonary effects of vasoactive drugs during pulmonary hypertension; however, since pulmonary hypertension produced by U46619 is an experimental model, care must be taken when extrapolating the results to the clinical situation.

The results of this study demonstrate that zaprinast is able to directly reduce elevated PV tone and not through secondary effects on other factors such as cardiac output or left atrial pressure. Additionally, compared to other pulmonary vasodilators, zaprinast had major pulmonary vasodilating effects at doses that maintained acceptable levels of systemic BP. These data indicate that it might be possible to use PDE-5 inhibitors in the clinical treatment of patients with pulmonary hypertension, although it should be noted that data from cats cannot necessarily be extrapolated to humans with this disorder. Two case reports showed encouraging results with the PDE-5 inhibitor sildenafil in patients with primary pulmonary hypertension, suggesting a role for PDE-5 inhibitors in the management of these patients.

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