Treatment with phosphodiesterase inhibitors type III and V: milrinone and sildenafil is an effective combination during thromboxane-induced acute pulmonary hypertension

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Objectives. To evaluate the effects of phosphodiesterase type III and V (PDEIII and PDEV) inhibition on pulmonary and systemic haemodynamics in a porcine model of acute pulmonary hypertension.

Methods. Twenty-four adult swine were anaesthetized with 1 MAC isoflurane and mechanically ventilated with an \( F_{\text{IO2}} \) of 100%. Micromanometer-tipped catheters were placed in the ascending aorta, pulmonary artery and right ventricle. Pulmonary flow was measured with a perivascular probe using transit time ultrasound. Pulmonary hypertension was induced with a continuous infusion of the thromboxane analogue, U46619. The animals were then randomized to four groups: Group 1 (n=6) received 50 mg of sildenafil (PDEV inhibitor) diluted in water via an orogastric tube; Group 2 (n=6) received 50 mg kg\(^{-1}\) of i.v. milrinone (PDEIII inhibitor); Group 3 (n=6) received sildenafil followed by milrinone; and Group 4 (n=6) received placebo via an orogastric tube.

Results. Pulmonary hypertension was achieved in all animals. Calculated pulmonary vascular resistance decreased by an average of 36% after sildenafil (\( P<0.05 \)), 41% after milrinone (\( P<0.05 \)), and 61% with both drugs combined (\( P<0.05 \)). Systemic vascular resistance decreased by 37% (\( P<0.05 \)) with milrinone alone, and 36% (\( P<0.05 \)) with milrinone and sildenafil combined but it was preserved in the sildenafil group. Cardiac output and right ventricular dP/dT were significantly improved after milrinone or both drugs combined, but not with sildenafil.

Conclusion. Milrinone and sildenafil are effective pulmonary vasodilators, with independent action and additive effect. Both drugs combined achieved a better haemodynamic profile, with greater pulmonary vasodilatation and increased contractility but without additional systemic vasodilatation. The systemic haemodynamic profile (systemic vasodilation, cardiac output, right ventricular dP/dT) is improved with milrinone but not with sildenafil.

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Acute intraoperative pulmonary hypertension (PHTN) can have significant impact on right ventricular (RV) function and can lead to haemodynamic decompensation and significant perioperative morbidity and mortality. Pulmonary vasorelaxation can be achieved with drugs that increase pulmonary smooth muscle cAMP or cGMP.1–4 The predominant pathway for inactivation of these cyclic nucleotides in the pulmonary vasculature is via phosphodiesterase enzymes type III (PDEIII) and type V (PDEV).5

Patients with chronic PHTN exhibit a lower response to agonist drugs. This is partly because of upregulation of both isoforms of phosphodiesterase which limit the duration of action of either cAMP or GMP.5,7 As a result, agents that block the activity of these enzymes have received significant interest for their potential therapeutic benefits. Milrinone is
a known inhibitor of PDEIII and is commonly utilized to improve heart function after cardiopulmonary bypass. Furthermore, this drug has been particularly useful in patients with concomitant PHTN.\(^8\)\(^9\)

The oral PDEV inhibitor, sildenafil, has shown to reduce pulmonary vascular resistance (PVR) in patients with chronic PHTN.\(^10\)\(^11\) Similarly, the preliminary use of i.v. sildenafil has been effective in reducing postoperative PHTN in children after heart surgery.\(^12\)\(^13\) In addition, we have shown in a porcine model that the pulmonary vascular effects of an i.v. sildenafil analogue are comparable with those of milrinone during acute PHTN.\(^14\)\(^15\)

A limitation to the use of sildenafil in the perioperative period is the relative unavailability of an i.v. preparation; it has been used in patients after cardiac surgery, but its use in an i.v. form is not widespread. Nonetheless, early clinical experience with sildenafil in patients with postcardiac surgery PHTN is encouraging.\(^16\)\(^-\)\(^20\) Accordingly, it is important to evaluate the interaction of sildenafil with other pulmonary vasodilators commonly used after cardiac surgery, such as PDEIII inhibitors. The purpose of this study was to compare the effects of the PDEV inhibitor, sildenafil, in its most available preparation, in combination with the PDEIII inhibitor, milrinone, during experimental PHTN.

### Methods

The protocol was approved by the University of Florida Institutional Animal Care and Use Committee. Animals were handled in accordance with guidelines established by the National Institutes of Health (NIH publication 85-23, revised 1985).

Twenty-four domestic swine weighing 50–55 kg were premedicated with i.m. ketamine (35 mg kg\(^{-1}\)) and anaesthetized with isoflurane in 100% oxygen. A tracheostomy was then performed and the animals mechanically ventilated at 12 bpm, with tidal volumes of 12 ml kg\(^{-1}\) to maintain an end-tidal carbon dioxide between 32 and 36 mm Hg. Anaesthesia and mechanical ventilation were maintained with the use of a Narkomed 4 anaesthesia machine (North American Dräger, Telford, PA). Pancuronium was utilized for muscle relaxation during the surgical preparation. A 7 French (Fr) pressure-tipped, flotation pulmonary artery catheter (Millar Instruments Inc., Houston, TX) was placed and advanced via the left internal jugular vein into the main pulmonary artery. All transducers were connected to a biomedical amplifier (Grass model 7D, Grass Instruments Co., Quincy, MA). The signals were digitized and continuously recorded at 200 Hz on a personal computer for later analysis (Sonometrics Corp., London, Ontario, Canada).

Maintenance of intravascular volume was accomplished with lactated Ringer’s solution administered by continuous infusion through a peripheral vein at a rate of 10 ml kg\(^{-1}\) h\(^{-1}\). Normothermia (pulmonary artery temperature of 37°C) was maintained by the application of a warming blanket. All animals were allowed to stabilize for 1 h after the surgical preparation before data collection.

### Haemodynamic measurements

Haemodynamic measurements included systemic arterial pressure, pulmonary artery pressure, central venous pressure, CO and RV \(\Delta p/\Delta t\). PVR and SVR were calculated using standard formulas (PVR=MPAP−PAOP/CO and SVR=MAP−CVP/CO; where PVR=pulmonary vascular resistance, MPAP=mean pulmonary artery pressure, PAOP=pulmonary artery occlusion pressure, CO=cardiac output, SVR=systemic vascular resistance, MAP=mean arterial pressure and CVP=central venous pressure).

### Drug preparation

Oral sildenafil 50 mg tablets (Pfizer Pharm., Sandwich, Kent, UK) were crushed and mixed with 50 ml of sterile water. This solution was then infused via an orogastric tube into the stomach. Injectable milrinone acetate (a gift from Sanofi-Synthelabo, Pharm, New York, NY), in a concentration of 1 mg ml\(^{-1}\), was administered undiluted. Before the study, 1 mg of the thromboxane A2 analogue, U46619 (Bio- mol Inc., St Louis, MO), was diluted in 20 ml of lactated Ringer’s solution.

After baseline measurements, a continuous i.v. infusion of U46619 was administered and slowly titrated (rate: 0.5–1.2 \(\mu\)g kg\(^{-1}\) min\(^{-1}\)) to achieve approximately a 2-fold elevation in MPAP. Once the target MPAP was achieved, the infusion of U46619 remained constant and sustained PHTN was maintained for 30 min. The animals were then randomized into four groups. Group 1 \((n=6)\) received 50 mg of sildenafil administered into the stomach via an orogastric tube.\(^19\)\(^-\)\(^21\) Group 2 \((n=6)\) received 50 mg kg\(^{-1}\) of milrinone i.v. over 5 min via an infusion pump (Medfusion 2010, Medexinc, Dublin, OH). Group 3 \((n=6)\) received sildenafil, followed 10 min later by i.v. milrinone. Group 4 \((n=6)\) received a placebo (a sugar tablet) via an orogastric tube diluted in sterile water. Data were monitored continuously and collected at baseline during stable PHTN, post drug administration at the lowest pulmonary artery pressure.
and 60 min after the administration of the drugs. Maximal drug effect, the lowest pulmonary artery pressure achieved by either drug (single or combination) in each group, was different for each group because of the different pharmacokinetics of the drugs (i.v. vs oral).

Statistical analysis
Values were expressed as mean (standard deviation). A two-way analysis of variance (ANOVA) was utilized, followed by Student Newman–Keuls test for multiple comparisons. A one-way ANOVA was utilized for baseline measurements to determine whether the four groups were comparable before the interventions. A P<0.05 was considered significant.

Results
Table 1 and Figure 3 show the measured haemodynamic data and Table 2 and Figures 1 and 2 show the calculated data in each one of the four groups of animals and in each one of the four time periods (baseline, PHTN, post drug and post 60 min).

Baseline
There were no significant differences between the four groups at baseline.

### Table 1 Measured haemodynamic data in each stage, in four treated groups

<table>
<thead>
<tr>
<th>Group</th>
<th>Baseline</th>
<th>PHTN</th>
<th>Post drug</th>
<th>60 min</th>
</tr>
</thead>
<tbody>
<tr>
<td>HR (bpm)</td>
<td>105 (11)</td>
<td>117 (9)</td>
<td>110 (7)</td>
<td>118 (7)</td>
</tr>
<tr>
<td></td>
<td>110 (7)</td>
<td>120 (12)</td>
<td>118 (10)</td>
<td>121 (5)</td>
</tr>
<tr>
<td></td>
<td>107 (7)</td>
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</tr>
<tr>
<td></td>
<td>100 (9)</td>
<td>123 (6)*</td>
<td>122 (10)*</td>
<td>127 (9)*</td>
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<td>MPAP (mm Hg)</td>
<td>15 (3)</td>
<td>32 (3)*</td>
<td>26 (3)*</td>
<td>33 (5)*</td>
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<tr>
<td></td>
<td>15 (5)</td>
<td>30 (5)*</td>
<td>24 (4)*</td>
<td>32 (5)*</td>
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<tr>
<td></td>
<td>17 (5)</td>
<td>37 (7)*</td>
<td>21 (4)*</td>
<td>30 (2)*</td>
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<tr>
<td></td>
<td>16 (4)</td>
<td>31 (8)*</td>
<td>30 (6)*</td>
<td>32 (5)*</td>
</tr>
<tr>
<td>MAP (mm Hg)</td>
<td>64 (7)</td>
<td>69 (13)</td>
<td>66 (9)</td>
<td>61 (11)</td>
</tr>
<tr>
<td></td>
<td>71 (10)</td>
<td>73 (4)</td>
<td>63 (4)*</td>
<td>68 (7)</td>
</tr>
<tr>
<td></td>
<td>70 (10)</td>
<td>71 (10)</td>
<td>61 (6)*</td>
<td>69 (8)</td>
</tr>
<tr>
<td></td>
<td>69 (11)</td>
<td>74 (15)</td>
<td>78 (10)</td>
<td>71 (9)</td>
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<tr>
<td>CVP (mm Hg)</td>
<td>5 (2)</td>
<td>9 (3)</td>
<td>7 (3)</td>
<td>7 (2)</td>
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<td>6 (3)</td>
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<td>6 (3)</td>
</tr>
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<td>PAOP (mm Hg)</td>
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<td>6 (3)</td>
<td>7 (3)</td>
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<td>5 (1)</td>
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<td></td>
<td>8 (3)</td>
<td>7 (2)</td>
<td>5 (3)</td>
<td>7 (3)</td>
</tr>
<tr>
<td>CO (litre min⁻¹)</td>
<td>2.9 (0.6)</td>
<td>1.7 (0.6)*</td>
<td>2.0 (0.5)*</td>
<td>2.1 (0.5)*</td>
</tr>
<tr>
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<td>2.9 (0.3)</td>
<td>1.8 (0.2)*</td>
<td>2.4 (0.3)*</td>
<td>2.0 (0.2)*</td>
</tr>
<tr>
<td></td>
<td>3.3 (0.2)</td>
<td>2.1 (0.3)*</td>
<td>2.8 (0.3)*</td>
<td>2.4 (0.3)*</td>
</tr>
<tr>
<td></td>
<td>2.7 (0.3)</td>
<td>1.9 (0.5)*</td>
<td>2.1 (0.3)*</td>
<td>2.0 (0.4)*</td>
</tr>
</tbody>
</table>

### Pulmonary hypertension
PHTN was achieved in all animals during the infusion of U46619, with a simultaneous and concomitant reduction in CO and RV dP/dT (Table 1 and Fig. 3).

### Maximal cardiovascular changes post drug administration (=Post drug)

The lowest MPAPs were achieved in Group 1 (sildenafil alone) after 20.8 (2.3) min, in Group 2 (milrinone alone) after 8.6 (1.7) min (P<0.05 for Group 2 vs Group 1 or 3) and in Group 3 (sildenafil with milrinone) after 17 (2.0) min.

### Table 2 The change (in percentage) in PVR and SVR and in each one of the groups

<table>
<thead>
<tr>
<th>Group</th>
<th>% Change in PVR</th>
<th>% Change in SVR</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>After PHTN</td>
<td>After PHTN</td>
</tr>
<tr>
<td>Group 1 (sildenafil)</td>
<td>36 (12)*</td>
<td>22 (8)*</td>
</tr>
<tr>
<td>Group 2 (milrinone)</td>
<td>41 (10)*</td>
<td>2 (4)</td>
</tr>
<tr>
<td>Group 3 (both)</td>
<td>61 (12)*</td>
<td>25 (10)</td>
</tr>
<tr>
<td>Group 4 (placebo)</td>
<td>5 (3)</td>
<td>1 (3)</td>
</tr>
</tbody>
</table>

### Fig 1 Changes in PVR in all four groups. All values represent mean (SEM). During the infusion of U46619, PVR increased more than 4-fold. Sildenafil (Group 1) decreased PVR from 1317±279 to 840±147 dynes cm⁻¹ s⁻⁵ and milrinone (Group 2) from 1022±222 to 600±132 dynes cm⁻¹ s⁻⁵. The combination of both drugs (Group 3) had an additive effect, decreasing PVR from 1104±276 to 428±57 dynes cm⁻¹ s⁻⁵. Pulmonary vasodilatation was short-lived, however, as by 60 min PVR had significantly increased. There were no significant changes after placebo (Group 4). PHTN, pulmonary hypertension. *P<0.05 compared with other sampling points; †P<0.05 compared with PHTN; ‡P<0.05 compared with 60 min.
Milorine, alone and in combination with sildenafil, decreased MAP and SVR. Milorine alone lowered MAP by 14% and SVR by 37% ($P<0.05$, for both). The combination of milorine and sildenafil also decreased MAP and SVR (by 14 and 36%, respectively), but was no different than the group receiving milorine alone (Table 1 and Fig. 2).

Similarly, the inotropic effects of milorine were also evident by an increase in RV dp/dT, whereas in the sildenafil and placebo groups, it remained unchanged (Fig. 3).

**Cardiovascular changes 60 min after drug administration (=post 60 min)**

The MPAP changes we observed disappeared by 60 min post drug administration and there were no significant effects on any of the groups. Sildenafil with (Group 3) or without milorine (Group 1) also caused significant changes in PVR after 60 min (24 and 22%, respectively), while milorine’s effect was shorter (2% decrease) after 1 h.

SVR was not significantly different from baseline at 60 min in Groups 2 and 3, indicating that the effect of milorine was short-lived. Milorine without (Group 2) or with sildenafil (Group 3) decreased SVR significantly only post drug administration and not after 60 min. Sixty minutes after the administration of milorine (Group 1), SVR decreased by 27%, accompanied by an increase in CO (Table 1 and Figs 1 and 2). RV dp/dT in the sildenafil only group (Group 1) and placebo group (Group 4) also remained decreased after 60 min and was not significantly different from the PHTN stage in the milorine groups (Groups 2 and 3) (Fig. 3).

**Discussion**

The results of this study demonstrate that during general anaesthesia, intragastric sildenafil, and i.v. milorine, produced comparable effects in the pulmonary circulation. More importantly, the effects of both drugs combined were clearly additive without a significant effect on the systemic circulation compared with milorine alone. Although we did not obtain blood sildenafil levels, our results clearly demonstrate a considerable effect from oral sildenafil, suggesting that significant gastric absorption occurs with general anaesthesia and mechanical ventilation as long as hypotension is not present.

Maximal drug effect, the lowest pulmonary artery pressure achieved by either drug (single or combination) in each group, was different for each group because of the different pharmacokinetics of the drugs (i.v. vs oral). Thus, the difference in time to peak effect on pulmonary vasculature that we found with sildenafil alone (vs milorine alone) is probably because of the pharmacokinetic effects when using different routes (oral vs i.v.) of administration. The dose of sildenafil given is consistent with several studies which utilized the same amount (1 mg kg$^{-1}$),19–21

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**Table 2.** PVR decreased significantly after each drug, with a simultaneous and concomitant increase in CO. The greatest effects on CO and PVR were seen with both drugs combined (Fig. 1 and Table 2). CO increased to baseline level with this combination (Table 1).

![Fig 2 Changes in SVR in all groups. All values represent mean (SEM). The systemic vasodilatory effect of milorine with or without sildenafil was evident. Milorine alone (Group 2) lowered SVR from 2800 (280) to 1766 (360) dynes cm$^{-1}$ s$^{-5}$. The decrease in SVR with milorine and sildenafil combined (Group 3) was similar to that with milorine alone (from 2400 (342) to 1542 (257) dynes cm$^{-1}$ s$^{-5}$). SVR remained unchanged after sildenafil (Group 1) or placebo (Group 4). PHTN, pulmonary hypertension. *$P<0.05$ compared with other sampling points; †$P<0.05$ compared with PHTN.**
The efficacy of sildenafil in achieving pulmonary vascular relaxation has been demonstrated in the acute and chronic setting. There is general agreement that the effects of sildenafil in the pulmonary circulation are a result of the inhibition of PDEV, thus enhancing intracellular cyclic GMP levels. Although negative inotropic effects of increased cyclic GMP have been described, there is little evidence that sildenafil causes decreased contractility. In fact, recent data suggest a cardioprotective effect of sildenafil against myocardial ischemia and reperfusion injury. No improvement in RV contractility was documented in our study with sildenafil, but no negative inotropic effects were found either.

After treating PHTN, the combination of the two drugs improved contractility more than using each drug alone. The reason that both drugs have a synergistic effect relates to the fact that increases in cyclic AMP (with milrinone) can be additive to cyclic GMP (with sildenafil), as each drug is working via different mechanisms in PDE inhibition.

Predictably, the administration of milrinone was associated with increased RV contractility and a significant effect on SVR. It is important to mention that we, similar to others, observed a relative degree of pulmonary selectivity after the administration of sildenafil. Furthermore, when administered in combination with milrinone, the decrease in PVR was greater than with either drug alone. SVR, however, was relatively preserved when compared with the group that received milrinone alone. This suggests that during acute PHTN, a PDEV inhibitor may provide additional pulmonary vasorelaxation when added to agents that increase cyclic AMP, without a significant risk for systemic hypotension. Thus, the combination of a PDEV and PDEIII inhibitor may provide a better haemodynamic profile, with the advantage of greater pulmonary vasodilatation and biventricular inotropism.

The perioperative treatment of acute PHTN with traditional agents such as nitrosodiators, beta adrenergic agonists and PDEIII inhibitors is limited by the risk of hypotension as a result of their systemic vasodilatory effects. Patients with preexisting PHTN seem to be at higher risk as they frequently require high doses of these agents.

Although inhaled nitric oxide is a potent selective pulmonary vasodilator, it has several limitations: it requires delivery through a closed system; rebound PHTN may occur upon withdrawal; and monitoring for toxicity is necessary. Additionally, the delivery systems for nitric oxide are not widely available in the USA, although nitric oxide is used more frequently clinically throughout Europe.

Several reports have recently described the use of oral sildenafil in cardiac surgical patients to facilitate the withdrawal of nitric oxide during placement of ventricular assist devices or in the postoperative period, suggesting a possible role for sildenafil in the treatment of perioperative PHTN.

The availability of an i.v. preparation of sildenafil for widespread clinical use is limited, because it is mainly used for clinical research. The suggested oral dose is 0.5–1 mg kg⁻¹. The balance between the benefits and disadvantages of i.v. vs oral administration is not in the recommended dose, as absorption from any mucus layer (sublingual, oral, gastric) is fast and similar to i.v. administration. The advantage is in the ability to titrate an infusion i.v. to the desired effect in a continuous fashion.

As this study demonstrated, the interaction of oral sildenafil with milrinone during acute pulmonary vasoconstriction has additive effects, without the risk of increased systemic vasodilation. This combination has the potential for creating better haemodynamic conditions in the post-bypass period, thus facilitating weaning from mechanical ventilation, inhaled nitric oxide and other inotropes and pulmonary vasodilators.

The fact that sildenafil has a longer effect on PVR and CO compared with milrinone, which lasted 60 min, gives this drug a clinical advantage in treating PHTN and maintaining a stable haemodynamic profile, in spite of the lack of inotropic effect on the RV.

In conclusion, this preliminary study demonstrated that the concomitant administration of sildenafil and milrinone was associated with greater pulmonary relaxation and a better haemodynamic profile than either drug alone. Future studies will better define the role of sildenafil and other PDEV inhibitors as part of a multimodal approach in the treatment of perioperative PHTN.

Acknowledgement
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