Prostacyclin and milrinone by aerosolization improve pulmonary hemodynamics in newborn lambs with experimental pulmonary hypertension

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Kumar VH, Swartz DD, Rashid N, Lakshminrusimha S, Ma C, Ryan RM, Morin FC III. Prostacyclin and milrinone by aerosolization improve pulmonary hemodynamics in newborn lambs with experimental pulmonary hypertension. J Appl Physiol 109: 677–684, 2010. First published July 8, 2010; doi:10.1152/japplphysiol.01082.2009.—Aerosolized prostacyclin (PGI2) produces selective pulmonary vasodilation in patients with pulmonary hypertension (PH). The response to PGI2 may be increased by phosphodiesterase type 3 inhibitors such as milrinone. We studied the dose response effects of aerosolized PGI2 and aerosolized milrinone both alone and in combination on pulmonary and systemic hemodynamics in newborn lambs with Nω-nitro-L-arginine methyl ester (L-NAME)-induced PH. We hypothesized that coaerosolization of PGI2 with milrinone would additively decrease pulmonary vascular resistance; pulmonary arterial pressure; pulmonary vascular resistance; pulmonary blood flow; persistent pulmonary hypertension of the newborn; systemic blood pressure.

PULMONARY HYPERTENSION (PH) of any etiology if untreated is a potentially fatal condition in children and adults (3). Nitric oxide (NO) and prostacyclin (PGI2) are used extensively as vasodilators in the short-term and long-term management of primary and secondary PH in children and adults (19, 32, 35–37). Lack of selectivity of intravenous PGI2 may lead to generalized vasodilation of systemic and pulmonary vessels, resulting in systemic hypotension. Persistent pulmonary hypertension of the newborn (PPHN) is a serious disorder of term and near-term infants with significant morbidity and mortality (33). Although inhaled nitric oxide (iNO) is commonly used to treat refractory cases of PPHN, 40% of infants do not respond to iNO (25). Prostacyclin analogs have been used in these infants with PPHN (4, 28) and found to be effective in infants not responding to iNO (13).

PGI2 increases cAMP by stimulating the enzyme adenylyl cyclase within the vascular smooth muscle resulting in vasodilation (17). Systemic hypotension and nonselective increase in perfusion of both ventilated and nonventilated units of the lung are particularly problematic with intravenously administered vasodilators such as PGI2 (22). Aerosolized PGI2 selectively dilates the pulmonary circulation and redistributes pulmonary blood flow away from nonventilated regions of the lung (21, 31). Aerosolized PGI2 has been shown to be at least as effective as iNO in decreasing PH in some animals and humans (9, 35). Because of its short biological half-life of 2–3 min at physiological pH (17), its vasodilatory effect on the lung vasculature wears off in <30 min after termination of nebulization (19).

Milrinone increases cAMP levels by inhibiting the enzyme phosphodiesterase type 3 (PDE 3) in cardiac and vascular smooth muscle. Milrinone is a positive inotrope and vasodilator and has been a valuable drug in the management of patients with congestive cardiac failure, shock, and following cardiac surgery (2, 6, 11, 23, 27). By increasing cAMP levels through different mechanisms, the combination of PGI2 and milrinone may enhance the relaxation of the pulmonary vascular bed and also prolong the vasorelaxant effects. We showed previously that milrinone enhances the relaxation to PGI2 and iloprost in pulmonary arteries isolated from lambs with PPHN (14). We also demonstrated that intravenously administered milrinone potentiates the vasodilatory effects of intratracheally instilled PGI2 in newborn lambs with ductal ligation-induced PPHN (24).

Although there are reports of aerosolized PGI2 being used anecdotally in human newborn infants (1, 4, 13), systematic studies on the dose response effect of aerosolized PGI2 in infants and children with PH is lacking. Unlike aerosolized milrinone, intravenous milrinone has been extensively studied in patients with PH associated with cardiac failure (2). Very few studies (11, 26) have addressed the hemodynamic effects of aerosolized milrinone in adult patients with PH, but none in the pediatric age group. As combining PGI2 and milrinone enhances relaxation of PAs (14) and also reduces pulmonary arterial pressure (PAP) in an in vivo model (24), coaerosolization may offer potential benefits. We studied the dose response of aerosolized PGI2 and milrinone on pulmonary and systemic hemodynamics in newborn lambs with experimental PH. We hypothesized that coaerosolization of PGI2 with milrinone would enhance the decrease in pulmonary vascular resistance (PVR), prolong the duration of action, and maintain the selec-

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tivity of the effect on the pulmonary vasculature. Thus we studied the agents in combination as well.

METHODS

The study was approved by the IACUC of the University at Buffalo. Sheep were purchased from Swartz family farm (Attica, NY).

Surgical preparation of the lamb. Time-dated pregnant sheep at 139 days gestation (term 145 days) were brought to the lab animal facility 24–72 h before surgery. After intravenous Pentothal (750 mg), the ewe was intubated and ventilated under general anesthesia (1–2% isoflurane). After partial exteriorization of the fetal head and neck via C-section, the carotid artery and jugular vein were catheterized. A left lateral thoracotomy was then performed in the fourth intercostal space on the fetus, and the pericardium was exposed. Ductus arteriosus was ligated to minimize variability in pulmonary blood flow (PBF) and PAP resulting from a dynamic shunt due to PH and/or administration of PGI2 or milrinone. An ultrasonic flow transducer (Transonics Systems, Ithaca, NY) was placed around the main pulmonary artery (PA) for measurement of pulmonary blood flow (PBF). Catheters were placed in the main PA and in the left atrium (LA) to measure PA and LA pressures, respectively. The lamb was then intubated with a cuffed endotracheal tube. To induce and maintain pulmonary hyper-tension by antagonizing the vasodilatory effects of endogenous nitric oxide (NO) and PGI2, Nω-nitro-l-arginine methyl ester (l-NAME) was administered at a dose of 25 mg/kg by a slow intravenous push over 2–3 min just before delivery of the lamb and freshly prepared indomethacin at a dose of 2 mg/kg was administered by slow intra-venous push within 5 min after birth. Lambs were maintained on l-NAME infusion at 10 mg·kg\(^{-1}\)·h\(^{-1}\) postdelivery. The lambs were delivered with an open chest and catheters and transducer in situ. The lambs were placed under a servo controlled infant warmer to maintain rectal temperature of 39°C and weighed immediately. Lambs were covered in a plastic bag to prevent heat loss. Ventilation was initiated with a time cycled pressure controlled Sechrist ventilator (Sechrist Industries, Anaheim, CA) at a rate of 60 breaths/min, PIP set to achieve adequate chest movement (21–26 cmH\(_2\)O), PEEP of 4–5 cmH\(_2\)O, I-time of 33% [I:E = 1:2] and FiO\(_2\) of 1. Intravenous fluids (10% dextrose + NaCl: 25 meq/l + KCl: 20 meq/l + NaHCO\(_3\): 10 meq/l) were administered continuously at 100 ml·kg\(^{-1}\)·day\(^{-1}\). Blood pressure, heart rate, temperature, and oxygen saturation were monitored continuously and arterial blood gases were obtained before and after aerosolization doses and as needed throughout the study. The lambs were well sedated with fentanyl administered as an infusion (3 μg·kg\(^{-1}\)·h\(^{-1}\)) with additional boluses of 5 μg/kg as needed. Lambs with persistent increased activity despite adequate sedation were paralyzed with pancuronium bromide (0.1 mg/kg every 3–4 h as needed). Lambs received normal saline or whole blood for low mean blood pressure of <35 mmHg and sodium bicarbonate for base deficit of >8 meq/l. Blood gases were done frequently during stabilization to maintain PaCO\(_2\): 35–55 mmHg and pH 7.35–7.45. Inspired oxygen was maintained at 100% throughout the study period. All catheters were connected to a physiological recorder (Gould Instrument Systems, Valley View, OH) for monitoring of systemic blood pressure (SBP), PAP, and LA pressure (LAP; mmHg). The flow probe was connected to a flow meter (Transonic Systems) for monitoring of PBF (ml/min). Pulmonary vascular resistance (PVR) was calculated using the standard formula (PAP – LAP)/PBF and corrected for body weight and expressed as millimeters Hg per milliliter per kilogram per minute. Hemodynamic data were also obtained from normal newborn lambs at similar gestational age (historic controls) instrumented for other reasons and compared them with PH lambs. Duration of action of aerosolized PGI2 with and without milrinone was defined as the time elapsed since the administration of the drugs for the PAP to decrease and then come back up to the preadministration baseline. Selectivity of the drug to the pulmonary vasculature was assessed by calculating the mean PAP-to-SBP ratio for the doses studied.

Delivery of prostacyclin and milrinone. The animals were allowed to stabilize on l-NAME infusion on 100% oxygen and documented to have pulmonary hypertension after birth (mean PAP > 50 mmHg). The first dose of the aerosolized drug was administered at 2–3 h after birth. The drugs were administered with the Aeroneb nebulizer system (Aeroneb Pro, Aerogen Mountain View, CA) placed in the inspiratory limb of a humidified ventilatory circuit connecting the ventilator to an endotracheal tube. The aerosol generator produces a fine particle, low-velocity aerosol and does not heat the medication during nebulization. The mass median aerodynamic diameter of the particles delivered by this nebulizer is 2.1 μm (SD ± 2.2) with the fine particle fraction (<5 μm) of 83% and residual volume of 0.3 ml (5). PGI2 (Sigma Chemicals, St. Louis, MO) and milrinone (Baxter Healthcare, Deerfield, IL) solutions were freshly prepared before the start of each experiment. PGI2 solution was prepared using glycerine buffer (pH 10.4) and milrinone using normal saline. The response for PGI2 was studied at doses of 2, 20, 100, 200, and 2000 mg·kg\(^{-1}\)·min\(^{-1}\) aerosolized in random order over 10 min in five newborn lambs. The response to milrinone was studied in sequentially increasing doses of 0.1, 1, and 10 μg·kg\(^{-1}\)·min\(^{-1}\) aerosolized over 10 min in the same lambs following PGI2. Aerosolization of both the drugs in combination was performed in a second set of lambs (n = 5) after analysis of dose response results from the earlier experiments. In the second set of experiments, PGI2 (20, 100, and 200 mg·kg\(^{-1}\)·min\(^{-1}\)) and milrinone (1 μg·kg\(^{-1}\)·min\(^{-1}\)) were administered alone and in combination (PGI2 20 + milrinone 1 μg·kg\(^{-1}\)·min\(^{-1}\); PGI2 100 + milrinone 1 μg·kg\(^{-1}\)·min\(^{-1}\); PGI2 200 + milrinone 1 μg·kg\(^{-1}\)·min\(^{-1}\)) and again aerosolized over 10 min via the aeroneb. PGI2 doses were administered at random followed by milrinone at 1 μg·kg\(^{-1}\)·min\(^{-1}\). The combination doses were then administered in sequentially increasing doses of 20, 100, and 200 of PGI2 with milrinone. The nebulized volume in all the experiments was 5 ml, which took about 10 min to nebulize with a residual volume of <0.3 ml. Blood gases were done pre- and postnebulization of the drugs. Fixed ventilator settings were maintained during the aerosolization period. A time interval of at least 30 min was maintained between doses to allow for the PAP to revert back to the prenebulization baseline.

We used the integrated pharmacokinetic/pharmacodynamic (PK/PD) approach to study the relationships between dose and effect in a statistical model accounting for variability to a given dose and among various doses of PGI2 and milrinone. Mathematically the graded concentration-effect relationship in a physiological system is represented by the sigmoidal E max equation: E = E\(_{\text{max}}\) × C/(C\(_{\text{IC50}}\) + E\(_{\text{max}}\)), where E\(_{\text{max}}\) is the maximum effect, C is the concentration (dose), E\(_{\text{IC50}}\) is the concentration of the drug that induces a response halfway between the baseline and maximum effect and s is the Hill coefficient (18). All data are expressed as mean ± SD with n representing the number of animals. The concentration-effect relationship E\(_{\text{max}}\) model was fitted by nonlinear mixed model procedure in SAS. The comparisons between drugs and doses were performed by mixed model with drug and doses as fixed effects and subject as a random effect. Statistical analysis was performed using SAS (Cary, NC). Differences among groups were analyzed by ANOVA and Student’s t-test. A P value of <0.05 was considered significant.

RESULTS

l-NAME plus indomethacin produced significant PH in newborn lambs (Table 1). The values in normal newborn lambs for the same hemodynamic parameters at similar gestational age are also shown in Table 1. Hemodynamic measurements were made following stabilization 2–3 h after birth in control lambs and in lambs with PH. Mean PAP and PVR were significantly higher and PBF significantly lower in lambs with PH compared with the control group (Table 1).
Aerosolized PGI2. PAP decreased significantly with increasing doses of PGI2 (P = 0.007; Table 2). The mean decrease in PAP after administration of PGI2 was significantly related to the increasing doses of PGI2 (Fig. 1A). The observed maximal decrease in PAP was 19.2 ± 4.4 mmHg (30 ± 6%). There was no significant correlation between pulmonary blood flow and increasing doses of PGI2 (Fig. 1B; Table 2). The mean decrease in PVR was significantly related to increasing doses of PGI2 (Fig. 1C). EC50 was 170 ± 53 ng·kg⁻¹·min⁻¹. Emax for PGI2 predicted by the concentration-effect model, on PVR was −0.47 ± 0.07 mmHg·ml⁻¹·kg⁻¹·min⁻¹, which was close to the observed maximal decrease in PVR of −0.41 ± 0.06 mmHg·ml⁻¹·kg⁻¹·min⁻¹. PAP-to-SBP ratio, a measure of selectivity to pulmonary vasculature decreased with increasing doses of PGI2 (P = 0.05; Fig. 3A), suggesting that aerosolized PGI2 was selective to the pulmonary vasculature.

Aerosolized milrinone. There was a significant decrease in PAP with increasing doses of milrinone (P = 0.002; Table 2). The mean change in PAP was significantly related to the increasing doses of milrinone (Fig. 2A). The observed maximal decrease in PAP was 7.6 ± 0.9 mmHg (14 ± 1%). There was no significant correlation between pulmonary blood flow and increasing doses of milrinone (Fig. 2B; Table 2). Aerosolized milrinone significantly reduced PVR (Table 2; P = 0.002). The mean change in PVR was significantly related to the increasing concentration of milrinone doses (Fig. 2C). EC50, the dose of milrinone that produces 50% of maximal decrease in PVR was 1.36 ± 0.77 μg·kg⁻¹·min⁻¹. Emax for milrinone on PVR was −0.34 ± 0.05 mmHg·ml⁻¹·kg⁻¹·min⁻¹, which was close to the observed maximal decrease in PVR of −0.30 ± 0.03 mmHg·ml⁻¹·kg⁻¹·min⁻¹ (−30 ± 4%). PAP-to-SBP ratio, a measure of pulmonary selectivity decreased with increasing doses of milrinone (P = 0.02; Fig. 3B), suggesting that aerosolized milrinone was selective to the pulmonary vasculature.

Although there was a clinical improvement in oxygenation, there was no significant increase in arterial PO2 postnebulization of PGI2 or milrinone (Table 4).

Combination studies. In the second set of experiments, PGI2 at doses of 20, 100 and 200 ng·kg⁻¹·min⁻¹ and milrinone at 1 μg·kg⁻¹·min⁻¹ when administered separately significantly reduced PAP (Table 3; P = 0.008). Aerosolization of PGI2 and milrinone together significantly reduced PAP (Table 3; Fig. 4A; P = 0.01) compared with either of the drugs alone. Milrinone by itself did not increase PBF but in combination with PGI2 significantly increased PBF (Table 3; Fig. 4B; P = 0.04). Combined aerosolization of PGI2 and milrinone significantly reduced PVR (Fig. 5; P = 0.003) compared with aerosolization of either of the drug alone (Table 3). The mean reduction in PVR by milrinone alone was 0.094 ± 0.020 and PGI2 alone at 20, 100, and 200 ng·kg⁻¹·min⁻¹ was 0.032 ± 0.009; 0.142 ± 0.034; and 0.158 ± 0.056 mmHg·ml⁻¹·kg⁻¹·min⁻¹, respectively. The combination of milrinone and PGI2 at 20, 100, and 200 ng·kg⁻¹·min⁻¹ resulted in a mean reduction of PVR by 0.107 ± 0.013; 0.228 ± 0.043 and 0.255 ± 0.40 mmHg·ml⁻¹·kg⁻¹·min⁻¹, respectively. EC50 for PGI2 when used in combination with milrinone (1 μg·kg⁻¹·min⁻¹), was 40 ± 16 ng·kg⁻¹·min⁻¹. The Emax for PVR was 0.31 ± 0.05 mmHg·ml⁻¹·kg⁻¹·min⁻¹.

The duration of action for PGI2 when aerosolized alone at 20, 100, and 200 ng·kg⁻¹·min⁻¹ was 9.42 ± 0.45; 9.52 ± 0.34 and 10.0 ± 0.35 min, respectively. The duration of action for the same doses of PGI2 when combined with 1 μg·kg⁻¹·min⁻¹ of milrinone was 17.5 ± 1.26; 19.0 ± 0.83 and 19.6 ± 1.20 min, respectively. Coaerosolization of PGI2 with milrinone signifi-
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decreases PAP and PVR in a dose-dependent manner in newborn lambs with PH. Aerosolized PGI₂ has been shown in a number of human studies to be beneficial in patients with adult respiratory distress syndrome (ARDS) (32), primary and secondary PH (16), and right ventricular dysfunction (10). Similar to our study, human studies have found a decrease in PAP and PVR with inhaled PGI₂ at doses of 50 to 100 ng·kg⁻¹·min⁻¹ in cardiac surgical patients with elevated PVR (9, 11). Aerosolized PGI₂ (85 ng·kg⁻¹·min⁻¹) reduced pulmonary pressures and improved right ventricular stroke volume in patients with PH undergoing cardiac surgery (8). This being an animal model, we were able to test a wide range of aerosolized PGI₂ from 2 to 1,000 ng·kg⁻¹·min⁻¹. The EC₅₀ for the doses tested was 170 (±53) ng·kg⁻¹·min⁻¹, which was comparable to the clinical therapeutic range reported in various studies.

Despite the high doses, aerosolized PGI₂ was selective to the pulmonary vasculature as determined by PAP-to-SBP ratio and a lack of significant decrease in SBP. Inhaled PGI₂ in hypoxicem patients with ARDS at doses up to 50 ng·kg⁻¹·min⁻¹ was found to be a selective pulmonary vasodilator with no significantly prolongs the duration of action of PGI₂ at the doses studied (P < 0.05; ANOVA). There was no significant decrease in the PAP-to-SBP ratio following coaerosolization of PGI₂ and milrinone, suggesting that the combination was not selective to the pulmonary vasculature (Fig. 3C). Combination of PGI₂ and milrinone did not produce any significant difference in ABG parameters such as Paco₂, pH, and PaCO₂ following aerosolization.

DISCUSSION

To our knowledge, this is the first study evaluating the dose response to aerosolized PGI₂ and milrinone in an animal model of experimental PH. We have shown that aerosolized PGI₂ decreases PAP and PVR in a dose-dependent manner in newborn lambs with PH. Aerosolized PGI₂ has been shown in a number of human studies to be beneficial in patients with adult respiratory distress syndrome (ARDS) (32), primary and secondary PH (16), and right ventricular dysfunction (10). Similar to our study, human studies have found a decrease in PAP and PVR with inhaled PGI₂ at doses of 50 to 100 ng·kg⁻¹·min⁻¹ in cardiac surgical patients with elevated PVR (9, 11). Aerosolized PGI₂ (85 ng·kg⁻¹·min⁻¹) reduced pulmonary pressures and improved right ventricular stroke volume in patients with PH undergoing cardiac surgery (8). This being an animal model, we were able to test a wide range of aerosolized PGI₂ from 2 to 1,000 ng·kg⁻¹·min⁻¹. The EC₅₀ for the doses tested was 170 (±53) ng·kg⁻¹·min⁻¹, which was comparable to the clinical therapeutic range reported in various studies.

Despite the high doses, aerosolized PGI₂ was selective to the pulmonary vasculature as determined by PAP-to-SBP ratio and a lack of significant decrease in SBP. Inhaled PGI₂ in hypoxicem patients with ARDS at doses up to 50 ng·kg⁻¹·min⁻¹ was found to be a selective pulmonary vasodilator with no

Fig. 1. Dose response effects of aerosolized PGI₂ on pulmonary arterial pressures (PAP; A), pulmonary blood flow (PBF; B), and pulmonary vascular resistance (PVR; C) in newborn lambs with nitro-L-arginine methyl ester (L-NAME)-induced pulmonary hypertension (PH). Y-axis represents the mean change in PAP, PBF, and PVR (raw data) with increasing doses of PGI₂ (doses studied: placebo, 2, 20, 100, 200, 500, and 1,000 ng·kg⁻¹·min⁻¹ aerosolized over 10 min). Data represent mean (±SE) from 5 newborn lambs with PH. PAP (*P = 0.007) and PVR (†P = 0.008) decreased significantly with increasing doses of PGI₂ (mixed modeling - E_max model). EC₅₀ for PGI₂: -170 ± 53 ng·kg⁻¹·min⁻¹; E_max: -0.47 ± 0.07 (C).

Fig. 2. Dose response effects of aerosolized milrinone on PAP (A), PBF (B), and PVR (C) in newborn lambs with PH. Y-axis represents the mean change in PAP, PBF, and PVR (raw data) with increasing doses of milrinone (0.1, 1, and 10 µg·kg⁻¹·min⁻¹ aerosolized over 10 min). Data represent mean (±SE) from 5 newborn lambs with PH. PAP and PVR decreased significantly with increasing doses of milrinone (*P = 0.002, mixed modeling - E_max model). EC₅₀ for milrinone: -1.36 ± 0.77 µg·kg⁻¹·min⁻¹; E_max: -0.34 ± 0.05 (C).
Aerosolization with increasing doses of milrinone produced a dose-dependent decrease in PAP and PVR in newborn lambs with PH. The fall in PAP was selective to the pulmonary vasculature. Two studies have addressed the hemodynamic effects of aerosolized milrinone in adult patients with PH (11, 26). However, no studies have addressed the role of aerosolized milrinone in infants and children. In adult patients with PH following cardiac surgery, milrinone aerosolization at two different concentrations of 0.5 and 1 mg/ml produced a selective reduction in PVR of 9 and 20% respectively (11). The maximal dose of milrinone in this clinical study was ~30–40% of the maximal dose used to our study. Selective vasodilation of the pulmonary vasculature was also noted during evaluation of heart transplant candidates with chronic heart failure (26). Aerosolized milrinone but not intravenous milrinone was a selective pulmonary vasodilator in a rat model of congestive heart failure (12). Repeated milrinone inhalations in 20-min intervals also caused a stable reduction in PAP and lung edema in this rat model, suggesting it may offer an effective pulmonary selective strategy for treatment of congestive heart failure in humans (12). While PAP and PVR were significantly lower when PGI2 and milrinone were aerosolized separately in our experiments, this was not accompanied by a corresponding increase in PBF, probably secondary to ductal ligation and prevention of left to right shunt.

In the second set of experiments, PGI2 (20, 100, and 200 ng·kg⁻¹·min⁻¹) and milrinone (1 µg·kg⁻¹·min⁻¹) produced a significant reduction in PAP and PVR when aerosolized independently in these lambs with PH. Aerosolization of milrinone with any of the three PGI2 doses resulted in an additional decrease in PAP and PVR greater than seen with either drug alone, suggesting that the drugs may act in synergy. PGI2 combined with milrinone produced an additional reduction in PVR of 8.3–9.9% across doses. The only other study of combined aerosolization was performed in adults with postoperative cardiac surgery patients with PH using one dose of each agent (11). In this study, aerosolized PGI2 with milrinone produced a reduction in PVR by an additional 8% compared with PGI2 alone. This is the first report of the results of aerosolization of milrinone combined with varying doses of aerosolized PGI2. Our data suggests that coaerosolization with a PDE3 inhibitor enhances the response in the pulmonary vascular bed across most of the clinically relevant doses of PGI2 (20–200 ng·kg⁻¹·min⁻¹). The addition of 1 µg·kg⁻¹·min⁻¹ of milrinone decreased the EC₅₀ of PGI2 from 170 (±53) to 40 (±16) ng·kg⁻¹·min⁻¹, suggesting that a lower dose of PGI2 could be used when aerosolized with milrinone. Aerosolized milrinone prolonged the duration of reduction in PAP to PGI2. Combination therapy seems to achieve both augmentation and prolongation of PGI2 action on the pulmonary vascular bed. Similar enhancement of duration and degree of pulmonary vasodilation with a combination of inhaled milrinone and PGI2 has been observed in cardiac postoperative patients with PH following discontinuation of inhalation (11). Amplification of pulmonary vasodilator response following administration of PDE inhibitors with PGI2, suggests that administering these drugs by aerosolization may be advantageous in the treatment of PH.

Aerosolization is said to deliver the drug to the site of desired action, thereby improving systemic oxygenation and minimizing systemic side effects. However, the effect on systemic oxygenation following PGI2 and milrinone was not demonstrated on SBP (30). Selectivity of aerosolized PGI2 to the pulmonary vasculature has been demonstrated in several studies to be comparable to that of iNO (9, 32) and is probably due to rapid inactivation at physiological pH. But PGI2 when reconstituted in a specific diluent has a pH of 10.4 to 10.8 and is increasingly unstable at lower pH. The effects of high pH when administered as an aerosol over a longer time period are not well studied. However, it has been used in the treatment of PH in adults with minimal side effects and inhalation of PGI2 over an 8-h period in healthy lambs did not produce major side effects or acute pulmonary toxicity (7).

**Fig. 3.** PAP-to-SBP ratio in newborn lambs with PH. Y-axis represents the ratio of PAP to SBP (raw data) with increasing doses of PGI2 (placebo, 2, 20, 100, 200, 500, and 1,000 ng·kg⁻¹·min⁻¹; A), milrinone (B), and combination of PGI2 and milrinone (C). Data represent mean (±SE) from 5 lambs. There was no difference in the ratio pre- and postaerosolization, suggesting that the combination was not selective to the pulmonary vasculature. The ratio, a measure of selectivity to pulmonary vasculature decreased with increasing doses of PGI2 (A; *P = 0.05) and milrinone (B; †P = 0.02; mixed modeling). The combination of PGI2 and milrinone was not selective to the pulmonary vasculature.

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Table 3. Hemodynamic variables following aerosolization of various doses of PGI2 and milrinone alone and in combination in newborn lambs with l-NAME-induced pulmonary hypertension

<table>
<thead>
<tr>
<th>Drug</th>
<th>Mean PAP, mmHg</th>
<th>PBF, ml·kg⁻¹·min⁻¹</th>
<th>PVR, mmHg·ml·kg⁻¹·min⁻¹</th>
<th>Mean SBP, mmHg</th>
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<tbody>
<tr>
<td></td>
<td>Pre</td>
<td>Post*</td>
<td>Pre</td>
<td>Post†</td>
</tr>
<tr>
<td>PGI2, ng·kg⁻¹·min⁻¹</td>
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<td></td>
<td></td>
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<tr>
<td>20</td>
<td>58</td>
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<td>55</td>
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<td>100</td>
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<td>200</td>
<td>57</td>
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<tr>
<td>Milrinone (M), µg·kg⁻¹·min⁻¹</td>
<td>1</td>
<td>56</td>
<td>± 10</td>
<td>52</td>
</tr>
<tr>
<td>PGI2 + Milrinone</td>
<td>20</td>
<td>+ M</td>
<td>59</td>
<td>± 7</td>
</tr>
<tr>
<td></td>
<td>100</td>
<td>+ M</td>
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<td>± 5</td>
</tr>
<tr>
<td></td>
<td>200</td>
<td>+ M</td>
<td>55</td>
<td>± 7</td>
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</table>

Data represent means ± SD from 5 newborn lambs with pulmonary hypertension. *PAP: PGI2, Post vs. Pre: P = 0.008; M, Post vs. Pre: P = 0.007; PGI2 + M, Post vs. Pre: P = 0.01; †PBF: PGI2, Post > Pre: P = 0.004; PGI2 + M, Post > Pre: P = 0.04. †PVR: PGI2, Post < Pre: P = 0.0001; M–Post < Pre: P = 0.004; PGI2 + M: Post < Pre: P = 0.003; mixed modeling procedure. Measured parameters, PAP, PBF, and SBP; Calculated parameters, PVR.

Table 4. Arterial blood gases pre- and postaerosolization of various doses of PGI2 and milrinone in newborn lambs with pharmacologically induced pulmonary hypertension

<table>
<thead>
<tr>
<th>Drug</th>
<th>Preaerosolization</th>
<th>Postaerosolization</th>
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<tbody>
<tr>
<td></td>
<td>pH</td>
<td>PaCO2</td>
</tr>
<tr>
<td>Diluent</td>
<td>5.76 ± 0.01</td>
<td>47 ± 1</td>
</tr>
<tr>
<td>PGI2, ng·kg⁻¹·min⁻¹</td>
<td>5.76 ± 0.01</td>
<td>43 ± 7</td>
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<tr>
<td></td>
<td>5.76 ± 0.01</td>
<td>43 ± 4</td>
</tr>
<tr>
<td></td>
<td>5.76 ± 0.01</td>
<td>43 ± 3</td>
</tr>
<tr>
<td></td>
<td>5.76 ± 0.01</td>
<td>42 ± 6</td>
</tr>
<tr>
<td></td>
<td>5.74 ± 0.02</td>
<td>45 ± 3</td>
</tr>
<tr>
<td></td>
<td>5.73 ± 0.02</td>
<td>45 ± 5</td>
</tr>
<tr>
<td>Milrinone, µg·kg⁻¹·min⁻¹</td>
<td>0.1</td>
<td>7.35 ± 0.02</td>
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<tr>
<td></td>
<td>1</td>
<td>7.35 ± 0.04</td>
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<tr>
<td></td>
<td>10</td>
<td>7.36 ± 0.03</td>
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</tbody>
</table>

Data represent means ± SD from 5 newborn lambs with pulmonary hypertension. PaCO2 and PaO2 are expressed in mmHg. All lambs ventilated in 100% FIO2 throughout the experiment. BD, base deficit.
selectivity in our experiments. This was a conscious decision on our part not to perform double thoracotomies (to measure cardiac output) in these sick newborn lambs. These issues may explain the nonselectivity of the combination of milrinone and PGI₂ or might also represent a significant limitation of the study.

The pharmacologically induced PH in newborn lambs is not the same as the clinical condition of PH in infants and children. We induced PH with a single dose of indomethacin, which has a longer half-life and a continuous infusion of L-NAME, a NOS inhibitor. The rationale for using both of these agents was to block endogenous PGI₂ and NO, the two major pathways contributing to pulmonary vasodilation, resulting in the physiological fall in PVR that occurs soon after birth. Indomethacin, by blocking endogenous PGI₂, also allowed us to study more accurately the effects of exogenously administered PGI₂ and milrinone on pulmonary hemodynamics. Blocking endogenous NO and PGI₂ production induced severe PH secondary to acute vasoconstriction in newborn lambs. Moreover the features that often complicate the clinical condition of PH such as parenchymal lung disease and vascular remodeling were absent in this model. Vasoconstrictors such as thromboxane and endothelin produced by the vascular endothelium along with the structural remodeling of the pulmonary vasculature contribute to PH in infants and children. However, a decrease in response to NO may also contribute to abnormal vasoactivity and excessive muscularization of the pulmonary vessels in PH (29). It is possible for our results to be affected by the interaction of the cAMP and cGMP systems via the PDE cross talk. As neither cGMP nor cAMP levels were measured either in the blood or in the pulmonary arteries, we can only speculate at this point regarding cross talk between the two signal transduction pathways. Sildenafil, a PDE5 inhibitor has been shown to exert antimitogenic action by activation of PKA by inhibition of PDE3 (20). In the absence of increasing cGMP by NOS inhibition in our model, it is unlikely for any significant PDE3 inhibition to have taken place; although a negative feedback (low cGMP potentiating PDE3) cannot be ruled out. However, in experiments involving milrinone and PGI₂ with milrinone, this effect on PDE3 should have been abolished. Increasing cAMP from PGI₂ alone might also have a modulating effect on the cGMP system as reported in literature (34).

In this acute model of PH lasting for several hours, there may be less time for smooth muscle proliferation and vascular remodeling to occur. Moreover newborn vasculature may behave differently relative to adult vasculature, which may go on to develop either atherosclerosis or primary PH. Although, the clinical responses were along expected lines in our model, cross talk among PDEs needs to be studied further.

Despite the above mentioned limitations, the study generates the pharmacodynamic data of exogenously administered PGI₂ and milrinone by aerosolized route in newborn lambs with PH. Augmenting the cAMP pathway with combination therapy is relatively unexplored in infants and children compared with agents that act on the cGMP pathway and this may have clinical implications. The study demonstrated the effectiveness of these agents in the newborn lung vasculature and the potential for exploring these agents via aerosolization. We found that aerosolized PGI₂ and aerosolized milrinone significantly reduce PVR in a dose-dependent manner in this model. PGI₂ and milrinone had synergistic effects on the pulmonary circulation when aerosolized in combination. Coaerosolization of milrinone with PGI₁ enhanced and prolonged the duration of action of PGI₂. Future studies evaluating the role of coaero-
solization of milrinone and PGI2 in infants and children with PH are warranted.

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DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the authors.

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