Pulmonary vascular effects of nitric oxide-cGMP augmentation in a model of chronic pulmonary hypertension in fetal and neonatal sheep

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Pulmonary vascular effects of nitric oxide-cGMP augmentation in a model of chronic pulmonary hypertension in fetal and neonatal sheep. Am J Physiol Lung Cell Mol Physiol 289: L798–L806, 2005. First published June 17, 2005; doi:10.1152/ajplung.00119.2005.—Persistent pulmonary hypertension of the newborn (PPHN) is partly due to impaired nitric oxide (NO)-cGMP signaling. BAY 41-2272 is a novel direct activator of soluble guanylate cyclase, but whether this drug may be an effective therapy for PPHN is unknown. We hypothesized that BAY 41-2272 would cause pulmonary vasodilation in a model of severe PPHN. To test this hypothesis, we compared the hemodynamic response of BAY 41-2272 to acetylcholine, an endothelium-dependent vasodilator, and sildenafil, a selective inhibitor of PDE5 in chronically instrumented fetal lambs at 1 and 5 days after partial ligation of the ductus arteriosus. After 9 days, we delivered the animals by cesarean section to measure their hemodynamic responses to inhaled NO (iNO), sildenafil, and BAY 41-2272 alone or combined with iNO. BAY 41-2272 caused marked pulmonary vasodilation, as characterized by a twofold increase in blood flow and a nearly 60% fall in PVR at day 1. Effectiveness of BAY 41-2272-induced pulmonary vasodilation increased during the development of pulmonary hypertension. Despite a similar effect at day 1, the pulmonary vasodilator response to BAY 41-2272 was greater than sildenafil at day 5. At birth, BAY 41-2272 dramatically reduced PVR and augmented the pulmonary vasodilation induced by iNO. We concluded that BAY 41-2272 causes potent pulmonary vasodilation in fetal and neonatal sheep with severe pulmonary hypertension. We speculate that BAY 41-2272 may provide a novel treatment for severe PPHN, especially in newborns with partial response to iNO therapy.

PERSISTENT PULMONARY HYPERTENSION of the newborn (PPHN) is a clinical syndrome characterized by abnormal pulmonary vascular tone, reactivity, and structure and sustained elevation of pulmonary vascular resistance (PVR) at birth leading to extrapulmonary right-to-left shunting of blood across the ductus arteriosus (DA) and foramen ovale and severe hypoxemia (15, 19, 27). Chronic partial ligation of the ductus arteriosus (DA) in fetal lambs causes marked elevation of PVR with abnormal pulmonary vasoreactivity, right ventricular hypertrophy (RVH), and structural remodeling of small pulmonary arteries, thereby providing a useful animal model for PPHN (3, 5, 24, 25).

The nitric oxide (NO)-cyclic 3′-5′-guanosine monophosphate (cGMP) cascade plays a major physiological role in the fetal and neonatal pulmonary circulation. NO is produced during conversion of L-arginine to L-citrulline by NO synthase (NOS) in endothelial cells and activates soluble guanylate cyclase (sGC) in vascular smooth-muscle cells to release cGMP. Past studies have shown that sGC is present and active early in the fetal lung (2, 16). Basal and stimulated NO release modulates pulmonary vasoregulation during late gestation. NOS antagonism increases PVR in near-term fetal lambs (1, 29). In addition, NOS inhibition selectively attenuates the pulmonary vascular response to acetylcholine, oxygen, shear stress, and myogenic response (1, 8, 22, 39). At birth, pretreatment with Nω-nitro-L-arginine, an NOS antagonist, reduces the fall in PVR and compromises the transition to neonatal circulation (1). Mechanisms that cause PPHN are uncertain, but previous studies suggest that impaired endothelial function with decreased NO production may contribute to PPHN (33, 41, 42).

Inhaled NO (iNO) is an effective therapy that improves gas exchange in neonates with severe pulmonary hypertension (7, 28, 31). However, past multicenter trials demonstrate that up to 40% of patients do not respond fully to iNO and require extracorporeal membrane oxygenation due to persistently elevated PVR and hypoxemia (7, 28, 31). These findings suggest that novel therapeutic strategies to stimulate pulmonary vasodilation or augment the response to iNO are still required to further improve outcomes in newborns with severe PPHN.

Direct pharmacological activators of sGC, such as BAY 41-2272, have been recently developed (36). BAY 41-2272 directly stimulates sGC on an NO-independent but heme-dependent site (36). Recent studies have shown that BAY 41-2272 causes potent vasodilation in the adult systemic and lung circulation (6, 10, 36, 37). Recently, BAY 41-2272 infusion has also been shown to cause potent and sustained pulmonary vasodilation in the fetus independently of endogenous NO release (9). Whether BAY 41-2272 is a potent vasodilator in the setting of chronic pulmonary hypertension has not been previously studied. In addition, the ability of BAY 41-2272 to augment the pulmonary vasodilation in responses to iNO in experimental PPHN is unknown.

Therefore, we hypothesized that therapeutic agents that cause vasodilation by directly stimulating sGC, such as BAY 41-2272, may provide an effective treatment of PPHN. To test this hypothesis, we studied the pulmonary vascular effects of BAY 41-2272 during the development of progressive pulmonary hypertension after partial ligation of the DA in chronically prepared, late-gestation fetal sheep. We report that BAY 41-2272 causes potent pulmonary vasodilation and improves ox-
ygenation at birth in sheep with experimental PPHN and that BAY 41-2272 augments the response to iNO.

METHODS

Surgical Preparation

All procedures were reviewed and approved by the Animal Care and Use Committee at the University of Colorado Health Sciences Center (Denver, CO). Surgery was performed between 125 and 130 days of gestation, according to previously published methods (4). Twelve mixed-breed (Columbia-Rambouillet) pregnant ewes were fasted for 48 h before surgery. Ewes were sedated with intramuscular buprenex (0.6 mg) and intravenous ketamine (60 mg) and diazepam (10 mg) and intratracheally intubated. Ewes were anesthetized with inhaled isoflurane (2–3%) and remained sedated but breathed spontaneously throughout surgery. Under sterile conditions, the left forelimb of the fetal lamb was delivered through a uterine incision. A skin incision was made under the left forelimb after local infiltration with 1% lidocaine. Polyvinyl catheters were inserted into the axillary artery and advanced into the ascending aorta (Ao) and the superior vena cava. A left axillary to sternal thoracotomy exposed the heart and great arteries. Polyvinyl catheters were inserted into the left pulmonary artery (LPA), the main pulmonary artery (MPA), and left atrium (LA) by direct puncture and secured into position with purse-string sutures, as previously described. A 6-mm ultrasonic flow transducer (Transonic Systems, Ithaca, NY) was placed around the LPA to measure blood flow to the left lung [LPA blood flow (QLPA)]. The DA (Transonic Systems, Ithaca, NY) was placed around the LPA to measure blood flow to the left lung [LPA blood flow (QLPA)]. The DA was exposed by blunt dissection. A cotton umbilical tape was placed around the DA and tightened around a right-angle surgical instrument to partially constrict the DA in a uniform manner. A catheter was placed in the amniotic cavity to serve as a pressure referent. The thoracotomy incision was closed in layers. The uteroplacental circulation was kept intact, and the fetus was gently replaced in the uterus. Ampicillin (500 mg) was added to the amniotic cavity before closure of the hysterotomy. Postoperatively, ewes were allowed to eat and drink ad libitum. All catheters were gently infused daily with 1–2 ml of normal (0.9%) saline with added heparin to maintain catheter patency.

Physiological Measurements

The Ao, MPA, and LA catheters were connected to a computer-monitored pressure transducer and recorder (Biopac Systems, Santa Barbara, CA). Pressures were referenced to amniotic pressure, and the pressure transducer was calibrated with a mercury manometer. The flow transducer cable was attached to an internally calibrated flow meter (Transonic Systems) for continuous measurements of QLPA. The absolute values of flow were determined from phasic blood flow signals as previously described (20). PVR in the left lung was calculated with the following equation: 

\[ \text{PVR} = \frac{\text{mmHg} \cdot \text{ml}^{-1} \cdot \text{min}^{-1}}{\text{mmHg}} = \left( \frac{\text{mean MPAP} - \text{mean LAP}}{\text{QLPA}} \right) \] 

where MPAP is mean pulmonary artery pressure and LAP is left atrial pressure. Arterial blood gas tensions, pH, hemoglobin, oxygen saturation, and methemoglobin were measured from blood samples that were drawn from the Ao catheter and measured at 39.5°C with a blood gas analyzer and hemoximeter (model OSM-3; Radiometer, Copenhagen, Denmark).

Study Drugs

Acetylcholine (A-6625; Sigma-Aldrich, St. Louis, MO) was dissolved in saline to achieve a concentration of 15 μg/ml immediately before use. BAY 41-2272 (kindly provided by Dr. Johannes-Peter Stasch, Bayer, Pharma Research, Wuppertal, Germany) was dissolved with 50% ethanol (1:1 vol/vol ethanol-saline) and diluted with saline to achieve a concentration of 500 μg/ml. Sildenafil (1 mg/ml, intravenous solution; Pfizer, Sandwich, UK) was diluted with normal saline for a final concentration of 100 μg/ml.

Experimental Design

Protocol 1: acute pulmonary hemodynamic effects of BAY 41-2272, acetylcholine, and sildenafil during the development of chronic pulmonary hypertension. The purpose of this protocol was to compare the effects of acute pulmonary administration of acetylcholine, an endothelium-dependent vasodilator, BAY 41-2272, a direct activator of sGC, and sildenafil, a selective inhibitor of cGMP-specific phosphodiesterase type 5 (PDE5), during the development of chronic PH. The studies were performed 1 and 5 days after partial ligation of the DA. Saline (0.1 ml/min) was first infused into the LPA catheter for at least 30 min, and baseline hemodynamic measurements were recorded every 10 min for QLPA, MPAP, aortic pressure (AoP), LAP, and heart rate (HR). After baseline measurements were stable for a 30-min period, acetylcholine (15 μg/ml), BAY 41-2272 (500 μg/ml), and sildenafil (100 μg/ml) were infused at 0.1 ml/min in random order into the LPA for 10 min. The dose of acetylcholine used in this study was based on past studies that demonstrate a doubling of blood flow without systemic effects. The BAY 41-2272 and sildenafil doses were selected from previous studies that demonstrated comparable twofold increase in pulmonary blood flow in fetal sheep (9). After each infusion, the catheter was subsequently flushed with saline (0.1 ml/min). Hemodynamic measurements were recorded for at least 30 min after the return to baseline values before the next drug infusion. Arterial blood gas tensions were obtained before and after each study period.

Protocol 2: independent and combined effects of iNO, BAY 41-2272, and sildenafil in newborn sheep with chronic pulmonary hypertension after birth. The purpose of this protocol was to compare the hemodynamic effects of BAY 41-2272 and sildenafil on the transitional pulmonary vasodilation and to determine whether the drugs would alter the pulmonary vasodilator response to iNO in experimental PPHN. Nine days after the initial surgery, cesarean-section deliveries were performed under inhalational isoflurane anesthesia to the ewe. The uterus was partially delivered through an abdominal incision, and the flow transducer cables and catheters were carefully freed from the maternal flank. After the injection of pancuronium bromide (0.1 mg/kg, inferior vena cava) to the fetus, a hysterotomy was performed, and the fetus was rapidly intubated. The fetus was extracted from the uterus, dried, and warmed. The animal was placed on a heating pad and ventilated with a time-cycled, pressure-limited mechanical ventilator (Infant Star 950; Infrasonics, San Diego, CA) with room air. Initial ventilator settings included rate of 30 breaths/min, a peak inspiratory pressure (PIP) of 35 cmH2O, a positive end-expiratory pressure of 6 cmH2O, and an inspiratory time (IT) of 1.0 s. Subsequent ventilator adjustments were made based on arterial blood gas values and chest wall excursion. Target blood gas parameters included achieving blood pH between 7.35 and 7.45, and arterial carbon dioxide pressure (Paco2) of 35–45 Torr. If Paco2 fell <35 Torr, PIP was carefully decreased to a minimum of 22 cmH2O. Ventilator rate and IT were gradually decreased thereafter if Paco2 remained below the target value after reaching a PIP of 22 cmH2O. Alternatively, if Paco2 increased >45 Torr, ventilator rate was increased by 5 breaths/min, and IT was decreased to maintain the inspiratory-to-expiratory ratio at 1:1. Hypotension, defined as mean arterial pressure <30 mmHg, was treated with a rapid infusion of normal (0.9%) saline (10 ml/kg over 5 min). Lambs were kept on a heating pad throughout the study. Saline (0.1 ml/min) was first infused into the LPA catheter for at least 30 min, and baseline hemodynamic measurements were recorded every 10 min (baseline period 1). These measurements included QLPA, MPAP, AoP, LAP, and HR. After baseline measurements were stable for a 30-min period, iNO (20 ppm) was delivered for 10 min. NO was stopped, and hemodynamic measurements were recorded until the return of these values to baseline. After a 30-min baseline period (baseline period 2), sildenafil (100 μg/ml) or BAY 41-2272 (500 μg/ml) was infused in random order at 0.1 ml/min into the LPA for 10 min. After sildenafil or BAY 41-2272 infusion was stopped, hemodynamic measurements were
recorded for at least 30 min after the return to baseline values before the next drug infusion (baseline period 3). The same protocol was repeated with sildenafil and BAY 41-2272 combined with iNO (20 ppm) for 10 min. Arterial blood gas tensions were obtained before and after each study period. At the end of the delivery study, sheep were killed with a large dose of pentobarbital sodium. Body weight was recorded, and the heart and lungs were rapidly removed in bloc through a midline thoracotomy. To assess the development of RVH after 9 days of DA compression, we weighed the free wall of the right ventricle and the left ventricle plus septum separately. RVH was expressed as the proportion of weights of the right ventricle and the left ventricle plus septum \( [RVH = RV/(LV + S)] \).

**Statistical Analysis**

Data are presented as means ± SE. Statistical analysis was performed with the Statview software package (SAS Institute, Cary, NC). Statistical comparisons were made by factorial and repeated-measures analysis of variance and Fisher’s protected least-significant-differences test for post hoc comparison. \( P < 0.05 \) was considered significant. In each experiment, \( n \) represents the number of different animals studied.

**RESULTS**

**Protocol 1: Acute Pulmonary Hemodynamic Effects of BAY 41-2272, Acetylcholine, and Sildenafil During the Development of Chronic Pulmonary Hypertension**

**Hemodynamic effects of partial constriction of DA.** Chronic partial constriction of DA increased mean PAP by 22% (from 57 ± 3 at day 1 to 70 ± 4 mmHg at day 5, \( P < 0.02 \), Fig. 1) and mean PVR by 32% (from 0.86 ± 0.07 at day 1 to 1.13 ± 0.15 mmHg·ml⁻¹·min⁻¹ at day 5, \( P < 0.05 \), Fig. 1). \( Q_{LPA} \) did not change during the study period (65 ± 3 at day 1 and 62 ± 5 ml/min at day 5).

**Fetal pulmonary hemodynamic response to acetylcholine during the development of pulmonary hypertension.** At day 1, acetylcholine infusion increased \( Q_{LPA} \) from 65 ± 3 to 91 ± 6 ml/min (\( P < 0.01 \), Fig. 1). Mean PAP did not change at this dose, and PVR decreased from 0.86 ± 0.07 to 0.63 ± 0.05 mmHg·ml⁻¹·min⁻¹ (\( P < 0.02 \), Fig. 1). However, the pulmonary vasodilator response to acetylcholine was abolished by day 5 (Fig. 1). Acetylcholine infusions did not alter arterial blood gas tensions or aortic and LA pressure at either time point (Tables 1 and 2).

**Fetal pulmonary hemodynamic response to sildenafil during the development of pulmonary hypertension.** At day 1, brief infusion of sildenafil caused a 117% rise in pulmonary \( Q_{LPA} \) (from 62 ± 3 to 135 ± 11 ml/min, \( P < 0.01 \), Fig. 2) and a 8% fall in MPAP (from 58 ± 3 to 53 ± 3 mmHg; not significant (NS), Fig. 2). These changes resulted in a 57% fall in PVR (from 0.91 ± 0.06 to 0.38 ± 0.03 mmHg·ml⁻¹·min⁻¹, \( P < 0.01 \), Fig. 2). Despite a progressive increase in PVR by day 5, sildenafil had a persistent pulmonary vasodilator effect that was greater than that observed at day 1 (Figs. 2 and 4). At day 5, sildenafil increased \( Q_{LPA} \) by 165% (from 63 ± 4 to 165 ± 6 ml/min, \( P < 0.01 \), Figs. 2 and 4), decreased MPAP by 11% (from 62 ± 4 to 53 ± 3 mmHg, NS, Fig. 2), and decreased PVR by 67% (from 1.12 ± 0.13 to 0.36 ± 0.04 mmHg·ml⁻¹·min⁻¹, NS, Figs. 2 and 4).

**Table 1. Blood gases, AoP, LAP, Hb, and HR after acetylcholine, sildenafil, and BAY 41-2272 infusion 1 day after DA ligation**

<table>
<thead>
<tr>
<th></th>
<th>Baseline</th>
<th>Acetylcholine</th>
<th>Sildenafil</th>
<th>BAY 41-2272</th>
</tr>
</thead>
<tbody>
<tr>
<td>pH</td>
<td>7.36 ± 0.02</td>
<td>7.35 ± 0.03</td>
<td>7.34 ± 0.02</td>
<td>7.33 ± 0.02</td>
</tr>
<tr>
<td>PO₂, Torr</td>
<td>47.6 ± 2.0</td>
<td>47.9 ± 1.9</td>
<td>49.9 ± 1.6</td>
<td>50.9 ± 1.9</td>
</tr>
<tr>
<td>SaO₂</td>
<td>97.4 ± 1.2</td>
<td>97.1 ± 1.2</td>
<td>99.2 ± 1.0</td>
<td>99.1 ± 0.8</td>
</tr>
<tr>
<td>Hb, g/dl</td>
<td>52.6 ± 4.0</td>
<td>51.4 ± 4.6</td>
<td>47.4 ± 2.4</td>
<td>47.4 ± 2.4</td>
</tr>
<tr>
<td>AoP, mmHg</td>
<td>39.1 ± 1.6</td>
<td>39.4 ± 1.4</td>
<td>36.0 ± 1.5</td>
<td>35.0 ± 1.3</td>
</tr>
<tr>
<td>LAP, mmHg</td>
<td>3.0 ± 0.4</td>
<td>3.6 ± 0.6</td>
<td>3.2 ± 0.5</td>
<td>3.5 ± 0.4</td>
</tr>
<tr>
<td>HR, bpm</td>
<td>176 ± 7</td>
<td>176 ± 8</td>
<td>178 ± 9</td>
<td>189 ± 4</td>
</tr>
</tbody>
</table>

Values are means ± SE. Blood gases, aortic and left atrial pressure, hemoglobin, and heart rate after acetylcholine (1.5 μg/min), sildenafil (10 μg/min), and BAY 41-2272 (50 μg/min) were infused in the left pulmonary artery catheter for 10 minutes at 0.1 ml/min. 1 day after partial ligation of the ductus arteriosus (DA). AoP, aortic pressure; LAP, left atrial pressure; Hb, hemoglobin; HR, heart rate.
mmHg⋅mL⁻¹⋅min⁻¹, P < 0.01, Figs. 2 and 4). Sildenafil infusion did not change arterial blood gas tensions, LA or Ao pressure (Tables 1 and 2).

**Fetal pulmonary hemodynamic response to BAY 41-2272 during the development of pulmonary hypertension.** BAY 41-2272 caused marked pulmonary vasodilatation in the chronically prepared fetal sheep with chronic pulmonary hypertension (Fig. 3). At day 1, brief infusion of BAY 41-2272 caused a 139% rise in Q̇LPA (from 65 ± 3 to 154 ± 14 mL/min, P < 0.01, Fig. 3) and an 11% fall in MPAP (from 58 ± 3 to 52 ± 3 mmHg; NS, Fig. 3). These changes resulted in a 61% fall in PVR (from 0.87 ± 0.05 to 0.34 ± 0.03 mmHg⋅mL⁻¹⋅min⁻¹, P < 0.01, Fig. 3). Despite a further increase in basal pulmonary vascular tone at day 5, BAY 41-2272 infusion had greater pulmonary vasodilator effects compared with day 1 (Figs. 3 and 4). This response included a 254% increase in Q̇LPA (from 59 ± 4 to 200 ± 14 mL/min, P < 0.01, Figs. 3 and 4), an 11% fall in MPAP (from 69 ± 4 to 62 ± 4 mmHg, NS, Figs. 3 and 4), and a 73% decrease in PVR (from 1.15 ± 0.14 to 0.29 ± 0.02 mmHg⋅mL⁻¹⋅min⁻¹, P < 0.01, Figs. 3 and 4). BAY 41-2272 infusion did not change arterial blood gas tensions or LA or Ao pressure (Tables 1 and 2). Comparisons between BAY 41-2272 and sildenafil demonstrated that BAY 41-2272 achieved a greater pulmonary vasodilatation at day 5 than sildenafil (P < 0.01, Fig. 4).

**Protocol 2: Independent and Combined Effects of iNO, BAY 41-2272, and Sildenafil in Newborn Sheep With Chronic Pulmonary Hypertension After Birth**

Treatment with iNO (20 ppm), sildenafil (10 μg/ml), and BAY 41-2272 (50 μg/ml) alone caused significant pulmonary vasodilation after cesarean-section delivery of hypertensive sheep, as reflected by changes in PVR, Q̇LPA, and MPAP (P < 0.01 compared with baseline, Figs. 5 and 6 and Table 3).

BAY 41-2272 induced greater pulmonary vasodilatation than iNO (P < 0.05, Fig. 5). In addition, treatment with BAY 41-2272 improved oxygenation as PaO₂ increased from 61.3 ± 5.5 Torr during baseline period to 95.7 ± 9.3 Torr with BAY 41-2272 (P < 0.02, Table 3). BAY 41-2272 augmented the pulmonary vasodilator response to iNO (Fig. 5, P < 0.01). As shown, BAY 41-2272 with iNO achieved lower PVR, higher Q̇LPA, and lower MPAP than either agent alone. In addition, PaO₂ increased to 105.6 ± 5.7 Torr during combined iNO and BAY 41-2272 therapy (P < 0.01, Table 3).

Sildenafil dramatically decreased PVR (Fig. 6) and improved oxygenation with a PaO₂ of 93.4 ± 9.1 Torr (P < 0.05, Table 3). Sildenafil combined with iNO decreased PVR to a greater extent than did iNO alone (PVR, 0.61 ± 0.06 mmHg⋅mL⁻¹⋅min⁻¹ for iNO and 0.37 ± 0.10 mmHg⋅mL⁻¹⋅min⁻¹ for sildenafil, P < 0.02, Fig. 6). PaO₂ increased to 100.4 ± 13.2 Torr during combined iNO and sildenafil therapy (P < 0.05, Table 3).

iNO did not have systemic effects (Table 3). Sildenafil and sildenafil combined with iNO reduced AoP by 7 and 8%, respectively (NS, Table 3). BAY 41-2272 and BAY 41-2272 combined with iNO reduced AoP by 9 and 11%, respectively (NS, Table 3). Arterial Pco₂, pH, hemoglobin, and HR did not change during infusion of the drugs.

Mean lamb weight at the time of study was 3,704 ± 376 g, and the ratio of the RV to LV + S was 0.69 ± 0.05 (n = 5).

**Table 2. Blood gases, AoP, LAP, Hb, and HR after acetylcholine, sildenafil, and BAY 41-2272 infusion 5 days after DA ligation**

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Baseline</th>
<th>Acetylcholine</th>
<th>Sildenafil</th>
<th>BAY 41-2272</th>
</tr>
</thead>
<tbody>
<tr>
<td>pH</td>
<td>7.37 ± 0.01</td>
<td>7.36 ± 0.01</td>
<td>7.36 ± 0.01</td>
<td>7.35 ± 0.01</td>
</tr>
<tr>
<td>Pco₂, Torr</td>
<td>50.0 ± 1.9</td>
<td>49.2 ± 0.7</td>
<td>50.3 ± 1.4</td>
<td>52.8 ± 1.3</td>
</tr>
<tr>
<td>Po₂, Torr</td>
<td>17.8 ± 0.7</td>
<td>18.2 ± 0.4</td>
<td>19.4 ± 1.1</td>
<td>17.8 ± 1.0</td>
</tr>
<tr>
<td>SaO₂</td>
<td>45.8 ± 3.3</td>
<td>50.6 ± 2.0</td>
<td>47.7 ± 3.2</td>
<td>45.1 ± 3.7</td>
</tr>
<tr>
<td>Hb, g/dl</td>
<td>6.4 ± 0.4</td>
<td>6.3 ± 0.3</td>
<td>6.3 ± 0.4</td>
<td>6.4 ± 0.4</td>
</tr>
<tr>
<td>AoP, mmHg</td>
<td>41.6 ± 2.1</td>
<td>40.0 ± 1.7</td>
<td>37.9 ± 1.1</td>
<td>37.3 ± 1.7</td>
</tr>
<tr>
<td>LAP, mmHg</td>
<td>4.0 ± 0.9</td>
<td>4.5 ± 1.0</td>
<td>4.8 ± 0.5</td>
<td>4.8 ± 0.8</td>
</tr>
<tr>
<td>HR, bpm</td>
<td>166 ± 8</td>
<td>153 ± 6</td>
<td>182 ± 6</td>
<td>183 ± 11</td>
</tr>
</tbody>
</table>

Values are means ± SE. Blood gases, AoP and LAP, Hb, and HR after acetylcholine (1.5 μg/min), sildenafil (10 μg/min), and BAY 41-2272 (50 μg/min) were infused in the left pulmonary artery catheter for 10 minutes at 0.1 ml/min, 5 days after partial ligation of the DA.

**Fig. 2. Hemodynamic response to sildenafil infusion (10 μg/min) 1 and 5 days after partial ligation of the ductus arteriosus.** Values are expressed as means ± SE. **P < 0.01 between baseline and sildenafil; #P < 0.05 and ##P < 0.01 between baseline day 1 and baseline day 5; ‡‡P < 0.05 and ††P < 0.03 between sildenafil day 1 and sildenafil day 5.

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DISCUSSION

To determine whether an NO-independent activator of sGC may provide a new and effective therapy for hypoxic newborns with PPHN, we studied the effects of BAY 41-2272 in fetal sheep with chronic pulmonary hypertension caused by partial constriction of the DA. We found that BAY 41-2272 markedly increased pulmonary blood flow by nearly twofold and reduced PVR by 61%. In contrast with the vasodilator response to acetylcholine, an endothelium-dependent agonist, pulmonary vasodilation to BAY 41-2272 persisted during progression of intrauterine pulmonary hypertension. At birth, BAY 41-2272 caused selective and potent pulmonary vasodilation in fetal sheep with PPHN, improved gas exchange, and augmented the pulmonary vasodilator response to inhaled NO.

These results support the hypothesis that direct activation of sGC by an NO-independent mechanism causes marked vasodilation during the development of pulmonary hypertension.

In this study, we explored three major targets of the NO-cGMP cascade (Fig. 7). As illustrated, NO mediates vasodilation by stimulating sGC in vascular smooth muscle cells. Enzyme activation by the binding of NO converts guanosine triphosphate to cGMP, which modulates the activity of cGMP-dependent kinases, cGMP-regulated phosphodiesterases, and cGMP-regulated ion channels, which regulate vasodilation (11). cGMP signaling is downregulated by PDE5 activity, which lowers intracellular cGMP content through the degradation of cGMP to 5’-GMP (11). As in many settings, acetylcholine stimulates endothelial NOS (eNOS) and increases...
endothelial NO release in fetal sheep (1). BAY 41-2272 directly stimulates sGC at a NO-independent but heme-dependent site (36). Sildenafil inhibits PDE5 and enhances NO-induced vasorelaxation by increasing vascular smooth muscle cGMP concentration (11).

This is the first report describing the pulmonary hemodynamic response to BAY 41-2272 in an experimental model of chronic pulmonary hypertension. Previous studies have shown that BAY 41-2272 is a potent vasodilator in the postnatal systemic circulation and older sheep with acute vasoconstriction (10, 36). After oral administration, BAY 41-2272 decreased blood pressure and improved mortality in hypertensive adult rats (36). In addition, Evgenov et al. (10) demonstrated that BAY 41-2272 is a potent pulmonary vasodilator in a model of acute pulmonary hypertension in juvenile lambs that was induced by infusion of the vasoconstrictor U-46619. Recently, we demonstrated that BAY 41-2272 causes potent and sustained pulmonary vasodilation in the developing lung (9). This current study extends the previous observation by demonstrating that BAY 41-2272 infusion caused persistent pulmonary vasodilation in an established experimental model of chronic pulmonary hypertension in newborn sheep.

In our study, chronic pulmonary hypertension was induced by partial compression of the DA in late-gestation fetal sheep. As previously described, DA ligation increases MPAP and PVR without causing sustained elevation of pulmonary blood flow or hypoxemia (22, 23, 38). In addition, chronic intratracheal pulmonary hypertension alters vasoreactivity, as reflected by downregulation of eNOS expression and altered endothelium-dependent vasodilation (22, 23, 33, 42). Over time, intratracheal pulmonary hypertension induces RVH and pulmonary vascular remodeling with increased smooth muscle cell hyperplasia, as observed in fatal human PPHN (3).

Multiple abnormalities in the NO-cGMP cascade contribute to mechanisms underlying endothelial dysfunction associated with chronic intratracheal pulmonary hypertension. Past studies have shown that chronic pulmonary hypertension decreases lung eNOS mRNA and protein expression and total NOS activity (42). eNOS activity is further impaired by altered heat shock protein 90-eNOS interaction (18), and increased superoxide generation may further limit NO bioactivity (34). In addition, in vitro studies demonstrated that sGC activity is impaired, resulting in a decreased generation of cGMP and reduced vascular relaxation to NO stimulation (38). Several studies have suggested that an increase in PDE5 activity could contribute to the pathophysiology of pulmonary hypertension.

Fig. 5. Hemodynamic response to inhaled nitric oxide (iNO, 1.5 μg/min), BAY 41-2272 infusion (BAY, 50 μg/min) alone or in combination with iNO (20 ppm) during delivery study in lambs after partial ligation of the ductus arteriosus. Values are expressed as means ± SE. *P < 0.02 and **P < 0.01 vs. baseline; #P < 0.05, ###P < 0.02, and ####P < 0.01 vs. iNO. BL, baseline.

Table 3. $Q_{LPA}$, MPAP, PVR, AoP, HR, blood gases, and Hb during delivery studies

<table>
<thead>
<tr>
<th></th>
<th>Baseline</th>
<th>iNO</th>
<th>Sildenafil</th>
<th>Sildenafil + iNO</th>
<th>BAY 41-2272</th>
<th>BAY 41-2272 + iNO</th>
</tr>
</thead>
<tbody>
<tr>
<td>$Q_{LPA}$, ml/min</td>
<td>62.3±5.5</td>
<td>96.3±15.3</td>
<td>106.0±3.1</td>
<td>138.7±14.0**</td>
<td>167.6±16.4**</td>
<td>168.0±24.9***</td>
</tr>
<tr>
<td>MPAP, mmHg</td>
<td>63.0±6.0</td>
<td>59.3±5.8</td>
<td>53.0±8.0</td>
<td>49.7±7.9</td>
<td>47.3±8.5</td>
<td>50.2±8.4</td>
</tr>
<tr>
<td>PVR, mmHg/ml/min</td>
<td>0.98±0.02</td>
<td>0.61±0.06***</td>
<td>0.49±0.08***</td>
<td>0.37±0.10***</td>
<td>0.42±0.10***</td>
<td>0.34±0.10***</td>
</tr>
<tr>
<td>AoP, mmHg</td>
<td>42.7±5.1</td>
<td>44.0±5.4</td>
<td>39.7±5.5</td>
<td>39.3±5.5</td>
<td>38.7±4.5</td>
<td>38.0±4.3</td>
</tr>
<tr>
<td>HR, bpm</td>
<td>165±5</td>
<td>163±14</td>
<td>172±14</td>
<td>170±14</td>
<td>167±18</td>
<td>187±10</td>
</tr>
<tr>
<td>pH</td>
<td>7.28±0.01</td>
<td>7.30±0.02</td>
<td>7.30±0.02</td>
<td>7.29±0.02</td>
<td>7.29±0.01</td>
<td>7.29±0.01</td>
</tr>
<tr>
<td>PAO$_2$, Torr</td>
<td>61.3±5.5</td>
<td>90.0±11.5*</td>
<td>93.4±9.1*</td>
<td>100.4±13.2*</td>
<td>95.7±9.3***</td>
<td>105.6±5.7***</td>
</tr>
<tr>
<td>PACO$_2$, Torr</td>
<td>56.5±5.1</td>
<td>53.4±2.0</td>
<td>54.6±1.5</td>
<td>56.4±0.4</td>
<td>58.7±2.2</td>
<td>56.3±2.1</td>
</tr>
<tr>
<td>Hb, g/dl</td>
<td>6.9±0.5</td>
<td>6.8±0.8</td>
<td>6.7±0.8</td>
<td>6.7±0.8</td>
<td>6.6±0.8</td>
<td>6.7±0.8</td>
</tr>
</tbody>
</table>

Values are means ± SE for left pulmonary artery blow flow ($Q_{LPA}$), mean pulmonary artery pressure (MPAP), pulmonary vascular resistance (PVR), AoP, HR, blood gases, and Hb during delivery studies. iNO, inhaled nitric oxide. *P < 0.05, **P < 0.02, ***P < 0.01 vs. baseline.
PDE5 activity is markedly elevated in fetal lamb with partial chronic DA ligation, suggesting that rapid cGMP hydrolysis may limit cGMP-dependent pulmonary vasodilation (12, 13). Thus chronic intrauterine pulmonary hypertension disrupts NO-cGMP signaling by decreasing eNOS expression and activity, altering sGC content and activity, and increasing PDE5 activity. Each pathway suggests potential alternate strategies to counteract the underlying pathophysiology of PPHN by treatment with exogenous NO, sGC activators, and PDE5 inhibitors.

In this model, BAY 41-2272 and sildenafil, but not acetylcholine, caused pulmonary vasodilation despite progressive increase in PVR. Interestingly, BAY 41-2272-induced pulmonary vasodilation was even greater at day 5 than at day 1. The mechanism of this enhanced effect is unclear. Mullershausen et al. (26) found that, in addition to direct stimulation of sGC, BAY 41-2272 may have some PDE5 inhibitor effects. In contrast, Stasch et al. (36) reported that BAY 41-2272 is an NO-independent sGC activator without any PDE5 inhibitory activity. Our previous study in the normal fetal sheep demonstrated that BAY 41-2272-induced pulmonary vasodilation was not blocked by nitro-L-arginine and the vasodilator effects of BAY 41-2272 were more sustained than those observed during treatment with sildenafil (9). Whether BAY 41-2272 at high doses can inhibit other phosphodiesterase isozymes is uncertain in our study (21). However, a role of additional phosphodiesterase isozymes in PPHN is currently unexplored and further studies are needed to fully examine the mechanisms responsible for this response. In addition, studies in pulmonary arteries isolated from fetal lambs with pulmonary hypertension demonstrated impaired relaxations to agents that stimulate endothelial NO production and cGMP production by sGC but normal relaxation to cGMP given exogenously or produced by particulate guanylate cyclase (38). Despite downregulation of lung sGC content and activity (38), our findings demonstrate persistent and potent pulmonary vasodilation by BAY 41-2272. We speculate that this effect may be partly due to its unique ability to activate sGC (36).

Several studies in perinatal animals and in human newborns demonstrated iNO as a potent and selective pulmonary vasodilator in transitional pulmonary circulation at birth, especially in the settings of impaired NO production, such as PPHN (7, 17, 28, 30, 31). However, some newborns have poor or partial response to iNO therapy. Mechanisms that contribute to this problem include poor lung inflation during mechanical ventilation, impaired cardiac function, and anatomic lung disease. In some cases, enhancement of the vascular response to iNO may be achieved via augmentation of the NO-cGMP cascade. Sildenafil causes pulmonary vasodilation in newborns and adults and has been proposed for primary treatment of pulmonary hypertension (32, 35, 40, 43). In animal models of acute pulmonary hypertension, intravenous sildenafil induces a potent pulmonary vasodilator effect (14, 35, 43) and nebulized sildenafil augments the iNO-induced pulmonary vasodilation...
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(14). In our study, BAY 41-2272 was at least as effective as iNO or sildenafil to reduce PVR in newborn sheep with chronic pulmonary hypertension. In addition, BAY 41-2272 augmented the pulmonary vasodilator effect to iNO. This data suggest that BAY 41-2272 may sensitize sGC to become more responsive to NO, as suggested by its effects on platelets (26). In this study, BAY 41-2272 and sildenafil did not decrease systemic arterial pressure; however, these agents were infused directly into the LPA. We suspect that these drugs may reduce systemic pressure if administered into the systemic circulation, as would likely occur in the clinical setting. Furthermore, this study examined only the acute effects of BAY 41-2272 and sildenafil in this model of PPHN. The vasodilator effect of BAY 41-2272 was sustained during prolonged infusions in the normal fetus (9), but whether continuous BAY 41-2272 infusion can sustain its effects in sheep with experimental PPHN remains unknown.

In conclusion, BAY 41-2272 causes potent pulmonary vasodilation in fetal sheep during the progressive increase of pulmonary hypertension in utero. Moreover, BAY 41-2272 causes selective and potent pulmonary vasodilatation and augments the pulmonary vasodilator response to iNO during transition at birth in sheep with chronic pulmonary hypertension. These observations suggest the therapeutic potential of BAY 41-2272 as an alternate or adjuvant therapy for severe neonatal pulmonary hypertension, leading to our speculation that BAY 41-2272 could provide a novel treatment or strategy for severe PPHN.

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