Effects of BAY 41–2272, a soluble guanylate cyclase activator, on pulmonary vascular reactivity in the ovine fetus

Philippe Deruelle,1,2 Theresa R. Grover,1 Laurent Storme,3 and Steven H. Abman1

1Pediatric Heart Lung Center, University of Colorado School of Medicine, Denver, Colorado; 2Faculté de Médecine, Université de Lille II; and 3Hôpital Jeanne de Flandre, Centre Hospitalier Regional Universitaire de Lille, Lille, France

Submitted 2 November 2004; accepted in final form 10 December 2004

Deruelle, Philippe, Theresa R. Grover, Laurent Storme, and Steven H. Abman. Effects of BAY 41–2272, a soluble guanylate cyclase activator, on pulmonary vascular reactivity in the ovine fetus. Am J Physiol Lung Cell Mol Physiol 288: L727–L733, 2005. First published December 17, 2004; doi:10.1152/ajplung.00409.2004.—Nitric oxide (NO)-cGMP signaling plays a critical role during the transition of the pulmonary circulation at birth. BAY 41–2272 is a novel NO-independent direct stimulator of soluble guanylate cyclase that causes vasodilation in systemic and local circulations. However, the hemodynamic effects of BAY 41–2272 have not been studied in the perinatal pulmonary circulation. We hypothesized that BAY 41–2272 causes potent and sustained fetal pulmonary vasodilation. We performed surgery on 14 fetal lambs (125–130 days gestation; term = 147 days) and placed catheters in the main pulmonary artery, aorta, and left atrium to measure pressures. An ultrasonic flow transducer was placed on the left pulmonary artery (LPA) to measure blood flow, and a catheter was placed in the LPA for drug infusion. Pulmonary vascular resistance (PVR) was calculated as pulmonary artery pressure minus left atrial pressure divided by LPA blood flow. BAY 41–2272 caused dose-related increases in pulmonary blood flow up to threefold above baseline and reduced PVR by 75% (P < 0.01). Prolonged infusion of BAY 41–2272 caused sustained pulmonary vasodilation throughout the 120-min infusion period. The pulmonary vasodilator effect of BAY 41–2272 was not attenuated by Nω-nitro-l-arginine, a NO synthase inhibitor. In addition, compared with sildenafil, a phosphodiesterase 5 inhibitor, the pulmonary vasodilator response to BAY 41–2272 was more prolonged. We conclude that BAY 41–2272 causes potent and sustained fetal pulmonary vasodilation independent of NO release. We speculate that BAY 41–2272 may have therapeutic potential for pulmonary hypertension associated with failure to circulatory adaptation at birth, especially in the setting of impaired NO production.

Address for reprint requests and other correspondence: S. H. Abman, Dept. of Pediatrics, B-395, Children’s Hospital, 1056 E. 19th Ave., Denver, CO 80218-1088 (E-mail: steven.abman@uchsc.edu).
muscle cell cGMP content, such as BAY 41–2272, may cause more potent and prolonged fetal pulmonary vasodilation and that the inability to sustain production of cGMP contributes to high PVR in the normal fetus.

To address this question, we examined the pulmonary hemodynamic response to BAY 41–2272 infusions and determined its mechanism of action in chronically prepared, late-gestation fetal lamb. We report that BAY 41–2272 is a potent pulmonary vasodilator in the fetus and speculate that this may be an effective strategy for the treatment of PPHN.

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 gestation, according to previously published methods (5). Fourteen 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 using 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 (Qp). 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. The ewe was allowed to recover from surgery for 48 h before fetal drug administration and hemodynamic studies.

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 pressures, and the pressure transducer was calibrated with a mercury manometer. The flow transducer cable was attached to an internally calibrated flowmeter (Transonic Systems) for continuous measurements of LPA blood flow (QLPA). The absolute values of flow were determined from phasic blood flow signals as previously described (21). PVR in the left lung was calculated with the following equation: 

$$PVR = \frac{\text{mean MPAP} - \text{mean LAP}}{Q_{LPA}}$$

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

BAY 41–2272 (kindly provided by Dr. J.-P. Stasch, Bayer AG, Pharma Research, Wuppertal, Germany) was dissolved with 50% ethanol (1 vol ethanol and 1 vol saline) and diluted with saline to achieve the different concentration used in this study (100 μg, 500 μg, 1 mg, and 2.5 mg/ml).
Table 1. Blood gases, AoPs, and HR after ethanol (50%, 1 ml) and BAY 41-2272 infused in the left pulmonary artery catheter for 10 min at 0.1 ml/min

<table>
<thead>
<tr>
<th></th>
<th>Baseline</th>
<th>Vehicle</th>
<th>BAY 41-2272 100 µg</th>
<th>BAY 41-2272 500 µg</th>
<th>BAY 41-2272 1 mg</th>
<th>BAY 41-2272 2.5 mg</th>
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<tbody>
<tr>
<td>pH</td>
<td>7.40±0.01</td>
<td>7.38±0.02</td>
<td>7.38±0.02</td>
<td>7.37±0.01</td>
<td>7.36±0.01</td>
<td>7.35±0.01</td>
</tr>
<tr>
<td>PCO₂, Torr</td>
<td>43.3±1.3</td>
<td>41.6±0.3</td>
<td>43.4±1.2</td>
<td>43.6±3.9</td>
<td>46.0±2.0</td>
<td>43.7±1.5</td>
</tr>
<tr>
<td>PO₂, Torr</td>
<td>19.9±0.6</td>
<td>19.7±1.0</td>
<td>20.8±0.2</td>
<td>20.1±0.5</td>
<td>18.2±0.8</td>
<td>19.0±0.6</td>
</tr>
<tr>
<td>AoP, mmHg</td>
<td>47.7±2.0</td>
<td>46.7±1.2</td>
<td>44.7±2.4</td>
<td>46.7±1.8</td>
<td>46.0±2.3</td>
<td>35.0±2.9*</td>
</tr>
<tr>
<td>LAP, mmHg</td>
<td>3.2±0.6</td>
<td>3.1±1.2</td>
<td>3.3±0.3</td>
<td>4.0±0.0</td>
<td>3.5±1.2</td>
<td>3.0±1.2</td>
</tr>
<tr>
<td>HR, beats/min</td>
<td>169±6.2</td>
<td>150±1</td>
<td>172±4</td>
<td>176±5</td>
<td>182±8</td>
<td>202±4*</td>
</tr>
</tbody>
</table>

Values are means ± SE. AoP, aortic pressure; LAP, left atrial pressure; HR, heart rate. *P < 0.01 compared with baseline value.

Nα-nitro-L-arginine (L-NA; 10 mg, Sigma, St. Louis, MO) was dissolved in normal saline plus 2–3 drops 1 M HCl and titrated with 1 M NaOH to achieve a pH of 7.4 (1 ml final vol). The drug was infused into the LPA by an infusion pump set to deliver 10 mg for a 10-min period. L-NA was prepared immediately before drug administration, and the dose chosen was based on previous studies that have demonstrated effective blockade of NOS activity during acetylcholine and flow-induced vasodilation for at least 4 h (10).

Sildenafil (1 mg/ml, intravenous solution, Pfizer, Sandwich, UK) was diluted with normal saline for a final concentration of 0.1 mg/ml.

Experimental Design

**Protocol 1: pulmonary hemodynamic effects of acute BAY 41–2272 infusion.** The purpose of this protocol was to determine the effects of acute intrapulmonary administration of BAY 41–2272 on fetal pulmonary hemodynamics and the dosage needed for optimal response. After a 48-h recovery period after surgery, 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 Q̇LPA, MPAP, AoP, LAP, and heart rate (HR). After baseline measurements were stable for a 30-min period, BAY 41–2272 was infused at one of several doses in random order (100 µg, 500 µg, 1 mg, and 2.5 mg) into the LPA for 10 min. 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. As BAY 41–2272 was diluted in 50% ethanol, we studied the hemodynamic response to ethanol (1 ml, 50%) to ensure that the response cannot be related to the solvent. Arterial blood gas tensions were obtained before and after each study period.

**Protocol 2: pulmonary hemodynamic effects of prolonged BAY 41–2272 infusion.** The purpose of this protocol was to investigate the effects of prolonged BAY 41–2272 infusion on fetal pulmonary circulation. Saline (0.1 ml/min) was first infused into the LPA catheter for at least 30 min. After 30 min of stable baseline measurements, BAY 41–2272 (1 mg/ml) was infused at 0.1 ml/min for 120 min into the LPA catheter (dose rate: 0.1 mg/min; total dose: 12 mg). Hemodynamic measurements were recorded every 10 min starting at the beginning of the infusion and continued for 60 min after drug infusion. Arterial blood gas tensions were obtained before, after 60 min of drug infusion, and at 30 min of the recovery period.

**Protocol 3: pulmonary hemodynamic effects of BAY 41–2272 after NOS inhibition.** The purpose of this protocol was to determine whether BAY 41–2272-induced pulmonary vasodilation was mediated through NO production. Protocol 2 was repeated after L-NA infusion. L-NA (10 mg over 10 min) was infused into the LPA catheter. The LPA catheter was then infused with saline for 20 min before starting the BAY 41–2272 infusion (1 mg/ml) at 0.1 ml/min for 120 min (dose rate: 0.1 mg/min; total dose: 12 mg). Hemodynamic measurements were recorded throughout the study period and 60 min after the BAY 41–2272 infusion was stopped.

**Fig. 2.** Pulmonary hemodynamic effects of prolonged infusion of BAY 41–2272. BAY 41–2272 (1 mg/ml at 0.1 mg/min, black rectangle) caused potent and sustained pulmonary vasodilation for the entire 120-min study period, which persisted for 30 min after termination of drug infusion. Values are expressed as means ± SE (n = 4). *P < 0.01 and §P < 0.05 compared with baseline value. PAP, pulmonary artery pressure.
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 using factorial and repeated-measures analysis of variance and Fisher’s protected least significant differences test. P < 0.05 was considered significant. In each experiment, n represents the number of different animals studied.

RESULTS

Protocol 1: Pulmonary Hemodynamic Effect of Acute BAY 41–2272 Infusion

BAY 41–2272 caused dose-related pulmonary vasodilation in the chronically prepared fetal lamb (Fig. 1). Brief infusion of BAY 41–2272 increased Qp and decreased PVR from baseline values at a threshold dose of 500 μg (P < 0.01, Fig. 1). At higher doses (1 and 2.5 mg), BAY 41–2272 progressively increased Qp and reduced PVR without altering blood gas tensions (P < 0.01). At 2.5 mg, BAY 41–2272 decreased MPA pressures and AoP and increased HR compared with baseline values and lower doses (P < 0.01, Fig. 1 and Table 1). Brief infusion of BAY 41–2272 did not alter blood gas tensions and LA pressures at the doses used in this study (Table 1).

Table 2. Blood gases, AoPs, and HR during baseline, after 60 min of drug infusion, and at 30 min of the recovery period for BAY 41–2272, BAY 41-2272 after NOS pretreatment, and sildenafil infusions

<table>
<thead>
<tr>
<th></th>
<th>BAY 41-2272</th>
<th>BAY 41-2272 + LNA</th>
<th>Sildenafil</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Baseline</td>
<td>During</td>
<td>Baseline</td>
</tr>
<tr>
<td>pH</td>
<td>7.38±0.01</td>
<td>7.35±0.01</td>
<td>7.34±0.01</td>
</tr>
<tr>
<td>PCO₂, Torr</td>
<td>45.0±1.3</td>
<td>43.2±2.3</td>
<td>47.5±3.4</td>
</tr>
<tr>
<td>P O₂, Torr</td>
<td>18.0±0.6</td>
<td>18.2±1.1</td>
<td>18.6±0.7</td>
</tr>
<tr>
<td>AoP, mmHg</td>
<td>41.8±1.5</td>
<td>39.5±2.2</td>
<td>45.8±2.3</td>
</tr>
<tr>
<td>LAP, mmHg</td>
<td>2.8±0.5</td>
<td>2.3±0.5</td>
<td>2.8±0.5</td>
</tr>
<tr>
<td>HR, beats/min</td>
<td>186±4</td>
<td>202±6</td>
<td>199±8</td>
</tr>
</tbody>
</table>

Values are means ± SE. *P < 0.05 compared with baseline value.
Prolonged (120 min) infusion of BAY 41–2272 caused sustained pulmonary vasodilation (Fig. 2). BAY 41–2272 infusion (0.1 mg/min) caused a nearly 3.5-fold rise in pulmonary blood flow (Qp: from 67 ± 8 to 242 ± 51 ml/min, P < 0.01; Fig. 2) and a 11% fall in MPA pressures (MPAP: from 46 ± 2 to 41 ± 2 mmHg, P < 0.05; Fig. 2). These changes resulted in a 75% fall in PVR (PVR: from 0.67 ± 0.06 to 0.17 ± 0.04 mmHg·ml⁻¹·min⁻¹, P < 0.01; Fig. 2). This vasodilator response was maximal 40 min after the beginning of the infusion and was sustained throughout the infusion period and persisted for 30 min after stopping drug infusion. Prolonged BAY 41–2272 infusion did not change arterial blood gas tensions but decreased AoP by 6% (P < 0.05; Table 2) and increased HR by 8% (not significant; Table 2).

Protocol 3: Pulmonary Hemodynamic Effects of BAY 41–2272 After NOS Inhibition

The response to BAY 41–2272 was not attenuated after NOS inhibition (Fig. 3). That is, after treatment with L-NA, which caused a marked rise in PVR, BAY 41–2272 still had potent and sustained vasodilator effects including a 247% increase in Qp (Qp: from 49.8 ± 6.5 to 196.5 ± 40.8 ml/min, P < 0.01; Fig. 3) and a 70% decrease in PVR (PVR: from 1.0 ± 0.28 to 0.28 ± 0.13 mmHg·ml⁻¹·min⁻¹, P < 0.01; Fig. 3A). Pretreatment with L-NA did not modify the effects of BAY 41–2272 on MPAP, AoP, and HR (Fig. 3B and Table 2). Blood gas tensions did not change during the study period (Table 2).

Protocol 4: Pulmonary Hemodynamic Effects of Prolonged Sildenafil Infusion

To compare the effects of PDE5 inhibition with BAY 41–2272, we infused sildenafil, a selective PDE5 inhibitor (0.1 mg/ml), into the LPA for 120 min. At this dose, sildenafil infusion caused a nearly threefold rise in pulmonary blood flow (Qp: from 74 ± 4 to 210 ± 23 ml/min, P < 0.01; Fig. 4) and a 60% fall in PVR (PVR: from 0.63 ± 0.05 to 0.25 ± 0.05 mmHg·ml⁻¹·min⁻¹, P < 0.01; Fig. 4). The maximal response and the return to baseline values were, respectively, obtained 40 and 110 min after the beginning of the infusion. There was no effect of sildenafil on MPAP, AoP, HR, and arterial blood gas tensions (Table 2). Comparisons of the pulmonary vascular responses between BAY 41–2272 and sildenafil demonstrated that pulmonary vasodilatation was better sustained and more prolonged during BAY 41–2272 than during sildenafil infusion (Fig. 4).

DISCUSSION

Although iNO is an effective therapy for hypoxemic newborns with severe pulmonary hypertension, responses are poor in up to 40% of patients. To determine whether a NO-independent activator of sGC can provide an alternate therapy, we studied the effects of BAY 41–2272 in the fetal lamb. We found that BAY 41–2272 markedly increased pulmonary blood flow by nearly 3.5-fold and reduced PVR by 75% and that the pulmonary vasodilator effect of BAY 41–2272 was not attenuated by NOS blockade. In addition, compared with sildenafil, a PDE5-selective inhibitor, the pulmonary vasodilator response of BAY 41–2272 was more sustained. These results support the hypothesis that direct activation of sGC by a NO-independent mechanism causes potent and sustained vasodilation in the developing lung. In addition, in contrast with many vasodilator stimuli, BAY 41–2272 causes potent and sustained pulmonary vasodilation in the fetus.

Our results are interesting because this is the first report describing the hemodynamic response to BAY 41–2272 in the developing pulmonary circulation. BAY 41–2272 directly stimulates sGC on a NO-independent but heme-dependent site (35). In vitro studies showed that BAY 41–2272 activates purified sGC up to 30-fold and is ~100-fold more potent than its analog YC-1 (35). After oral administration, BAY 41–2272...
increased production of vasoconstrictors or PDE inhibition greater than with these other agents. Mechanisms that sustain increased blood pressure and improved mortality in hypertensive rats (35). Recently, in a model of acute pulmonary hypertension in juvenile lambs (mean weight = 19 ± 0.4 kg), Egenov et al. (13) demonstrated that BAY 41–2272 is a potent pulmonary vasodilator. Although BAY 41–2272 induces a fall in blood pressure, the pulmonary effects were significantly greater than the systemic effects (13). In our study, BAY 41–2272 infusion caused potent and sustained falls in PVR but systemic effects were observed at higher doses and prolonged infusion.

The NO-cGMP cascade is one of the major physiological pathways in the fetal and neonatal pulmonary circulation. NO is produced during conversion of L-arginine to L-citrulline by the NOS in endothelial cells and activates sGC in vascular smooth muscle cells to release cGMP. Past studies showed that sGC is present and active early in the fetal lung (4, 17). Basal and stimulated NO release modulates pulmonary vasoregulation during late gestation. NOS antagonism increases PVR in near-term fetal lambs (3, 29). In addition, NOS inhibition selectively attenuates pulmonary vascular response to acetylcholine, oxygen, shear stress, and myogenic response (3, 10, 23, 38). At birth, pretreatment with L-NA reduces the fall in PVR and compromises the transition to neonatal circulation (3). Pathological conditions support the potential importance of NO and sGC in the regulation of the perinatal pulmonary circulation. Endothelial NOS and sGC activities and expression are altered in lamb models of persistent pulmonary hypertension and congenital diaphragmatic hernia (7, 27, 33, 41, 43, 44).

Stasch et al. (35) reported BAY 41–2272 as a NO-independent sGC activator without any PDE5 inhibitory activity. Our data agree with these findings as BAY 41–2272-induced vasodilatation was not blocked by L-NA, suggesting a NO-independent mechanism. However, with higher doses, Muller-shausen et al. (25) found that in addition to direct stimulation of sGC, BAY 41–2272 may have some PDE5 inhibitor effects as well. In addition, BAY 41–2272 may sensitize sGC to become more responsive to NO (25). For example, BAY 41–2272 augments and prolongs pulmonary vasodilatation induced by iNO (13). Whether BAY 41–2272 at high doses can inhibit phosphodiesterase isoforms other than PDE5 is uncertain (22). In our study, the vasodilator effects of BAY 41–2272 were more sustained than those observed during treatment with the PDE5 inhibitor sildenafil. Further studies are needed to fully examine the mechanisms responsible for this response.

We found that BAY 41–2272 caused a prolonged and sustained vasodilation in the ovine fetal lung. This response is different from several endothelium-dependent agonists (acetylcholine, bradykinin, histamine, tolazoline, oxygen, or shear stress), which are unable to sustain vasodilation (1, 2, 5, 6). Conversely, direct stimulators of vascular smooth muscle cells such as 8-bromo-cGMP, atrial natriuretic peptide, and iNO can produce a sustained pulmonary vasodilatation (2, 18). Norepinephrine and estradiol, probably related to NO and lemakalin, a direct K+-ATP channel agonist, are also able to maintain a pulmonary vasorelaxant response, but the estradiol response occurred after 24–48 h of treatment (11, 16, 28). Nevertheless, in our findings, the BAY 41–2272 vasodilator response was greater than with these other agents. Mechanisms that sustain fetal pulmonary vasodilation are unknown but may include increased production of vasocostritors or PDE inhibition activity. BQ-123 and phosphoramidon, two endothelin-1 antagonists, augment and prolong the increase in flow during acute ductus arteriosus compression (15). Dipyridamole, a cGMP phosphodiesterase inhibitor, prolongs iNO-induced pulmonary vasodilation in the ovine transitional circulation (46). However, neither endothelin-1 blockade nor dipyridamole treatment completely inhibited this vasoconstrictor response in the fetal lung.

Increase cGMP production activates cGMP-dependent protein kinase (PKG). PKG induces the phosphorylation of phosphodiesterases that downregulate cGMP production. Inhibition of phosphodiesterases by a PDE5 inhibitor such as sildenafil enhances NO-induced vasorelaxation by increasing vascular smooth muscle cGMP concentration (14). Sildenafil causes vasodilation both in adult and neonatal pulmonary circulation (31, 34, 42, 45). Sildenafil may be useful in a newborn after cardiac surgery in association with iNO (37) and has been proposed for children with pulmonary hypertension. In our study, BAY 41–2272 infusion induced a more sustained pulmonary vasodilation than sildenafil infusion. This observation suggests that the therapeutic potential of BAY 41–2272 may be at least equivalent to sildenafil in the setting of severe pulmonary hypertension.

In conclusion, we found that BAY 41–2272 induces a potent and sustained vasorelaxant response in the fetal pulmonary circulation. The mechanism by which BAY 41–2272 causes pulmonary vasodilatation is independent of NO release. The vasodilator response to BAY 41–2272 is greater and more sustained than the previously studied fetal pulmonary vasodilators. We speculate that BAY 41–2272 could improve conditions associated with failure to circulatory adaptation at birth. However, further investigations are necessary to evaluate and examine the systemic effects of BAY 41–2272 in these pathological conditions.

ACKNOWLEDGMENTS

We thank Dr. J.-P. Stasch and Bayer Healthcare for providing the BAY 41–2272 compound.

GRANTS

This work was supported in part by National Institutes of Health Grants HL-68702 and HL-57144 to S. H. Abman, Bourse Lavoisier, Collège National des Gynécologues Obstétriciens Français, and Société Française de Médecine Périmatate (to P. Deruelle).

REFERENCES


