Soluble Guanylate Cyclase Activator Reverses Acute Pulmonary Hypertension and Augments the Pulmonary Vasodilator Response to Inhaled Nitric Oxide in Awake Lambs

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Background—Inhaled nitric oxide (NO) is a potent and selective pulmonary vasodilator, which induces cGMP synthesis by activating soluble guanylate cyclase (sGC) in ventilated lung regions. Carbon monoxide (CO) (reviewed in Ichinose et al1 ) also activates sGC and relaxes vascular smooth muscle (reviewed in Ryter and Otterbein2 ), though to a lesser degree. Analogs of CO to the perfusate increases lung cGMP content and improves cardiac output (PH) of various etiologies. Inhaled NO produces selective pulmonary vasodilation in well-ventilated lung regions by activating soluble guanylate cyclase (sGC) and increasing the synthesis of cGMP in pulmonary vascular smooth muscle cells (reviewed in Ichinose et al1 ). Carbon monoxide (CO) also activates sGC and relaxes vascular smooth muscle (reviewed in Ryter and Otterbein2 ), though to a lesser degree than does NO.3,4 In isolated rat lungs, addition of exogenous CO to the perfusate increases lung cGMP content and reverses pulmonary vasoconstriction induced by U-46619, a stable endoperoxide analogue of thromboxane A2.5

Direct pharmacological stimulators of sGC have been recently developed based on the indazole derivative YC-1, an NO-independent and heme-dependent sGC activator, which also sensitizes sGC to NO and inhibits phosphodiesterase-5 (PDE5).3,6 In addition, in the presence of YC-1, CO stimulates purified sGC to a similar extent as does NO.7 BAY 41-2272 {3-(4-amino-5-cyclopropylpyrimidine-2-yl)-1-(2-fluorobenzyl)-1H-pyrazolo[3,4-b]pyridine} is 100-fold more potent than YC-1 in stimulating sGC in vitro. Concentrations of BAY 41-2272 as low as 10 to 100 nmol/L greatly enhance the sensitivity of purified sGC to NO.8 Whereas in initial studies BAY 41-2272 did not inhibit PDE5 in concentrations up to 10 μmol/L,6,8 a more recent report has shown that BAY 41-2272 inhibits PDE5 with an IC50 of 3 μmol/L.9 Administration of BAY 41-2272 decreases mean arterial pressure (MAP) in spontaneously hypertensive rats and produces systemic and renal vasodilation in dogs with congestive heart failure.9,10 However, its effects on the pulmonary circulation remain to be fully elucidated.

The aim of the present study was to test the hypothesis that direct stimulation of sGC by BAY 41-2272 would produce pulmonary vasodilation and augment the pulmonary responses to inhaled NO or CO.

Methods and Results—In awake, instrumented lambs, the thromboxane analogue U-46619 was intravenously administered to increase mean pulmonary arterial pressure to 35 mm Hg. Intravenous infusion of BAY 41-2272 (0.03, 0.1, and 0.3 mg · kg⁻¹ · h⁻¹) reduced mean pulmonary arterial pressure and pulmonary vascular resistance and increased transpulmonary cGMP release in a dose-dependent manner. Larger doses of BAY 41-2272 also produced systemic vasodilation and elevated the cardiac index. Nω-nitro-L-arginine methyl ester abolished the systemic but not the pulmonary vasodilator effects of BAY 41-2272. Furthermore, infusing BAY 41-2272 at 0.1 mg · kg⁻¹ · h⁻¹ potentiated and prolonged the pulmonary vasodilation induced by inhaled NO (2, 10, and 20 ppm). In contrast, inhaled CO (50, 250, and 500 ppm) had no effect on U-46619—induced pulmonary vasoconstriction before or during administration of BAY 41-2272.

Conclusions—In lambs with acute pulmonary hypertension, BAY 41-2272 is a potent pulmonary vasodilator that augments and prolongs the pulmonary vasodilator response to inhaled NO. Direct pharmacological stimulation of sGC, either alone or in combination with inhaled NO, may provide a novel approach for the treatment of pulmonary hypertension. (Circulation. 2004;110:2253-2259.)

Key Words: hypertension, pulmonary ▪ enzymes ▪ vasodilation ▪ nitric oxide ▪ hemodynamics
pulmonary vasodilation in lambs with pharmacologically induced acute PH. We also sought to examine whether or not the drug would alter pulmonary vasodilator responsiveness to the inhalation of NO or CO.

Methods

Our investigation conformed to the Guide for the Care and Use of Laboratory Animals published by the US National Institutes of Health and was approved by the Subcommittee on Research Animal Care of the Massachusetts General Hospital.

Instrumentation and Hemodynamic Measurements

Twenty-seven lambs weighing 19.0±0.4 kg (mean±SEM) were anesthetized with an intramuscular injection of ketamine (15 mg/kg) and supplemental intravenous injections of thiopental (2 to 4 mg/kg). The animals were instrumented with a 7F pulmonary thermal dilution catheter (Edwards Lifesciences), a polyvinyl chloride catheter (1.5-mm internal diameter) in the left common carotid artery, and an 8-mm cuffed tracheostomy tube (SIMS Portex), as described previously. After emergence from anesthesia, the lambs were allowed at least 3 hours for recovery. Cardiac output, MAP, mean pulmonary arterial pressure (PAP), pulmonary capillary wedge pressure (PCWP), and central venous pressure (CVP) were measured using the methods described below (data not shown). With the use of volumetrically calibrated flowmeters, NO gas (800 ppm in N2, INO Therapeutics Inc) or CO gas (1000 ppm in N2, BOC Gases) was administered through a custom-made breathing circuit, as described previously. The inspired gas concentrations were monitored, and FiO2 was maintained at 0.5. The NO concentration in ambient air was 4 ppb.

Experimental Protocols

During the experiments, the lambs were awake and breathed spontaneously while receiving an intravenous infusion of lactated Ringer’s solution (10 mL·kg⁻¹·h⁻¹). All measurements and samples were obtained at baseline and before and at the end of each treatment.

After baseline measurements in 7 lambs, PAP was increased to 35 mm Hg by an intravenous infusion of U-46619 (dissolved in lactated Ringer’s solution; 1.2±0.1 μg · kg⁻¹ · min⁻¹, Cayman Chemical). After a 30-minute stabilization period, the lambs received BAY 41-2272 (Alexis Biochemicals) dissolved in dimethyl sulfoxide/Cremophor EL (Sigma-Aldrich)/saline (2/4/94, vol/vol/vol). BAY 41-2272 was infused intravenously in incremental doses of 0.03, 0.1, and 0.3 mg · kg⁻¹ · h⁻¹ for 30 minutes at each dose. In pilot experiments, the vehicle had no hemodynamic effects. After reconstitution in the vehicle, BAY 41-2272 concentrations were found to be stable for at least 24 hours at 22°C, as measured using the methods described below (data not shown). Eight lambs received N⁵-nitro-L-arginine methyl ester (L-NNAME) dissolved in saline and administered intravenously (25 mg/kg plus 5 mg · kg⁻¹ · h⁻¹). The dose of L-NNAME was chosen because similar doses inhibited the systemic vasodilator responses to administrations of acetylcholine, sildenafil, or endotoxin in sheep. In L-NNAME–treated lambs, PAP was increased to 35 mm Hg with an intravenous infusion of U-46619 (0.4±0.1 μg · kg⁻¹ · min⁻¹). After a 30-minute period of stable PH, BAY 41-2272 was infused intravenously at 0.1 mg · kg⁻¹ · h⁻¹ for 30 minutes. In 8 lambs, PAP was increased to 35 mm Hg with an intravenous infusion of U-46619 (1.2±0.1 μg · kg⁻¹ · min⁻¹). After a 30-minute period of stable PH, NO gas was administered via inhalation in random sequence at 2, 10, and 20 ppm. At each dose level, NO was inhaled for 10 minutes followed by a 15-minute NO-free period. All hemodynamic parameters returned to pretreatment values during the latter period. A continuous intravenous infusion of BAY 41-2272 at 0.1 mg · kg⁻¹ · h⁻¹ was then commenced. Thirty minutes later, the dose of U-46619 was adjusted to 2.0±0.1 μg · kg⁻¹ · min⁻¹ to increase PAP to 35 mm Hg. After a 30-minute stabilization period, NO was inhaled again at the same concentrations and in the same order as given before BAY 41-2272. In 4 lambs, PAP was increased to 35 mm Hg with an intravenous infusion of U-46619 (1.1±0.1 μg · kg⁻¹ · min⁻¹). After a 30-minute period of stable PH, NO gas was administered via inhalation in random sequence at 50, 250, and 500 ppm. At each dose level, CO was inhaled for 10 minutes followed by a 15-minute CO-free period. A continuous intravenous infusion of BAY 41-2272 at 0.1 mg · kg⁻¹ · h⁻¹ was then commenced. Thirty minutes later, the dose of U-46619 was adjusted to 1.9±0.1 μg · kg⁻¹ · min⁻¹ to increase PAP to 35 mm Hg. After a 30-minute stabilization period, CO was inhaled again at the same concentrations and in the same order as given before BAY 41-2272.

Blood Gases and Transpulmonary cGMP Release

Blood samples were simultaneously obtained (at baseline and at the end of each treatment) from the carotid and pulmonary arteries and analyzed for pH, P0₂, PCO₂, O₂ saturation, and the concentrations of hemoglobin, methemoglobin, and carboxyhemoglobin. The alveolar-arterial oxygen tension gradient [P(A-a)O₂] and venous admixture (Qs/Qt) were calculated using standard equations. In addition, blood was collected in sample tubes containing sodium citrate and 3-isobutyl-1-methylxanthine (0.5 mmol/L final concentration). The mixture was centrifuged at 4°C (200g, 10 minutes). Arterial and mixed venous plasma concentrations of cGMP were determined by radioimmunoassay (BT-300, Biomedical Technologies). The quantity of cGMP released by the lung during each treatment was calculated as the product of cardiac output times the difference between the arterial and mixed venous plasma cGMP concentrations.

Measurement of BAY 41-2272 Concentrations

Samples were subjected to high-performance liquid chromatography performed on a 2300 HTLC system (Cohesive Technologies) with a Lichrocart Purospher Star RP-18e column (55×4 mm internal diameter, 3 μm particle size; Merck KGaA) at a flow rate of 1 mL/min. The mobile phase consisted of 10 mmol/L ammonium acetate (pH 3.0) and acetonitrile. A linear gradient from 20% to 85% acetonitrile (vol/vol) within 1 minute was applied. Tandem mass spectrometry was performed on an API 3000 triple-quadrupole mass spectrometer (PE Sciex) connected to the 2300HTLC system through a Turboionspray interface. The lower limit for quantification of BAY 41-2272 was 0.5 μg/L.

Data Analysis

Data are expressed as mean±SEM. The half-time of the reversal of pulmonary vasodilation (t½) was determined by measuring the elapsed time from the termination of NO inhalation to the time when the PAP had returned to a value halfway between the value recorded at the end of NO inhalation and baseline PH. Percent changes of selected hemodynamic variables were calculated as the difference between the baseline PH value and the value recorded at the end of each treatment divided by the baseline PH value. The effects of each BAY 41-2272 dose and the effects of NO or CO inhaled alone or during BAY 41-2272 administration were tested with a repeated-measures ANOVA. A Dunnett adjustment was used for comparisons with baseline PH, and a Tukey adjustment was applied for between-group comparisons. A paired t test with a Bonferroni adjustment was used to compare percent changes of selected hemodynamic variables. Linear regression analysis was used to test the trends between the doses or the plasma concentrations of BAY 41-2272 and the outcomes. A value of P<0.05 was considered statistically significant.

Results

Effects of Incremental Doses of BAY 41-2272

As shown in Table 1 and Figure 1, intravenous infusion of incremental doses of BAY 41-2272 (30 minutes each) re-
duced PAP and PVRI in a dose-dependent manner ($P<0.001$). The maximal level of pulmonary vasodilation was attained within $\approx 15$ minutes after the start of each dose. When infused at 0.1 and 0.3 mg $\cdot$ kg$^{-1}$ $\cdot$ h$^{-1}$, BAY 41-2272 also decreased PCWP, MAP, SVRI, and PVRI/SVRI and increased CI and SVI ($P<0.05$). At all dose levels, BAY 41-2272 induced greater percent reductions of PAP and PVRI than of MAP and SVRI ($P<0.01$). In addition, BAY 41-2272 infused at 0.3 mg $\cdot$ kg$^{-1}$ $\cdot$ h$^{-1}$ decreased RVSWI and P(A-a)O$_2$ and increased PaO$_2$/FiO$_2$ ($P<0.05$). No significant changes in CVP and HR were noted during administration of BAY 41-2272 (data not shown). BAY 41-2272 significantly in-

### Table 1. Effects of Incremental Doses of Intravenously Infused BAY 41-2272 in Lambs With U-46619–Induced Acute PH

<table>
<thead>
<tr>
<th>Variable</th>
<th>Baseline PH</th>
<th>0.03</th>
<th>0.1</th>
<th>0.3</th>
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<tbody>
<tr>
<td>PAP</td>
<td>34.9±0.6</td>
<td>32.0±0.9*</td>
<td>28.7±1.0*</td>
<td>24.1±0.9*</td>
</tr>
<tr>
<td>PCWP</td>
<td>13.6±0.6</td>
<td>12.7±0.6</td>
<td>11.0±0.4*</td>
<td>9.9±0.4*</td>
</tr>
<tr>
<td>CI</td>
<td>4.3±0.2</td>
<td>4.8±0.2</td>
<td>5.3±0.2*</td>
<td>6.1±0.2*</td>
</tr>
<tr>
<td>PVRI</td>
<td>400±11</td>
<td>324±20*</td>
<td>273±22*</td>
<td>190±14*</td>
</tr>
<tr>
<td>MAP</td>
<td>109±3</td>
<td>108±3</td>
<td>102±3*</td>
<td>96±3*</td>
</tr>
<tr>
<td>SVRI</td>
<td>1874±90</td>
<td>1668±97</td>
<td>1423±93*</td>
<td>1141±48*</td>
</tr>
<tr>
<td>PVRI/SVRI</td>
<td>0.22±0.01</td>
<td>0.20±0.01</td>
<td>0.19±0.01*</td>
<td>0.17±0.01*</td>
</tr>
<tr>
<td>SVI</td>
<td>36.2±2.6</td>
<td>39.0±2.3</td>
<td>40.4±2.3*</td>
<td>43.6±2.7*</td>
</tr>
<tr>
<td>RSVWI</td>
<td>12.1±1.0</td>
<td>11.5±0.9</td>
<td>10.3±0.8</td>
<td>9.8±0.6*</td>
</tr>
<tr>
<td>Pao$_2$/FiO$_2$</td>
<td>306±49</td>
<td>292±47</td>
<td>373±54</td>
<td>405±30*</td>
</tr>
<tr>
<td>P(A-a)O$_2$</td>
<td>169±25</td>
<td>177±24</td>
<td>138±26</td>
<td>120±15*</td>
</tr>
<tr>
<td>O$_2$/Qt</td>
<td>0.14±0.02</td>
<td>0.15±0.03</td>
<td>0.14±0.02</td>
<td>0.13±0.01</td>
</tr>
<tr>
<td>Plasma BAY 41–2272</td>
<td>NA</td>
<td>2.0±0.3*</td>
<td>6.5±0.6*</td>
<td>22.5±3.4*</td>
</tr>
</tbody>
</table>

MAP is measured in mm Hg; PCWP, in mm Hg; CI, in L $\cdot$ min$^{-1}$ $\cdot$ m$^{-2}$; PVRI, in dyne $\cdot$ s $\cdot$ cm$^{-5}$ $\cdot$ m$^{-2}$; MAP, in mm Hg; SVRI, in dyne $\cdot$ s $\cdot$ cm$^{-5}$ $\cdot$ m$^{-2}$; SVI, in mL $\cdot$ beat$^{-1}$ $\cdot$ m$^{-2}$; RVSWI, in g $\cdot$ m $\cdot$ m$^{-2}$; Pao$_2$/FiO$_2$, in mm Hg; P(A-a)O$_2$, in mm Hg; and plasma concentration of BAY 41-2272, in $\mu$g/L. Data are mean±SEM, n=7. Abbreviations are as defined in text.

$*P<0.05$ vs baseline PH.

Figure 1. Percent changes (Δ) of MAP, PAP, SVRI, and PVRI; transpulmonary cGMP release; and relations between plasma BAY 41-2272 concentrations and ΔMAP, ΔPAP, ΔSVRI, ΔPVRI, or transpulmonary cGMP release during intravenous infusion of incremental doses of BAY 41-2272 in lambs with U-46619–induced acute PH. Data are mean±SEM (n=7) or individual values; $*P<0.05$ vs baseline PH; †$P<0.01$ vs ΔMAP at corresponding dose of BAY 41-2272; ‡$P<0.01$ vs ΔSVRI at corresponding dose of BAY 41-2272. Abbreviations are as defined in text.
creased transpulmonary cGMP release beginning at a dose of 0.1 mg · kg⁻¹ · h⁻¹ (P<0.001). The plasma BAY 41-2272 levels were correlated with transpulmonary cGMP release (r=0.65, P<0.05), as well as with the percent reductions of PAP (r=0.86, P<0.001), PVRI (r=0.83, P<0.001), MAP (r=0.59, P<0.05), and SVRI (r=0.7, P<0.05).

Effects of Combined Administration of BAY 41-2272 and L-NAME
As shown in Figure 2, BAY 41-2272 infused at 0.1 mg · kg⁻¹ · h⁻¹ markedly reduced PAP, PVRI, MAP, and SVRI (P<0.05). Pretreatment with L-NAME abolished the systemic but not the pulmonary vasodilation induced by BAY 41-2272. Furthermore, L-NAME had no effect on the ability of BAY 41-2272 to augment transpulmonary cGMP release, which increased from 8.1±1.8 to 14.4±2.5 μmol/min after BAY 41-2272 alone (P<0.05) and from 6.4±1.4 to 14.1±1.7 μmol/min after administration of the combination of L-NAME and BAY 41-2272 (P<0.05).

Effects of NO Inhaled Alone or in Combination With BAY 41-2272
As shown in Table 2 and Figure 3, inhaled NO produced a dose-dependent, selective pulmonary vasodilation. After NO was discontinued, PAP rapidly returned to baseline PH with a t½ <1 minute. Inhaled NO at 20 ppm also increased PaO₂/FIO₂ and reduced P(A-a)O₂ and Qs/Qt (P<0.05). Inhalation of 10 and 20 ppm NO increased transpulmonary cGMP release (P<0.01), whereas there were no significant changes of arterial methemoglobin concentrations (data not shown).

Administration of BAY 41-2272 markedly enhanced the reductions of PAP, PVRI, and PVRI/SVRI induced by inhaled NO (P<0.05). After NO was discontinued, the persistence of the pulmonary vasodilation (as reflected by t½)
during BAY 41-2272 infusion was greater than that before the infusion ($P<0.05$). In the presence of BAY 41-2272, NO inhaled at 10 and 20 ppm produced minor reductions of PCWP and SVRI and increments in CI and SVI ($P<0.05$ versus baseline PH) and reduced RVSWI to a greater extent than did inhaled NO alone ($P<0.05$), whereas MAP, HR, and CVP remained unchanged (data for HR and CVP not shown). Moreover, during BAY 41-2272 infusion, inhalation of 10 and 20 ppm NO augmented $\text{PaO}_2/\text{FiO}_2$ and reduced $\text{P(A-a)O}_2$ and $\text{Qs/Qt}$ ($P<0.01$ versus baseline PH). The coadministration of BAY 41-2272 and inhaled NO increased transpulmonary cGMP release to a greater extent than did inhaled NO alone ($P<0.05$). During the period of NO administrations, the mean plasma BAY 41-2272 concentrations remained at a stable level.

**Effects of CO Inhaled Alone or in Combination With BAY 41-2272**

As illustrated in Figure 4 and the online-only Data Supplement Table, inhalation of CO alone or after administration of BAY 41-2272 had no vasodilator effect on the U-46619–induced pulmonary vasoconstriction. Systemic hemodynamics, lung gas exchange, and transpulmonary cGMP release were also unchanged by CO inhalation. Arterial concentrations of carboxyhemoglobin gradually rose from 1.0±0.2% to 4.7±0.4% ($P<0.01$) and from 1.4±0.1% to 5.2±0.2% ($P<0.01$), respectively, after breathing 500 ppm CO alone or in combination with BAY 41-2272. The mean plasma levels of BAY 41-2272 remained stable during the period of CO administrations.

**Discussion**

Our study revealed that in conscious lambs, intravenous administration of the novel sGC activator BAY 41-2272 counteracted the U-46619–induced acute PH and increased transpulmonary cGMP release. Furthermore, BAY 41-2272 both enhanced and prolonged the pulmonary vasodilator response to inhaled NO. In contrast, CO inhaled alone or during the infusion of BAY 41-2272 did not attenuate acute PH but produced carboxyhemoglobinemia.

Consistent with its ability to directly activate sGC,$^{6,8}$ BAY 41-2272 caused dose-dependent reductions of PAP and PVRI, as well as a marked increase of pulmonary cGMP release into arterial blood. Although higher doses of BAY 41-2272 also induced systemic vasodilation, the pulmonary vasodilatory effects prevailed: PVRI/SVRI decreased, and the percent reductions of PAP and PVRI were significantly greater than the corresponding changes in MAP and SVRI. Although it is likely that the increments in SVI and CI induced by BAY 41-2272 were due to reduced right and left ventricular afterload, as well as enhanced venous return, a positive inotropic effect of BAY 41-2272 cannot be excluded.$^{10}$ Plasma BAY 41-2272 levels were correlated with the magnitude of the pulmonary and systemic vasodilation, as well as the rate of transpulmonary cGMP release. Our findings support the concept of using direct activators of sGC in therapy for PH. However, an important limitation of these drugs is systemic hypotension, similar to what we observed with the intravenous infusion of high doses of BAY 41-2272 in sheep. Further studies of the efficacy and toxicity of sGC activators in pulmonary vascular disorders are warranted.

Pretreatment with the NO synthase inhibitor L-NAME had no effect on the pulmonary vasodilation or transpulmonary cGMP release induced by BAY 41-2272. These findings suggest that, in the pulmonary vasculature, BAY 41-2272 acts independently of endogenous NO production, and they are consistent with the ability of this agent to directly stimulate sGC.$^{6,8}$ In contrast, L-NAME abolished the systemic vasodilation induced by BAY 41-2272, suggesting that endogenous NO is required for the systemic vasodilator response to BAY 41-2272. The mechanisms responsible for the difference in the systemic and pulmonary vasomotor responses to BAY 41-2272 in the presence of L-NAME are unknown and merit further investigation.

The vasodilator effects of BAY 41-2272 were similar to those we previously reported for sildenafil, a PDE5 inhibitor,
in our lamb model of PH. Recently, Mullershausen and colleagues have proposed that, in addition to sGC stimulation, the vasodilator effects of BAY 41-2272 may be attributable to PDE5 inhibition with IC\textsubscript{50} values of 3 \(\mu\)mol/L. However, the plasma concentrations of BAY 41-2272 that were achieved in sheep with infusion at the highest drug dose (62±10 \(\mu\)mol/L) appear to be too low to inhibit PDE5. Moreover, the pulmonary vasodilator effects of BAY 41-2272 were not inhibited by L-NAME, whereas we previously found that pretreatment with L-NAME completely blocked the pulmonary vasodilation produced by sildenafil. Taken together, it is unlikely that PDE5 inhibition can account for the pulmonary vasodilation induced by BAY 41-2272 in the present investigation.

Inhaled NO selectively dilates the pulmonary vasculature and improves oxygenation by reducing intrapulmonary blood shunting, particularly in lung injury. Limitations of NO inhalation as long-term therapy for PH include the short duration of pulmonary vasodilation after NO is discontinued, the development of methemoglobinemia after inhalation of high concentrations of NO gas, and the observation that not all PH patients respond to inhaled NO. We hypothesized that agents that sensitize sGC to NO would augment and prolong the pulmonary vasodilator effects of inhaled NO. We observed that infusion of BAY 41-2272 enhanced and prolonged the pulmonary vasodilator response to inhaled NO and was associated with a further increase in transpulmonary cGMP release. Moreover, BAY 41-2272 enabled a lower concentration of NO (10 ppm) to augment systemic oxygenation, suggesting that the drug sensitized the pulmonary vascular sGC to NO gas, reaching well-ventilated lung areas and thereby improving the matching of ventilation and perfusion. Our findings suggest the possibility that in the clinical setting, the ability of BAY 41-2272 to augment the efficacy of inhaled NO may result in an increased number of patients with PH responding to low concentrations of NO. In addition, prolongation of the vasodilator effects of NO by BAY 41-2272 may potentially facilitate long-term therapy with intermittently inhaled NO. Moreover, in patients with ventilation-perfusion mismatch, such as that associated with acute lung injury, administration of BAY 41-2272 may augment the ability of inhaled NO to increase systemic oxygenation.

Investigations with purified enzyme and isolated platelets have suggested that YC-1 markedly enhances the ability of CO, as well as of NO, to activate sGC. To examine the ability of direct sGC activators to augment the vasodilator response to CO in vivo, we evaluated inhalation of CO in the presence or absence of BAY 41-2272. In agreement with a recent study in fetal lambs, we found no changes in PAP, PVRI, or the rate of transpulmonary cGMP release in lambs with U-46619-induced acute PH (n=4). Values for NO inhalation alone (NO group) or in combination with BAY 41-2272 (BAY+NO group) are added for comparison (n=8). Data are mean±SEM; *\(P<0.01\) vs baseline PH. Abbreviations are as defined in text.

Figure 4. Percent changes (\(\Delta\)) of PAP and PVRI and transpulmonary cGMP release during CO inhalation alone (CO group) or in combination with BAY 41-2272 (BAY+CO group) in lambs with U-46619-induced acute PH (n=4). Values for NO inhalation alone (NO group) or in combination with BAY 41-2272 (BAY+NO group) are added for comparison (n=8). Data are mean±SEM; *\(P<0.01\) vs baseline PH. Abbreviations are as defined in text.
inhaling 50 to 500 ppm CO. Moreover, coadministration of BAY 41-2272 at a dose sufficient to augment the pulmonary vasodilator response to inhaled CO did not enable CO to dilate the pulmonary vasculature. Although we did not observe any toxic effects of breathing CO, a 10-minute inhalation of 500 ppm CO increased circulating carboxyhemoglobin concentrations to ≈5%. Thus, if observations in sheep can be extrapolated to humans, inhalation of CO at safe concentrations, alone or in combination with BAY 41-2272 is unlikely to be an effective therapeutic approach to PH. However, it remains possible that BAY 41-2272 may enhance other benefits of exogenous CO, such as an antiinflammatory effect.217

In conclusion, BAY 41-2272 reverses acute PH in lambs, most likely through an NO synthase–independent increase of pulmonary cGMP production. Moreover, BAY 41-2272 administration augments and prolongs the pulmonary vasodilator response to inhaled NO. In contrast, inhalation of CO fails to attenuate acute PH, even in the presence of BAY 41-2272. The present study provides strong evidence that the direct pharmacological stimulators of sGC, either alone or in combination with exogenous NO, may be an effective therapeutic intervention in PH.

Acknowledgments
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Disclosure
The Massachusetts General Hospital has licensed patents covering the inhalation of nitric oxide to INO Therapeutics Inc, a division of Linde Gas Therapeutics, and Dr Zapol receives a portion of the royalties. Drs Zapol and Bloch are members of the Scientific Advisory Board of INO Therapeutics Inc.

References
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ONLINE DATA SUPPLEMENT

A Soluble Guanylate Cyclase Activator Reverses Acute Pulmonary Hypertension and Augments the Pulmonary Vasodilator Response to Inhaled Nitric Oxide in Awake Lambs

Table 3. Effects of Inhaled Carbon Monoxide (CO) Administered Alone or in Combination with Intravenously-Infused BAY 41-2272 in Lambs with U-46619-induced Acute Pulmonary Hypertension (PH).

<table>
<thead>
<tr>
<th>Variable</th>
<th>Intervention</th>
<th>Baseline PH</th>
<th>Inhaled CO (ppm)</th>
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</thead>
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<td></td>
<td></td>
<td></td>
<td>50</td>
</tr>
<tr>
<td>PAP</td>
<td>CO</td>
<td>2330±130</td>
<td>2215±168</td>
</tr>
<tr>
<td></td>
<td>BAY+CO</td>
<td>2199±141</td>
<td>2149±127</td>
</tr>
<tr>
<td>PCWP</td>
<td>CO</td>
<td>482±32</td>
<td>476±41</td>
</tr>
<tr>
<td></td>
<td>BAY+CO</td>
<td>479±21</td>
<td>469±29</td>
</tr>
<tr>
<td>CI</td>
<td>CO</td>
<td>44.3±4.7</td>
<td>43.0±4.5</td>
</tr>
<tr>
<td></td>
<td>BAY+CO</td>
<td>43.0±5.3</td>
<td>42.0±5.7</td>
</tr>
<tr>
<td>PVRI/SVRI</td>
<td>CO</td>
<td>0.21±0.01</td>
<td>0.21±0.01</td>
</tr>
<tr>
<td></td>
<td>BAY+CO</td>
<td>0.22±0.01</td>
<td>0.22±0.01</td>
</tr>
<tr>
<td>SVI</td>
<td>CO</td>
<td>15.2±1.0</td>
<td>14.5±1.3</td>
</tr>
<tr>
<td></td>
<td>BAY+CO</td>
<td>14.2±1.7</td>
<td>14.4±1.9</td>
</tr>
<tr>
<td>RVSWI</td>
<td>CO</td>
<td>346±53</td>
<td>345±60</td>
</tr>
<tr>
<td></td>
<td>BAY+CO</td>
<td>360±54</td>
<td>347±44</td>
</tr>
<tr>
<td>PaO2/FiO2</td>
<td>CO</td>
<td>154±26</td>
<td>156±29</td>
</tr>
<tr>
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<td>BAY+CO</td>
<td>147±27</td>
<td>152±22</td>
</tr>
<tr>
<td>P(A-a)O2</td>
<td>CO</td>
<td>0.12±0.02</td>
<td>0.12±0.03</td>
</tr>
<tr>
<td></td>
<td>BAY+CO</td>
<td>0.12±0.02</td>
<td>0.11±0.01</td>
</tr>
<tr>
<td>Qs/Qt</td>
<td>BAY+CO</td>
<td>7.9±0.7</td>
<td>8.4±2.0</td>
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</table>
CO group, inhaled CO alone; BAY+CO group, a combination of intravenously infused BAY 41-2272 and inhaled CO; PAP, mean pulmonary arterial pressure (mmHg); PCWP, pulmonary capillary wedge pressure (mmHg); CI, cardiac index (l·min⁻¹·m⁻²); PVRI, pulmonary vascular resistance index (dyne·sec·cm⁻⁵·m⁻²); MAP, mean arterial pressure (mmHg); SVRI, systemic vascular resistance index (dyne·sec·cm⁻⁵·m⁻²); PVRI/SVRI, PVRI-to-SVRI ratio; SVI, stroke volume index (ml·beat⁻¹·m⁻²); RVSWI, right ventricle stroke work index (g·m·m⁻²); PaO₂/FiO₂, ratio of arterial oxygen tension-to-inspired oxygen fraction (mmHg); P(A-a)O₂, alveolar-arterial oxygen tension gradient (mmHg); Qs/Qt, venous admixture; Plasma BAY, plasma concentration of BAY 41-2272 (µg/L). Data are means ± SEM, n = 4.