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Effects of Intravenous Zaprinast and Inhaled Nitric Oxide on Pulmonary Hemodynamics and Gas Exchange in an Ovine Model of Acute Respiratory Distress Syndrome

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Background: Inhaled nitric oxide (NO) selectively dilates the pulmonary vasculature and improves gas exchange in acute respiratory distress syndrome. Because of the very short half-life of NO, inhaled NO is administered continuously. Intravenous Zaprinast (2-o-propoxyphenyl-8-azapurin-6-one), a cyclic guanosine monophosphate phosphodiesterase inhibitor, increases the efficacy and prolongs the duration of action of

inhaled NO in models of acute pulmonary hypertension. Its efficacy in lung injury models is uncertain. The authors hypothesized that the use of intravenous Zaprinast would have similar beneficial effects when used in combination with inhaled NO to improve oxygenation and dilate the pulmonary vasculature in a diffuse model of acute lung injury.

Methods: The authors studied two groups of sheep with lung injury produced by saline lavage. In the first group, 0, 5, 10, and 20 ppm of inhaled NO were administered in a random order before and after an intravenous Zaprinast infusion (2 mg/kg bolus followed by 0.1 mg · kg⁻¹ · min⁻¹). In the second group, inhaled NO was administered at the same concentrations before and after an intravenous infusion of Zaprinast solvent (0.05 NaOH).

Results: After lavage, inhaled NO decreased pulmonary arterial pressure and resistance with no systemic hemodynamic effects, increased arterial oxygen partial pressure, and decreased venous admixture (all $P < 0.05$). The intravenous administration of Zaprinast alone decreased pulmonary artery pressure but worsened gas exchange ($P < 0.05$). Zaprinast infusion abolished the beneficial ability of inhaled NO to improve pulmonary gas exchange and reduce pulmonary artery pressure ($P < 0.05$ vs. control).

Conclusions: This study suggests that nonselective vasodilation induced by intravenously administered Zaprinast at the dose used in our study not only worsens gas exchange, but also abolishes the beneficial effects of inhaled NO. (Key words: Lavage lung injury; phosphodiesterase inhibitor; pulmonary circulation; sheep.)

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THE acute respiratory distress syndrome is characterized by intrapulmonary shunting that results in arterial hypoxemia¹ and by acute pulmonary arterial hypertension² caused by vasoconstriction and widespread occlusion of the pulmonary microvasculature.² Pulmonary arterial hypertension contributes to pulmonary edema³ and can cause right ventricular dysfunction.⁴ Vasodilators reduce pulmonary resistance and pulmonary capillary pressure,^{2,5,6} thereby improving right ventricular function and possibly promoting the resolution of pulmonary edema.⁵ However, intravenous vasodilators can induce systemic hypotension, sometimes producing right ventricular ischemia and heart failure,^{2,6,7} and because of

diffuse pulmonary vasodilation, can worsen ventilation-perfusion mismatching.^{6,8} Inhaled nitric oxide (NO) has been shown to selectively vasodilate ventilated lung regions and improve venous admixture (\dot{Q}_{VA}/\dot{Q}_T) in many models of pulmonary hypertension and lung injury.^{8,9}

Nitric oxide diffuses into vascular smooth muscle and mediates vasodilation by stimulating soluble guanylate cyclase to produce guanosine 3'-5' cyclic monophosphate (cGMP), thereby causing smooth muscle relaxation.¹⁰ In the circulation, NO is rapidly inactivated by hemoglobin, significantly restricting the hemodynamic effects of inhaled NO to the pulmonary vasculature.¹¹ Because of the very short biologic half-life of this molecule, inhaled NO needs to be administered continuously. The potential toxicity of NO and its metabolites (particularly in conjunction with increased fraction of inspired oxygen [F_{iO_2}] in injured lungs) is unknown and might restrict prolonged clinical use. In addition, many patients have a minimal or absent pulmonary vasodilator response to inhaled NO.¹² Devising new methods to increase responsiveness to inhaled NO might improve the efficacy of the therapy.

Zaprinast (M&B 22948; 2-*o*-propoxyphenyl-8-azapurin-6-one; Rhône-Poulenc Rorer, Dagenham, Essex, United Kingdom) selectively inhibits the hydrolysis of cGMP with minimal effects on the breakdown of adenosine 3',5'-cyclic monophosphate (cAMP) in vascular smooth muscle cells and in vascular rings.¹³⁻¹⁶ Zaprinast potentiates the vasodilator action of inhaled NO and intravenously administered nitrovasodilators in newborn lambs¹⁷ and in the isolated pulmonary lobar artery of the cat.¹⁸ Intravenous administration of Zaprinast prolongs the duration of action of inhaled NO in an ovine model of pulmonary hypertension induced by U46619.¹⁹ We hypothesized that the intravenous administration of Zaprinast might have similar effects when inhaled NO is coadministered in a lung injury model. Accordingly, the current study tested the effect of intravenous Zaprinast administration on the ability of inhaled NO to produce pulmonary vasodilation and alter gas exchange in anesthetized sheep after the induction of acute lung injury by lung lavage.

Materials and Methods

All studies were approved by the Massachusetts General Hospital Subcommittee on Research Animal Care. Suffolk lambs weighing 28-30 kg were anesthetized with pentobarbital sodium (30 mg/kg administered as an

intravenous bolus), their tracheas were intubated, and their lungs were mechanically ventilated at 15 breaths/min and 15 ml/kg tidal volume with a large animal respirator (Harvard Apparatus, Natick, MA). F_{iO_2} was continuously monitored (Hudson Ventronics Division, Temecula, CA) and was maintained at 0.9 throughout the experiment. Positive end-expiratory pressure of 5 cm H₂O was applied to the lungs. Anesthesia was maintained by continuous infusion of pentobarbital sodium (2 mg · kg⁻¹ · min⁻¹ intravenously), and muscular paralysis was obtained by administering pancuronium (0.1 mg/kg intravenously) every 2 h. Core body temperature was maintained at 37-39°C with an external heater.

Animal Preparation

A 7-French thermodilution pulmonary artery catheter (Edwards Laboratory, Santa Anna, CA) was placed *via* the right external jugular vein through an 8-French introducer (Cordis, Miami, FL) to measure pulmonary hemodynamics, cardiac output, core temperature, and to obtain mixed venous blood samples. The femoral artery was cannulated with a polyvinyl chloride catheter (8-mm ID), which was advanced 20 cm into the aorta for continuous arterial pressure monitoring and arterial blood sampling. A sterile left thoracotomy *via* the fifth intercostal space was performed to place a left atrial catheter using a 19-gauge catheter (Intracath; Deseret Medical, Inc., Sandy, UT). A tracheostomy was performed, and an 8.0-mm ID cuffed tracheostomy tube (Portex, Keene, NH) was inserted.

Hemodynamic Measurements and Calculations

Systemic arterial pressure, pulmonary arterial pressure (PAP), left atrial pressure, central venous pressure, and pulmonary capillary wedge pressure were measured continuously by calibrated pressure transducers (Cobe Laboratories, Lakewood, CO), zeroed at the midthorax level. After amplification of pressure signals (Model 7700; Hewlett Packard, Palo Alto, CA), the values were recorded on an eight-channel recorder (Western Graph Tec, Inc., Irvine, CA). Mean measurements were obtained at end expiration. Thermodilution cardiac output was measured using a cardiac output computer (Model 9520A; American Edwards Laboratories, Irvine, CA) as the average of three determinations after injection of 5 ml 0°C Ringer's lactate solution. Pulmonary vascular resistance and systemic vascular resistance were computed using standard formulas. Arterial (P_{aO_2} and P_{aCO_2}) and mixed venous (P_{vO_2} and P_{vCO_2}) blood gas (O_2 and

CO₂) tensions and pH were measured using a blood gas analyzer (Model 238; Ciba-Corning Diagnostic Ltd, Halstead, United Kingdom).

Lung Lavage Technique

Baseline data were collected after a 1-h stabilization period, which followed the thoracotomy. Bilateral lung lavages then were performed with 0.5% (vol/vol) polyoxyethylene-sorbitan monooleate (Tween 80; Sigma Chemical, St. Louis, MO) in 37°C saline in the following manner. The tracheostomy tube was replaced with a cuffed left double-lumen endotracheal tube (39 French; Sheridan Catheter Inc., Argyle, NY) modified to fit the sheep's trachea. After animals breathed at F_IO₂ 1.0 for 10 min, one lung was ventilated at 8 ml/kg while the opposite lung was deaerated for 5 min and then filled with 500 ml lavage solution and maintained in apnea for 5 min. To achieve a uniform distribution of lavage solution, the sheep's position was changed periodically from supine to lateral. The lavage fluid then was drained by gravity and suctioning. The lavaged lung was manually reinflated and after reexpansion was mechanically ventilated. The same procedure was performed for the opposite lung. Each lung was lavaged twice. At the end of the lavage periods, the double-lumen tube was exchanged for an 8.0-mm ID cuffed tracheostomy tube, and both lungs were ventilated simultaneously.

Experimental Protocol

The studies were performed in 15 healthy sheep that fulfilled the following exclusion criteria evaluated 1 h after thoracotomy and immediately before lung lavage: core temperature < 40.2°C, leukocyte count 3,500–10,000 cells/μl, and mean PAP < 20 mmHg. $\dot{Q}_{VA}/\dot{Q}_T \leq 17\%$ was considered normal for supine anesthetized sheep.²⁰ Two groups were studied (fig. 1): the Zaprinast group (n = 10) and the control group (n = 5).

In the Zaprinast group, after a 1-h stabilization period following the lung lavage procedure, inhaled NO at 1, 5, 10, and 20 ppm was administered in random order. At each dose, NO was administered for 10 min followed by a 10-min NO-free period. A loading dose of Zaprinast (2 mg/kg intravenously over 5 min) was then administered, followed by a continuous infusion (0.1 mg · kg⁻¹ · min⁻¹ intravenously). After a 30-min stabilization period, inhaled NO was administered at the same concentration and in the same order as before the Zaprinast infusion. Arterial and venous blood gas tensions were measured at baseline and then every 10 min. Arterial blood samples for the determination of plasma [cGMP] were withdrawn at baseline and at the end of each NO administration period in seven animals.

In the control group, after a 1-h stabilization period following the lung lavage procedure, inhaled NO at 1, 5, 10, and 20 ppm in random order was administered. At each dose, NO was administered for 10 min followed by a 10-min NO-free period. The Zaprinast solvent (NaOH 0.05 N in saline) then was administered at the same volume as used in the Zaprinast group (see above). After a 30-min stabilization period, inhaled NO was administered at the same concentrations and in the same order as before the solvent administration. Arterial and venous blood gas tensions were measured at baseline and then every 10 min. Arterial blood samples for the determination of plasma [cGMP] were withdrawn at baseline and at the end of each NO administration period in three animals.

Determination of Arterial Plasma Cyclic Guanosine Monophosphate Concentration

Cyclic guanosine monophosphate levels were determined using ¹²⁵I radioimmunoassay (Biomedical Technologies, Stoughton, MA) according to the methodology of Harper and Brooker.²¹ Briefly, 10 μl of 50 mM 3-isobutyl-1-methylxanthine (IBmx) was added to 1 ml of c

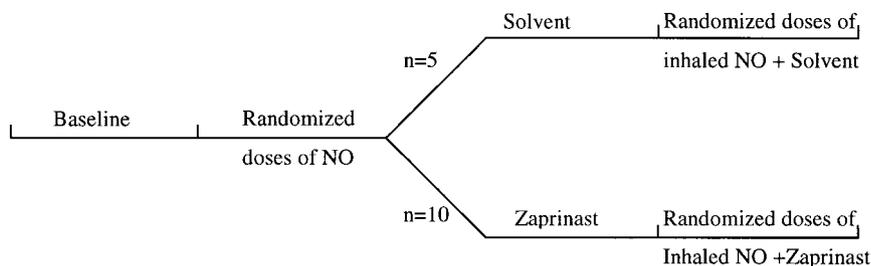


Fig. 1. Schema summarizing the experimental protocol. After a 1-h stabilization period (baseline) after the lung lavage procedure, inhaled NO at 1, 5, 10, and 20 ppm was administered in random order. A loading dose of Zaprinast followed by a continuous infusion (0.1 mg · kg⁻¹ · min⁻¹ intravenously; n = 10) or solvent alone (control group, n = 5) was then administered. After a 30-min stabilization period, inhaled NO was administered again at the same concentrations and in the same order as before the Zaprinast or solvent infusion.

trated blood, and the mixture was then centrifuged at 2,500g at 4°C for 10 min. The supernatant was diluted with acetate buffer and acetylated with an acetic anhydride and triethylamine mixture. Subsequently, cGMP concentrations in the samples were determined based on competitive binding of sample and known amounts of ¹²⁵I-labeled cGMP for a specific antibody. All measurements were performed in duplicate. Intraassay and inter-assay quality were controlled by measuring a known amount of cGMP. The cGMP concentration in the blood samples was expressed as picomoles per milliliter of plasma.

Drug Administration

Pentobarbital sodium (Schein, Port Washington, NY), pancuronium bromide (Gensia Pharmaceuticals, Irvine, CA), and Zaprinast (Rhône-Poulenc Rorer) were infused intravenously. Zaprinast was dissolved in saline with NaOH (0.05 N). NO was obtained from Airco (Murray Hill, NJ) as a mixture of 800 ppm NO in nitrogen. Less than 1% of the stock NO gas was present as NO₂. Using volumetrically calibrated flowmeters, varying quantities of NO mixed with N₂ were substituted for pure N₂ to keep the F_IO₂ constant at 0.9 throughout the experiment. The NO, N₂, and O₂ were mixed in a 5-l reservoir bag immediately before delivery at a fresh gas flow rate of 6 l/min to the ventilator. Inhaled NO concentration was continuously monitored by a chemiluminescence NO-NO_x analyzer (model 14A; Thermo Environmental Instruments, Franklin, MA). Exhaled gas was scavenged by continuous aspiration of the expiratory limb of the ventilator.

Statistical Analysis

The effects of inhaled NO were compared with the averaged steady state condition before and after each inhalation period. All values are expressed as mean ± SE. Linear regression analysis was used to examine the association between two variables. The data were analyzed using an analysis of variance for repeated measures followed by Student *t* test *post hoc* analysis. *P* < 0.05 was the criterion for statistical significance.

Results

Baseline Values

Lung lavage dramatically reduced the Pa_O₂ (from 504 ± 19 to 71 ± 9 mmHg) and increased Q_{VA}/Q_T (from 7.4 ± 1.1% to 46.5 ± 3.1%). Mean PAP and pulmonary vascular

resistance increased from 19 ± 1 to 22 ± 1 mmHg (*P* < 0.01) and from 233 ± 24 to 419 ± 54 dynes · s · cm⁻⁵ (*P* < 0.001), respectively. Mean systemic arterial pressure and systemic vascular resistance decreased (105 ± 3 vs. 86 ± 6 mmHg, *P* < 0.05, and 2,413 ± 148 vs. 2061 ± 191 dynes · s · cm⁻⁵, *P* = 0.06, respectively). Left atrial pressure, central venous pressure, cardiac output, and plasma [cGMP] did not change significantly after lung lavage. The baseline values before and after lung lavage were similar in both the control and Zaprinast groups (table 1).

Dose-Response Study of Intermittent Nitric Oxide Inhalation Alone

The effects of inhaled NO alone on gas exchange and hemodynamics were identical in the control and Zaprinast groups. After lung lavage, inhalation of NO increased Pa_O₂ and decreased Q_{VA}/Q_T (table 2). Inhaled NO also decreased pulmonary artery resistance without any effect on systemic vascular resistance. The maximum reduction of PAP was achieved at 5 ppm (table 2). Arterial plasma [cGMP] significantly increased in proportion to the administered dose of NO (fig. 2). Thus, there was no correlation between plasma cGMP levels and alterations of Pa_O₂, Q_{VA}/Q_T, or PAP.

Effect of Zaprinast Infusion

Compared with control, Zaprinast infusion significantly decreased pulmonary vascular resistance. Systemic vascular resistance seemed to decrease but without reaching statistical significance. Arterial oxygenation was significantly worsened by Zaprinast infusion. The Pa_O₂ decreased from 83 ± 16 to 52 ± 5 mmHg (*P* < 0.05) and Q_{VA}/Q_T increased from 41.8 ± 4.1% to 49.4 ± 2.1% (table 3; *P* < 0.05). Baseline plasma [cGMP] was increased during the infusion of Zaprinast from 15 ± 2 to 42 ± 8 pmol/ml (*P* < 0.01), whereas [cGMP] was unchanged during the infusion of Zaprinast solvent alone (fig. 2).

Dose-Response Study of Intermittent Nitric Oxide Inhalation during Zaprinast or Solvent Infusion

The Zaprinast infusion abolished all of the effects of NO inhalation on the measured gas exchange and hemodynamic variables (figs. 3 and 4). The infusion of the Zaprinast solvent did not alter the effects of inhaled NO.

Discussion

This study demonstrates that a continuous intravenous infusion of Zaprinast, a cGMP-specific phosphodiester-

Table 1. Baseline Values before and after Lung Lavage

	Control Group (n = 5)		Zaprinast Group (n = 10)	
	Before LL	After LL	Before LL	After LL
Pa _{O₂} (mmHg)	502 ± 13	60 ± 11†	504 ± 29	77 ± 13†
Q _{VA} /Q _T (%)	6.7 ± 1.1	49 ± 6.2†	7.7 ± 1.5	45 ± 3.6†
LAP (mmHg)	9 ± 1	9 ± 1	9 ± 1	10 ± 1
CVP (mmHg)	8 ± 1	9 ± 1	8 ± 1	9 ± 1
Mean PAP (mmHg)	18 ± 1	23 ± 1*	19.3 ± 1	22 ± 1*
CO (l/min)	3.1 ± 0.2	2.9 ± 0.4	3.5 ± 0.2	3.1 ± 0.2
PVR (dynes · s · cm ⁻⁵)	225 ± 27	422 ± 77*	237 ± 27	417 ± 30*
Mean SAP (mmHg)	99 ± 8	86 ± 8	108 ± 3	86 ± 9
SVR (dynes · s · cm ⁻⁵)	2,473 ± 280	2,121 ± 224	2,383 ± 183	2,203 ± 272
cGMP (pmol/ml)	22.2 ± 1.4 (n = 3)	26.5 ± 1.9	18.3 ± 3.1 (n = 7)	15.4 ± 1.5

There was no significant difference between the baseline values of both groups either before or after lung lavage. Data are expressed as mean ± SEM.

* $P < 0.05$.

† $P < 0.01$.

LL = lung lavage; Pa_{O₂} = arterial blood oxygen tension; Q_{VA}/Q_T = venous admixture; LAP = left atrial pressure; CVP = central venous pressure; PAP = pulmonary arterial pressure; SAP = systemic arterial pressure; CO = cardiac output; PVR = pulmonary vascular resistance; SVR = systemic vascular resistance; cGMP = arterial plasma cyclic guanosine monophosphate concentration.

ase inhibitor, produces diffuse vasodilation and exerted a detrimental effect on oxygenation in an ovine lung injury model. Rather than augmenting the localized vasodilatory effects of inhaled NO, intravenous Zaprinast, at the dose examined in this study, entirely abolished the beneficial effects of inhaled NO on oxygenation and PAP.

Inhaled NO produces selective pulmonary vasodilation in a variety of lung models and clinical scenarios of acute lung injury. Because of its route of administration and its very short duration of action, inhaled NO acts preferen-

tially on vasoconstricted pulmonary vessels, shifting perfusion from nonventilated to well-ventilated areas and thus reducing venous admixture. Importantly, a considerable number of patients who receive inhaled NO therapy do not respond by either pulmonary vasodilation or improvement of systemic oxygenation. The reported rate of nonresponse ranges from 30% to 45%, depending on the threshold value chosen to define hyporesponsiveness.^{12,22} Undoubtedly, many factors are important in determining the response to NO inhalation. Suggested factors include the presence of sepsis,^{12,23} the presence

Table 2. Effect of Inhaled Nitric Oxide on Hemodynamics and Gas Exchange

	Baseline after LL	NO			
		1 ppm	5 ppm	10 ppm	20 ppm
pH	7.44 ± 0.07	7.45 ± 0.06	7.46 ± 0.06	7.45 ± 0.06	7.46 ± 0.06
Pa _{CO₂} (mmHg)	33 ± 6	33 ± 7	32 ± 6	32 ± 7	32 ± 7
Pa _{O₂} (mmHg)	71 ± 12	84 ± 8	88 ± 9*	91 ± 8*	92 ± 10†
Q _{VA} /Q _T (%)	46.3 ± 4.5	42.5 ± 3.6	41.4 ± 3*	39 ± 3.1†	40 ± 3.1†
LAP (mmHg)	8 ± 1	9 ± 1	9 ± 1	9 ± 1	8 ± 1
CVP (mmHg)	9 ± 1	9 ± 1	9 ± 1	9 ± 1	9 ± 1
Mean PAP (mmHg)	23 ± 1	21 ± 1	20 ± 1*	19 ± 1*	18 ± 1†
PVR (dynes · s · cm ⁻⁵)	396 ± 46	325 ± 31	293 ± 26*	272 ± 27†	279 ± 26†
Mean SAP (mmHg)	90 ± 5	96 ± 4	96 ± 5	99 ± 3	95 ± 4
SVR (dynes · s · cm ⁻⁵)	2176 ± 256	2152 ± 201	2120 ± 224	2200 ± 337	2192 ± 342
CO (l/min)	3.1 ± 0.2	3.2 ± 0.1	3.0 ± 0.1	3.0 ± 0.1	3.1 ± 0.3
cGMP (pmol/ml)	17.2 ± 1.8	24.3 ± 3.5	33.5 ± 3.9*	47.7 ± 5.2†	64 ± 8.4†

First dose-response trials from both groups pooled (before Zaprinast or solvent administration, n = 15). Nitric oxide doses were administered in random order.

See table 1 for list of abbreviations.

* $P < 0.05$ versus baseline.

† $P < 0.01$ versus baseline.

INHALED NO AND ZAPRINAST IN OVINE ARDS

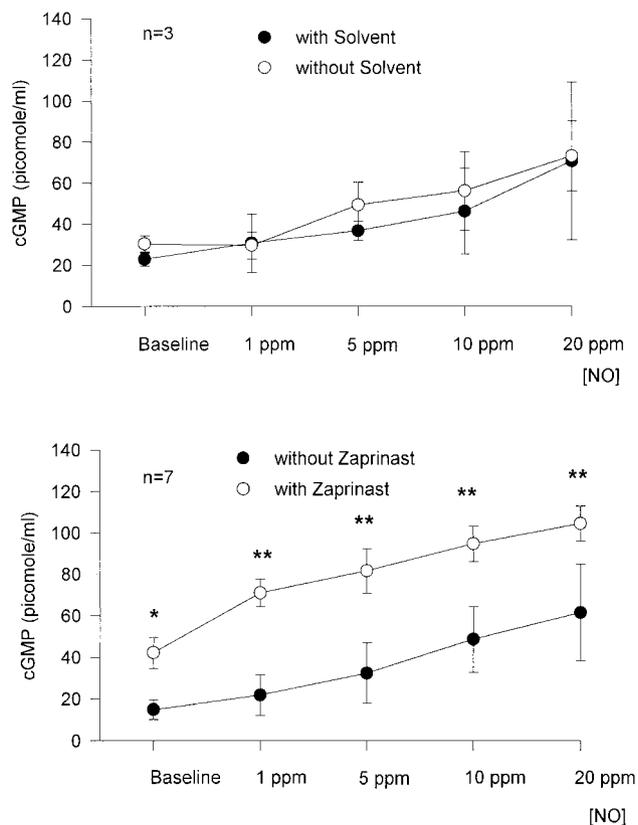


Fig. 2. Influence of Zaprinast on plasma cyclic guanosine monophosphate (cGMP) levels at baseline and during nitric oxide (NO) gas inhalation (second dose-response trial: 1, 5, 10, and 20 ppm in random order). The administration of solvent (NaOH 0.05 M in saline) did not change cGMP levels. Values are reported as mean \pm SE. * $P < 0.05$, ** $P < 0.01$ compared with control.

of high levels of endogenously produced NO, and the opposing pulmonary vasoconstrictor action of exogenous and endogenous catecholamines.²⁴ Increased vascular production of \dot{O}_2^- , as observed in systemic nitrate tolerance, also may contribute to hyporesponsiveness to NO.²⁵

Nitric oxide exerts many of its actions by activating guanylate cyclase and stimulating the local production of cGMP.²⁶ Increased tissue cGMP concentrations correlate well with smooth muscle relaxation.¹⁰ Because cGMP is hydrolyzed by phosphodiesterases, inhibition of phosphodiesterases could increase the efficacy and duration of the action of inhaled NO. This hypothesis has been tested in a variety of experimental models. In awake lambs with U46619-induced pulmonary arterial hypertension, intravenous infusion of Zaprinast increased the duration of action of inhaled NO and accentuated the NO-induced reduction of pulmonary vascular resistance. With administration of Zaprinast, the half-time of pulmonary vasodilation after discontinuing a 4-min 40-ppm NO inhalation trial was increased from 1–2 min to 10–12 min.¹⁵ Potentiation of the effects of NO by another phosphodiesterase inhibitor, dipyridamole, has been reported in the ovine fetal pulmonary circulation.^{27,28}

Such interactions between phosphodiesterase inhibitors and inhaled NO are becoming increasingly important in clinical situations. The ability of dipyridamole to augment the efficacy of inhaled NO has been reported in pediatric patients with pulmonary hypertension by Ziegler *et al.*²⁹ In 50% of patients, combining 0.6 mg/kg intravenous dipyridamole with 20 ppm NO decreased pulmonary vascular resistance by 20% more than the use of either drug alone. Dipyridamole therapy has been used to reduce the rebound pulmonary hypertension that sometimes occurs during the withdrawal of inhaled NO therapy, thereby blunting the sudden decrease of [cGMP] that may occur after the withdrawal of NO.^{30,31} Phosphodiesterase inhibitors such as sildenafil currently are being used to treat erectile dysfunction in millions of patients.³² In addition, the use of sildenafil to reduce rebound pulmonary hypertension during the withdrawal

Table 3. Effect of Zaprinast Infusion on Hemodynamics and Gas Exchange

	Control Group (n = 5)		Zaprinast Group (n = 10)	
	Before	After	Before	After
PaO ₂ (mmHg)	75 \pm 11	70 \pm 10	83 \pm 16	52 \pm 5*
Q _{VA} /Q _T (%)	40.2 \pm 4.3	42.2 \pm 4.4	41.8 \pm 4.1	49.4 \pm 2.1*
LAP (mmHg)	9 \pm 1	9 \pm 1	9 \pm 1	9 \pm 1
CVP (mmHg)	9 \pm 1	10 \pm 1	10 \pm 1	9 \pm 1
PAP (mmHg)	23 \pm 2	24 \pm 1	23 \pm 1	18 \pm 1
PVR (dynes \cdot s \cdot cm ⁻⁵)	329 \pm 24	390 \pm 58	356 \pm 56	189 \pm 29*
SAP (mmHg)	93 \pm 7	85 \pm 11	101 \pm 3	80 \pm 5
SVR (dynes \cdot s \cdot cm ⁻⁵)	2112 \pm 131	2072 \pm 301	2128 \pm 141	1496 \pm 159
CO (l/min)	3.2 \pm 0.2	3.0 \pm 0.4	3.6 \pm 0.2	4.0 \pm 0.3

See table 1 for list of abbreviations.

* $P < 0.05$.

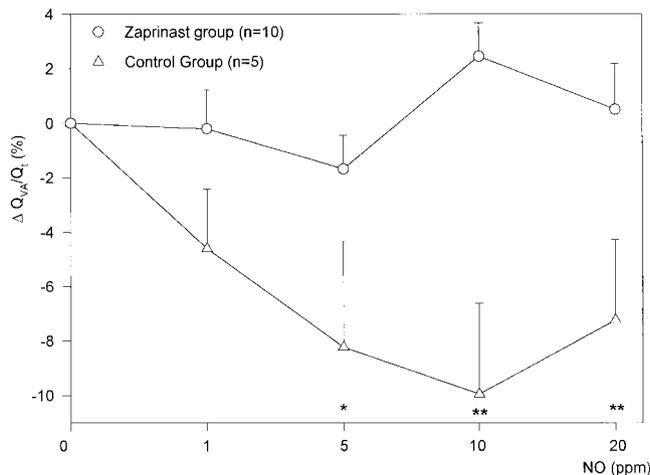


Fig. 3. Absolute change of venous admixture ($\Delta\dot{Q}_{VA}/\dot{Q}_T$) during nitric oxide (NO) gas inhalation (second dose-response trial: 1, 5, 10, and 20 ppm in random order) during intravenous administration of Zaprinast (Zaprinast group) or solvent (NaOH 0.05 M in saline, Control group). Values are reported as mean \pm SE. * $P < 0.05$, ** $P < 0.01$ compared with control.

of inhaled NO has been described.³³ Despite such widespread interest and use, the effects of specific cGMP phosphodiesterase inhibitors on gas exchange in various pulmonary diseases, and in combination with inhaled NO, remain to be fully investigated.

In vitro, Zaprinast inhibits cGMP-specific phosphodiesterase, increases cGMP levels, and relaxes vascular smooth muscle.^{34,35} In the current study, the infusion of Zaprinast alone caused a significant increase of plasma cGMP concentrations. It was previously reported that Zaprinast increased plasma cGMP levels in anesthetized rats when infused at $1 \text{ mg} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$ for 30 min,¹⁸ but not in an ovine pulmonary hypertension model when infused at the same dose used in the present study.¹⁹ Because Zaprinast inhibits the breakdown but not the synthesis of cGMP, these differences probably reflect differing basal rates of endogenous cGMP synthesis in the different models. Reduced cGMP synthesis would be expected to reduce the effects of Zaprinast. For example, the vasorelaxation induced by Zaprinast in precontracted pulmonary arteries of the rat has been reported to be reduced in the absence of endothelium or in the presence of the NO synthase inhibitor, L-NAME.³⁶

The administration of inhaled NO increased plasma [cGMP] in a dose-dependent manner. Because we randomized the order of NO inhalation and separated each administration with a period of NO-free breathing, this increase would not simply reflect the accumulation of cGMP over time. When Zaprinast was infused during NO

administration, plasma cGMP concentrations were greater than those at identical inhaled NO levels without Zaprinast (fig. 2).

In the present study, Zaprinast increased venous admixture and reduced PaO_2 . Moreover, Zaprinast abolished the beneficial effects of inhaled NO on oxygenation. Nonselective vasodilation of pulmonary vessels by Zaprinast aggravated intrapulmonary shunting and prevented further vasodilation by inhaled NO. Because further vasodilation was not evident, the addition of inhaled NO to Zaprinast was unable to affect gas exchange. Intravenous infusion of nonspecific vasodilators, such as nitroglycerin or nitroprusside, also produces diffuse pulmonary vasodilation and can worsen systemic arterial oxygenation.^{2,6,37} Whether these intravenous vasodilators also decrease the efficacy of inhaled NO has not been well studied.

It is possible that the effects of Zaprinast that were observed were dose- or route-dependent. The dose of Zaprinast chosen in the current study was identical to that used in previous studies.¹⁹ This dose does not produce significant systemic vasodilation and preserves the pulmonary vasodilator selectivity of inhaled NO during acute pulmonary hypertension.¹⁹ Lower doses of Zaprinast might produce less complete pulmonary vasodilation and preserve some pulmonary vasoconstriction, and also the relatively selective effect of inhaled NO on

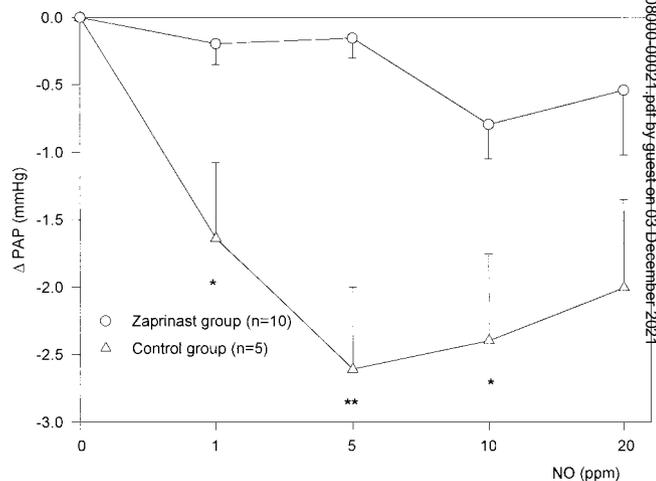


Fig. 4. Absolute change of pulmonary artery pressure (ΔPAP) during inhalation of nitric oxide (NO) gas (second dose-response trial: 1, 5, 10, and 20 ppm in random order) with coadministration of Zaprinast (Zaprinast group) or solvent (NaOH 0.05 M in saline, Control group). Values are reported as mean \pm SE. * $P < 0.05$, ** $P < 0.01$ compared with control.

well-ventilated lung regions. It also is possible that the route of administration of Zaprinst (e.g., systemic *vs.* inhaled) may alter the effects of the drug on the pulmonary circulation. In a recent study by Ichinose *et al.*,³⁸ aerosolized Zaprinst selectively dilated the pulmonary circulation and increased the potency and duration of action of inhaled NO in awake lambs with U46619-induced pulmonary hypertension. The effects of Zaprinst also may depend on the basal level of endogenous NO synthesis, because the drug amplifies cGMP concentrations by inhibiting the breakdown of cGMP, rather than by stimulating endogenous *de novo* production of the messenger. Lastly, the preexisting degree of vasoconstriction of the pulmonary vasculature is undoubtedly important. In our previous study,¹⁹ the initial pulmonary vasodilation induced by Zaprinst was countered by increasing the infusion rate of U46619 to obtain the same degree of pulmonary vasoconstriction as observed at baseline (a mean PAP of 30 mmHg). In our current study, the degree of pulmonary vasoconstriction was very mild (mean PAP, 22–23 mmHg) and was reversed by the Zaprinst infusion.

Lung lavage was used to mimic some of the major clinical features of acute respiratory distress syndrome.²⁰ Bilateral lavage induced systemic arterial hypoxemia, increased venous admixture, and produced mild pulmonary arterial hypertension. The major cause of the impaired oxygenation produced by lung lavage is believed to be reduced lung surfactant activity. The degree of pulmonary hypertension observed after lavage in this study was mild because lung lavage primarily induces epithelial rather than endothelial or vascular damage.²⁰ The differing effects of Zaprinst in the current model from those observed in models of acute pulmonary hypertension suggest that the results of combining phosphodiesterase inhibitor therapy with inhaled NO may depend on baseline pulmonary vascular tone and perhaps vary in different disease states and different types of lung injury.

In conclusion, inhalation of NO for brief periods improved gas exchange and decreased PAP in an ovine model of acute respiratory distress syndrome induced by lung lavage. Intravenous administration of Zaprinst decreased PAP but worsened gas exchange, suggesting that Zaprinst induced diffuse vasodilation of poorly ventilated regions. Moreover, Zaprinst abolished the beneficial effects observed with NO inhalation alone. Further investigation of the interactions between phosphodiesterase inhibitors and inhaled NO appears warranted.

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