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Original Paper

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PGI_2 Aerosol versus Nitric Oxide for Selective Pulmonary Vasodilation in Hypoxic Pulmonary Vasoconstriction

Key Words
Prostacyclin
Aerosol
Nitric oxide
Pulmonary hypertension

Abstract

Intravenous prostacyclin (PGI_2) is a potent pulmonary vasodilator in pulmonary hypertension. However, dose-dependent systemic vasodilation, an increase in intrapulmonary shunt and hypoxemia limit its clinical application. Recently, inhaled nitric oxide (NO) has been reported to elicit selective pulmonary vasodilation, but its clinical use is restricted by its potential toxicity; furthermore, the feasibility of NO application in clinical practice seems difficult. Therefore, we investigated the effects of PGI_2 aerosol on pulmonary and systemic circulation and compared the hemodynamic effects to those of inhaled NO. In 6 dogs, ventilation with a hypoxic gas mixture (FiO_2 0.09–0.11) increased pulmonary vascular resistance (PVR) by 196% (HPV). Aerosolization of a PGI_2 solution at a concentration of 430 ng/ml reduced hypoxia-induced increase of pulmonary artery pressure by 48% and PVR by 52% within 6–10 min without systemic vasodilation. The administered dose of PGI_2 was 0.87 ± 0.26 ng/kg/min. In 2 dogs, doubling the PGI_2 concentration (860 ng/ml) did not enhance the vasodilatory effect. After termination of PGI_2 inhalation, HPV was restored within 10–15 min. Inhaled NO (50 ppm) decreased the HPV-induced increase in PAP by 76% and in PVR by 73% within 5–10 min. Clinically relevant systemic vasodilation was not observed. It is concluded that inhalation of aerosolized PGI_2 leads to selective pulmonary vasodilation in hypoxia-induced pulmonary hypertension. Aerosolized PGI_2 at a concentration of 430 ng/ml was less potent than NO (50 ppm). However, due to the lack of known toxicity and its uncomplicated mode of application, inhaled PGI_2 may be one alternative to inhaled NO in the treatment of acute pulmonary hypertension.

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Prostacyclin (PGI_2), an arachidonic acid metabolite, is a potent pulmonary vasodilator [1]. It has been successfully given intravenously to decrease pulmonary artery pressure (PAP) in primary pulmonary hypertension [2], in patients with ARDS [3], and in various experimental models of acute pulmonary hypertension [4–6]. The vasodilatory effect of PGI_2 is, however, not restricted to the pulmonary circulation. When infused intravenously, PGI_2 leads to dose-dependent systemic vasodilation and hypotension. Furthermore, intrapulmonary shunt and venous admixture increase dose-dependently; consequently, arterial oxygen content decreases and the net effect of intravenous PGI_2 on oxygen delivery depends on the ability to increase cardiac output. For these reasons, the clinical use of intravenous PGI_2 to treat pulmonary hypertension is limited [1–3, 7].

Recently, inhaled gaseous nitric oxide (NO), considered to be identical with endothelial derived relaxing factor [8], has been described to act as a selective pulmonary vasodilator in pulmonary hypertension [9, 10]. In awake and spontaneously breathing lambs, inhaled NO reduced acute pulmonary hypertension induced by hypoxia or by intravenous infusion of the stable endoperoxide analogue U-46619 without being associated with systemic vasodilation. Experimental studies have demonstrated that inhaled NO improves the ventilation/perfusion mismatch in hypoxia-induced pulmonary hypertension and is efficient in reversing pulmonary hypertension due to sepsis [11, 12].

In mechanically ventilated sheep inhalation of NO (20 ppm) had no effect on pulmonary gas exchange but decreased blood flow to unventilated and poorly ventilated areas of the lung (ventilation/perfusion ratio <0.1) and consequently reduced ventilation/perfusion mismatch [11]. In addition to its effects on pulmonary circulation and arterial oxygenation inhaled NO exerts dilation of the bronchial smooth muscles which might improve pulmonary ventilation/perfusion ratio [13]. Inhaled NO had no effect on pulmonary hemodynamics or gas exchange when applied during normoxia (FiO_2 0.21) [11].

In experimetal models of sepsis using infusion of endotoxin [14] or group B streptococcus [12] inhalation of NO diminished pulmonary vasoconstriction. In the latter study in ventilated piglets inhalation of 150 ppm of NO reversed pulmonary hypertension during the early (<1 h) and late phase of sepsis (2 to 6 h) whereas ventilation/perfusion mismatch was not counteracted in the late phase of sepsis [12]. More recently inhaled NO was therapeutically applied in man. In a restricted number of patients, inhaled NO proved efficient in lowering PAP; however, in contrast to intravenous PGI_2, inhaled NO caused no systemic vasodilation [7, 15, 16].

In the most recent human studies low doses of inhaled nitric oxide (20 to 80 ppm) decreased pulmonary artery pressure in hypoxia-induced pulmonary hypertension in volunteers [17], in cardiac patients before and after cardiopulmonary bypass [18] and in children with pulmonary hypertension due to congenital heart disease [19].

In adults with respiratory distress syndrome (ARDS) inhalation of NO (5 to 36 ppm) reduced pulmonary artery pressure and intrapulmonary shunting and improved arterial oxygenation for up to 53 days without tachyphylaxis and noticeable side effects. Though in the same study intravenous prostacyclin (4 ng/kg/min) lowered pulmonary artery pressure as well, systemic arterial pressure fell and intrapulmonary shunt increased [20].

Finally, NO (10 ppm) inhaled for 30 minutes by an infant after cardiac surgery improved gas exchange permanently while pulmonary hypertension was resolved permanently [21]. Furthermore, enhanced resolu-

tion of pneumonic infiltrates was observed upon NO inhalation [22].

Despite the convincing evidence that inhaled NO leads to selective pulmonary vasodilation and hence may be useful to treat pulmonary hypertension, it should be noted that NO itself is a radical with potential cytotoxic effects. Although severe acute lung injury and methemoglobinemia are observed after inhalation of gas mixtures containing high concentrations of NO, there is little evidence for pulmonary toxicity at low concentrations (<100 ppm) of NO inhaled for short periods of time in different species [10]. However, little is known about the toxicity of long-term breathing of NO at low concentrations and the effect of NO on lungs with pre-existing acute or chronic disease. As long as the absence of long-term toxicity of NO has not been demonstrated, its routine clinical application for selective pulmonary vasodilation is restricted [10]. Furthermore, for the application of gaseous NO, a specially designed ventilator and an expensive chemiluminescence analyzer for continuous monitoring of the inspiratory NO and NO_x concentrations are mandatory; hence it is questionable whether NO inhalation will become clinical routine.

In view of these limitations of both inhaled NO and intravenous PGI_2, the present study was designed to investigate the effect of PGI_2 inhaled as an aerosol on hypoxic pulmonary vasoconstriction in dogs. We hypothesized that inhaled PGI_2 may lead to selective pulmonary vasodilation via direct relaxation of pulmonary vascular smooth muscle cells. Vasodilatory effects on both the pulmonary and the systemic circulation were assessed. To evaluate the relative potency of inhaled PGI_2 at the concentration used, its vasodilatory effect was compared to that of inhaled NO at a concentration (50 ppm) known to cause a nearly maximal vasodilatory effect in HPV-induced pulmonary hypertension [10]. We aimed to answer the question whether inhaled PGI_2 induces pulmonary vasodilation without causing systemic hypotension.

Methods

Animal Preparation

The studies were performed in 6 foxhounds of either sex (mean body weight 27.6 ± 1.9 kg). All animals received care in compliance with the 'Guide for the Care and Use of Laboratory Animals' [NIH Publ. No. 85-23, revised 1985]. The study was approved by the institutional animal care and use committee.

After premedication with propiomazine (1.5 mg/kg i.m., Combelen®, Bayer AG, Leverkusen, FRG), anesthesia was induced by intravenous injection of pentobarbital (20 mg/kg, Nembutal®, Ceva, Bad Segeberg, FRG), piritramide (0.75 mg/kg, Dipidolor®, Janssen, Neuss, FRG) and alcuronium (0.25 mg/kg, Alloferin®, Roche, Grenzach-Whylen, FRG), and maintained by continuous intravenous infusion of pentobarbital (5 $mg \cdot kg^{-1} \cdot h^{-1}$). After termination of the surgical preparation an additional infusion of piritramide (150 $\mu g \cdot kg^{-1} \cdot h^{-1}$) and alcuronium (75 $\mu g \cdot kg^{-1} \cdot h^{-1}$) was started. Fluid losses were replaced by intravenous infusion of Ringer's solution (5 $ml \cdot kg^{-1} \cdot h^{-1}$). A warming pad was used to keep core body temperature between 35.5 and 37 °C. The dogs were endotracheally intubated and mechanically ventilated at a rate of 12 cycles/min using a FiO_2 of 0.5 (Servo 900B, Siemens-Elema, Solna, Sweden). Tidal volume (V_T) was adjusted to maintain an arterial P_{CO_2} of 35–40 mm Hg.

Fluid-filled catheters (PP270, Portex, Hythe, UK) were positioned in the descending aorta and superior caval vein via the left femoral artery and the right external jugular vein to measure mean arterial (MAP) and central venous pressure (CVP), respectively. A Swan-Ganz catheter (model 93A-431-7.5F G, Baxter Healthcare Corp., Santa Ana, Calif., USA) was inserted into the pulmonary artery via the right external jugular vein to measure mean PAP and cardiac output (CO). After median sternotomy and pericardiotomy prewarmed, precalibrated tip manometers (PC 370, Millar Instruments, Houston, Tex., USA) were inserted into the right ventricle (RV) via a stab incision at the apex of the RV and into the left ventricle (LV) via the right carotid artery. In four dogs an ultrasonic flow probe (Transonic Systems Inc., Ithaca, N.Y., USA) was placed around the pulmonary artery. At the end of the surgical preparation, the pericardium was closed with running suture to avoid constraint to the

myocardium. Thereafter, a chest tube was inserted, the thorax was closed air-tight, and the lungs were completely reinflated. Subsequently, the dogs were turned to the left lateral decubitus position.

Administration of NO

NO was obtained in 50-liter cylinders as a precise mixture of 200 ppm NO in nitrogen (Linde AG, Unterschleissheim, FRG). During inspiration the NO/N_2 mixture was introduced into the inspiratory gas using a T-tube connected to the endotracheal tube. A magnetic valve (Servo Nebulizer 945, Siemens, Erlangen, FRG) triggered by the electronic regulation system of the ventilator was used for insufflation of NO exclusively during inspiration. The inspiratory NO flow was varied using a precise pressure reduction valve (SP 750.0183.M, Kuhnke, Malente, FRG) and the NO pressure was monitored with an electronic manometer (Type 352-P, Debro GmbH, Meerbusch, FRG). The correct inspiratory NO concentration was produced by varying the NO admixture to the inspired gas mixture at constant tidal volumes (T_V). The FiO_2 was maintained constant during administration of the NO/N_2 mixture by increasing the inspiratory oxygen concentration proximal to the NO insufflation port. The inspired oxygen concentration (FiO_2) was continuously monitored distal to the NO insufflation port (Oxydig, Dräger AG, Lübeck, FRG). Expired gas was scavenged and discarded. Prior to the experiments the complete application system (ventilator, pressure reduction valve, electronic manometer) had been calibrated to achieve the desired inspiratory NO concentration at different T_V and oxygen concentrations using a chemoluminescence NO/NO_x analyzer (Beckman, Model 951 A, Beckman Instruments, Munich, FRG). The inspiratory NO concentration at a given system setting varied no more than 3% during repeated calibration measurements.

To avoid oxidation of NO to NO_2, the critical contact time of NO and O_2 during inspiration [10] has been kept short by using a short inspiration time (1.65 s) and by introducing NO into the inspiratory gas mixture very close to the endotracheal tube (19 cm). The concentrations of NO_2 measured at the endotracheal tube during calibration were below 1 ppm.

Administration of PGI_2 Aerosol

For inhalation of PGI_2 as aerosol, a jet nebulizer (Servo Nebulizer 945, Siemens, FRG) was connected to a Siemens 900B ventilator. The nebulizer chamber (Intersurgical Ltd, Twickenham, UK) was located 16 cm from the endotracheal tube. The jet flow supplied to the nebulizer chamber during inspiration was kept constant in all experiments at a flow rate of 14 liters/min. This high flow rate was chosen because the fraction of particles with a diameter of $< 2\mu m$, which are likely to settle in the alveolar region of the lung, increases with flow rate. T_V and total inspiratory flow rate were maintained constant during aerosolization by decreasing the inspiratory flow from the ventilator. Total T_V was monitored (CO_2 analyzer 930, Siemens-Elema).

PGI_2 was supplied as the sodium salt of epoprostenol (Flolan®, Wellcome, London, UK) dissolved in glycine buffer of pH 10.5 at a concentration of 10 μg/ml; the solution was prepared on the day of the experiment and stored on ice until use. Prior to inhalation, PGI_2 was further diluted with normal saline to a concentration of 430 ng/ml and a total volume of 10 ml of this solution was filled into the nebulizer chamber. The dosage of PGI_2 was based on pilot experiments in which this concentration has proven to be effective in lowering PAP. To assess the actual amount of PGI_2 nebulized, the total volume of the PGI_2 solution in the nebulizer chamber was measured prior to and immediately after inhalation (volume nebulized × concentration/duration of inhalation).

6-keto-Prostaglandin-$F_{1\alpha}$ (6-keto-$PGF_{1\alpha}$), the stable metabolite of PGI_2, was measured by radioimmunoassay (antibody: Pasteur Diagnostic, Marne-la-Coquette, France; ^{3}H-PG's: Amersham Buchler, Braunschweig, FRG) in unextracted arterial plasma before and after PGI_2 inhalation in 3 dogs.

To exclude that the vasodilatory effect of PGI_2 aerosol was mediated by uptake of PGI_2 into the blood, the effects of PGI_2 infused at a rate of 1 and 2 ng/kg/min were compared to those of inhaled PGI_2 in 2 dogs.

Measurements

Hemodynamics. All measurements were performed with the dogs in left lateral position. MAP, PAP, and CVP were recorded (Astromed MT 9500, Astro-Med, Inc., West Warwick, R.I., USA) using Statham P23Db transducers (Gould-Statham, Oxnard, Calif., USA) referred to the right atrium. ECG and ventricular pressures (LVP, RVP) were sampled every 4 min, digitized in real-time (A/D converter C1000, Cosima Corp., Salem, Oreg., USA) and stored for evaluation using a PC/AT 386 computer system. Data were analyzed with interactive software ('Meduse', H. Zeintl, Neckargemünd, FRG). All parameters were evaluated at end-expiration using the average of three consecutive heart beats.

In the RV and LV, maximal (RVP_{max}, LVP_{max}) and end-diastolic pressures (RVEDP, LVEDP) were assessed. CO was obtained from triplicate thermodilution measurements at end-expiration (REF-1™, Ejection Fraction/Cardiac Output Computer, Baxter Healthcare, Santa Ana, Calif., USA).

The following parameters were calculated:

$$CI = 10 \cdot CO \cdot BW^{-0.75}$$

(according to Holt et al. [23])

$$PVR = (PAP_{mea} - LVEDP) \cdot 79.9 \cdot CO^{-1}$$

$$SVR = (MAP_{mea} - RVEDP) \cdot 79.9 \cdot CO^{-1}$$

where CI = cardiac index, CO = cardiac output, BW = body weight, PVR = pulmonary vascular resistance, LVEDP = LV end-diastolic pressure, SVR = systemic vascular resistance, RVEDP = right ventricular end-diastolic pressure, HR = heart rate.

Lung Function. P_{O_2}, P_{CO_2} and pH (ABL 300, Radiometer, Copenhagen, Denmark), as well as arterial (SaO_2) and mixed venous oxygen saturation (S_vO_2) and arterial methemoglobin fraction (CO-Oximeter 282, Instrumentation Laboratory Inc., Lexington, Mass., USA) were analyzed. End-tidal CO_2 concentration and T_v were monitored using a CO_2 analyzer (CO_2 analyzer 930, Siemens-Elema), airway pressures (P_{peak}, P_{plat}) and total thoracic compliance (C_{tot}) were assessed (Lung Mechanics Calculator 940, Siemens-Elema).

Experimental Protocol

After termination of surgery the dogs were isovolumically hemodiluted with 6% dextran 60 (Makrodex®, 6%, Schiwa, Glandorf, FRG) to a hematocrit of 27–30% in order to achieve identical values of hematocrit and hemoglobin in all animals. The withdrawn blood was used to replace blood samples taken for laboratory analysis. Hematocrit and hemoglobin did not change significantly during the experiment.

Control measurements were performed 30 min after termination of surgery under stable hemodynamic conditions (control). Thereafter, hypoxia was induced by decreasing the FiO_2 to 0.09–0.11 to achieve a PaO_2 of near 40 mm Hg. The inspired FiO_2 was monitored and $PaCO_2$ was maintained constant by adjusting V_T. Following a period of stable hemodynamics, baseline recordings were obtained during hypoxia (HPV). Subsequently, either NO at a concentration of 50 ppm or PGI_2 at a concentration of 430 ng/ml were administered in random order. Measurements were obtained after 10 min of NO or PGI_2 application, respectively (NO, PGI). Subsequently, the administration of either NO or PGI_2 was terminated and a further measurement under hypoxic conditions was performed (post-NO, post-PGI) 10–15 min thereafter.

Statistical Analysis

Data are reported as mean ± SD. Statistical analysis was performed using a repeated measures analysis of variance (rANOVA). If the f value was significant ($p < 0.05$), the following time points were compared by paired t test: control vs. HPV, HPV vs. NO, HPV vs. PGI_2 as well as the difference between HPV and NO versus the difference between HPV and PGI_2. Bonferroni correction was applied as appropriate. Differences were considered significant for $p < 0.05$.

Results

Hemodynamics

The effects of NO and PGI_2 on hypoxia-induced changes in pulmonary and systemic hemodynamics are summarized in figure 1. Aerosolization of the solvent (glycine buffer, pH 10.3) and normal saline did not exert effects on systemic or pulmonary hemodynamics.

Hypoxia. Ventilation with a hypoxic gas mixture (FiO_2 0.09–0.11) produced a significant increase in PAP (+57%, $p < 0.001$), PVR (+196%, $p < 0.001$), heart rate (control 99 ± 11, HPV 135 ± 30, $p < 0.01$) and CI (control 2.3 ± 0.3, HPV 3.0 ± 0.5, $p < 0.05$) as well as a reduction in SVR (–26%, $p < 0.05$) in all dogs (fig. 1). The degree of hypoxic pulmonary vasoconstriction as assessed by PVR remained constant during basic HPV, post-NO and post-PGI. Right and left ventricular preload (RVEDP, LVEDP) remained constant.

Nitric Oxide. When NO was added to the hypoxic gas mixture, PAP was significantly reduced in all dogs (–28%, $p < 0.001$ vs. HPV) but did not reach baseline levels (FiO_2 0.5). NO attenuated the HPV-induced increase in PAP and PVR by 76 and 73%, respectively (fig. 1). A minor reduction in MAP (–5%, $p < 0.05$ vs. HPV) was observed, however, there was no change in calculated SVR (fig. 1). The maximal pulmonary vasodilating effect of NO occurred within 7 ± 1.3 min (fig. 2). After termination of NO inhalation, all hemody-

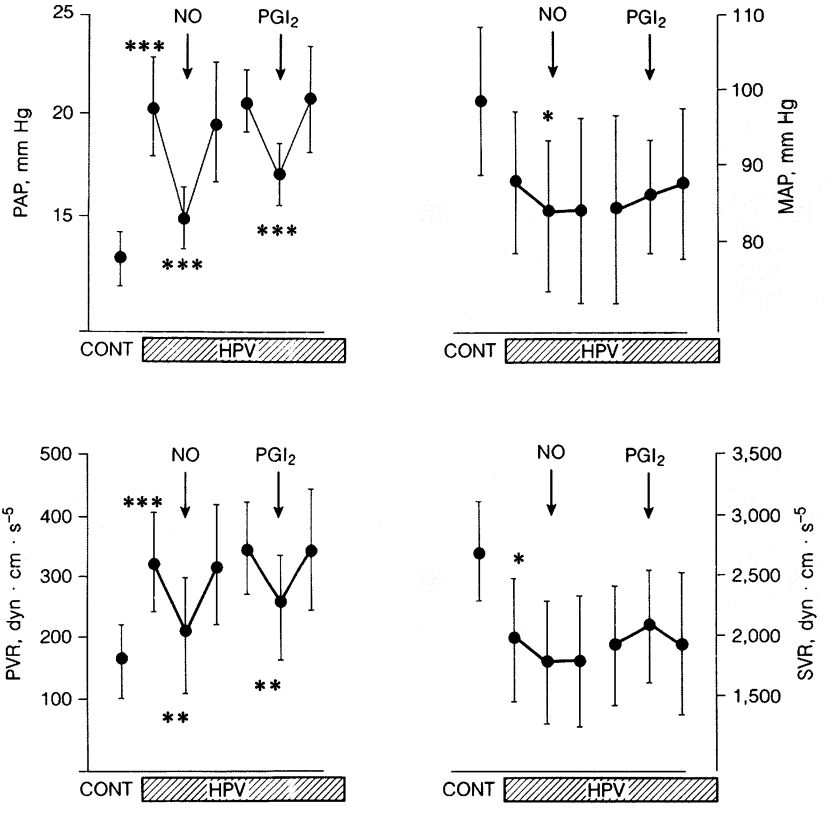

Fig. 1. Effects of inhalation of NO (n = 6) and PGI_2 (n = 6) during hypoxia on PAP, MAP, PVR and SVR. Inhalation of NO and PGI_2 decreased PAP and PVR reversibly while MAP and SVR remained unchanged. Data are given as mean ± SD. CONT = Control before hypoxia. * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$ versus preceding measurement.

namic variables returned to pre-NO values. During inhalation of NO, HR, CI, LVEDP and RVEDP remained unchanged.

Mean methemoglobin levels increased from 0.8 ± 0.2 to 1.2 ± 0.3% after NO inhalation ($p < 0.05$) and returned to baseline values after termination of NO application.

Prostacyclin. Inhalation of PGI_2 aerosol during HPV decreased PAP and PVR by 17 and 32%, respectively (fig. 1). The hypoxia-induced increase in PAP was reduced by 48% and the increase in PVR by 52%. No significant changes occurred in MAP (–2 ± 5 mm Hg), SVR (+83 ± 124 dyn·cm·s^{-5}) (fig. 1), CI (–0.25 ± 0.27 liter/min) and RVEDP. During PGI_2 inhalation, HR was slightly reduced (HPV 139 ± 27, PGI 127 ± 27, $p < 0.05$). The maximal pulmonary vasodilating effect of PGI_2 was achieved within 8 ± 1 min (fig. 2). The decrease in PAP induced by PGI_2 was smaller compared to the effect of NO in all dogs ($p < 0.02$). After termination of PGI_2 administration, hypoxic pulmonary vasoconstriction was restored within 10–15 min.

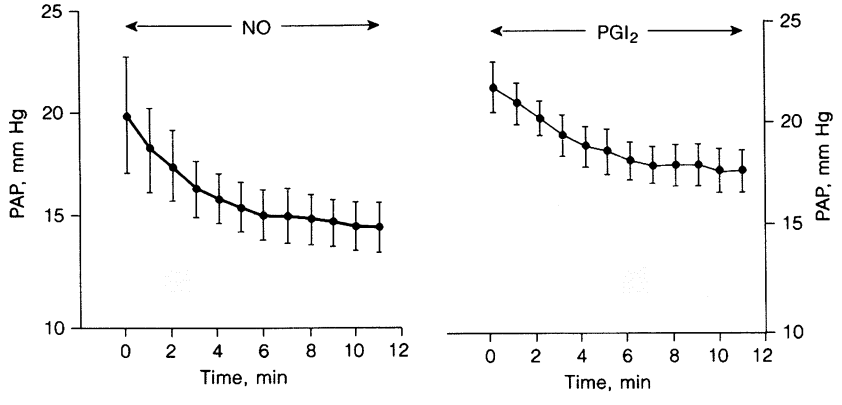

Fig. 2. Sequential changes of PAP during inhalation of NO (n = 6) and PGI_2 (n = 5). NO and PGI_2 led to an immediate decrease of PAP that reached its maximum within 10 min with both substances; the magnitude of PAP and PVR change was greater during NO than during PGI_2 inhalation ($p < 0.05$). Data are given as mean ± SD.

Table 1. Blood gas and respiratory parameters at control, at baseline HPV and during NO and PGI_2 inhalation

Variables	Control	HPV	HPV + NO	Post-NO	HPV	HPV + PGI_2	Post-PGI_2
PaO_2, mm Hg	186±65	41±9.6[a]	42.4±9.2	41.1±9.6	40.8±9.7	45.1±13.1[c]	39.6±5.2
PvO_2, mm Hg	46.1±3.0	27.6±5.3[b]	28.4±5.0	27.8±5.2	27.1±5.2	29.0±5.4[c]	26.6±3.5
$PaCO_2$, mm Hg	38.9±2.0	37.6±2.8	37.2±2.1	35.3±2.9	36.3±2.9	33.5±4.5	36.5±6.6
pH art	7.36±0.06	7.36±0.03	7.36±0.03	7.37±0.04	7.38±0.04	7.39±0.05	7.36±0.05
COM, ml/cm H_2O	59.5±18.4	52.8±14.3	53.5±15.0	50.8±13.5	50.3±14.8	51.0±12.2	50.7±14.1
AWP_{plat}, mbar	12.0±2.2	11.7±2.7	11.0±1.7	12.2±2.6	12.5±3.0	11.7±1.8	11.8±3.1

Data are given as mean ± SD. PaO_2 = Arterial O_2 tension; PvO_2 = mixed venous O_2 tension; $PaCO_2$ = arterial CO_2 tension; pH art = arterial pH; COM = total compliance of lung and thoracic wall; AWP_{plat} = plateau airway pressure at end inspiration.
[a] $p < 0.05$; [b] $p < 0.01$ HPV vs. control; [c] $p < 0.05$ PGI_2 vs. HPV.

The mean effective dose of PGI_2 administered to the lungs was 0.87 ± 0.26 ng/kg/min. Intravenous infusion of PGI_2 at comparable infusion rates (1 and 2 ng/kg/min) in 2 dogs had neither effects on pulmonary nor systemic hemodynamics (data not shown).

Plasma concentrations of 6-keto-$PGF_{1\alpha}$ measured in 3 dogs before, during and after PGI_2 inhalation remained unchanged (HPV 94 ± 50 pg/ml, PGI 112 ± 20 pg/ml, post-PGI 103 ± 9 pg/ml).

Lung Function

Blood gas parameters and lung mechanics are summarized in table 1. Ventilation with the hypoxic gas mixture (FiO_2 0.09–0.11) re-

sulted in a decrease of mean arterial P_{O_2} from 186 ± 65 mm Hg and mean PvO_2 from 46.1 ± 3.0 to 41 ± 9.6 mm Hg and 27.6 ± 5.3 mm Hg, respectively. Arterial and mixed venous P_{O_2} remained constant during ventilation with the hypoxic gas mixture. There was no significant change in arterial P_{CO_2} throughout the experiments. Mean arterial pH was maintained between 7.36 and 7.39. PaO_2 and PvO_2 increased slightly and reversibly only during PGI_2 inhalation. There was no change in airway pressures (P_{peak}, P_{plat}). Total thoracic compliance slightly decreased after induction of hypoxia, but remained constant during NO and PGI_2 administration.

Discussion

The main finding of this study was that inhaled PGI_2 selectively attenuated hypoxia-induced pulmonary hypertension without causing systemic vasodilation. Both PGI_2 aerosol and NO gas reduced hypoxia-induced increase in PVR within minutes without affecting systemic vascular resistance or CI; the effects on the pulmonary vessels were reversible upon termination of either drug within 10–15 min.

PGI_2 is the main metabolite of arachidonic acid produced by endothelial cells in different species. It is a potent dilator of both arteries and veins [24]. The vasodilatory effect is endothelium independent and is mediated by stimulation of specific receptors on membranes of vascular smooth muscle cells [25]. Intracellulary PGI_2 acts by increasing the concentration of cyclic adenosine monophosphate (cAMP) [25]. PGI_2 has a rapid onset of action and a short half-life (2–3 min) when infused intravenously. Due to the lack of a carrier mechanism to transfer PGI_2 across the cell membrane, it is, unlike most other prostaglandins, not metabolized by the lung and retains its pulmonary vasodilatory properties [26]. Therefore, and because of its lack of known toxicity, PGI_2 aerosol was chosen for inhalation.

Since the first description of the dilating effects on pulmonary and systemic vessels of intravenous PGI_2 in dogs [1], it has been successfully applied as pulmonary vasodilator in animal models of acute pulmonary hypertension [4, 5, 27, 28] and in patients with pulmonary hypertension of various etiologies [2, 29]. At doses effective in reducing PVR, intravenous PGI_2 did, however, induce systemic vasodilation and an increase in cardiac output. In addition, intravenous PGI_2 was shown to attenuate HPV and to increase venous admixture; consequently, arterial oxygen content decreases and the net effect of intravenous PGI_2 on oxygen delivery depends on the ability to increase cardiac output [4, 30].

In this study, the inhalation of PGI_2 aerosol reduced the hypoxia-induced increase in PAP and PVR without eliciting systemic vasodilation or changes of CI. The finding that inhaled PGI_2 did not completely reverse HPV is in agreement with the results of previous studies, in which PGI_2 given intravenously also failed to completely abolish the hypoxia-induced increase in PVR [5, 28].

In contrast to intravenous PGI_2, PGI_2 aerosol did not increase cardiac output. Therefore, the calculated PVR, which was used in this study to assess pulmonary vascular tone and that is influenced by changes of cardiac output, may truly reflect PVR changes.

In contrast to the decrease in PaO_2 occurring during intravenous infusion of PGI_2, the inhalation of PGI_2 produced a small and reversible increase in PaO_2 and PvO_2. This finding cannot be easily explained from our data. One may speculate that PGI_2 inhalation led to a reduction in V_a/Q mismatches as it has been described during NO inhalation in

patients with ARDS [7]. However, in this study, NO did not affect either arterial or mixed venous oxygenation during inhalation. The effect of inhaled PGI_2 on pulmonary ventilation/perfusion ratio has to be assessed in further studies using more appropriate techniques (e.g. multiple inert gas elimination technique).

The mean dose of PGI_2 aerosolized was 0.87 ± 0.26 mg/kg/min. Doubling the concentration of the PGI_2 solution (860 ng/ml) at constant inspiratory flow, and hence presumably doubling the dose administered, did not enhance the effect on pulmonary or systemic hemodynamics. So far, we have not yet established the minimal effective dosage of inhaled PGI_2, therefore we cannot exclude that a higher concentration of the PGI_2 solution might have a more pronounced effect. Furthermore, it cannot be excluded that inhalation of PGI_2 at higher concentrations might affect systemic hemodynamics. In patients with bronchial asthma, systemic vasodilation has been reported following the short-time inhalation (maximal time of inhalation 3 min) of considerably higher doses of PGI_2 (200–400 μg) [31].

It should be noted that the dose of PGI_2 administered was assessed by measuring the volume of the PGI_2 solution in the nebulizer chamber before and after inhalation; this dose may, however, not reflect the actual amount of PGI_2 inhaled as it does not account for losses in the nebulizer, the ventilator tubing, the endotracheal tube and the extrapulmonary airways. Using radiolabeled aerosol, it has been demonstrated that during mechanical ventilation only a small fraction of a given dose of aerosol (0.37–3.68%) is actually deposited in the lung [32]. Therefore, the effective dose of inhaled PGI_2 may be considerably lower than calculated. Furthermore, it must be emphasized that the actual output of nebulizers is influenced by the type of nebulizer used, by the volume initially placed in the nebulizer and by the ventilator setting [33].

Still, the dose of PGI_2 aerosol effective in reducing HPV in this study is considerably lower than the dose of intravenously given PGI_2 necessary to lower PAP in different models of experimental pulmonary hypertension (249 ± 69 ng/kg/min [4], 79 ± 5 μg/kg/min [5]) or in patients with pulmonary hypertension of various origin (7.1–35.0 ng/kg/min [3, 29]. In 2 dogs the intravenous infusion of PGI_2 at a rate of 1 and 2 ng/kg/min – comparably to the inhaled dose – neither caused pulmonary nor systemic vasodilation and had no effect on CO (data not shown).

Our findings indicate that aerosolized PGI_2 at the concentration used in this study acts predominantly on smooth muscle cells in the lung vessels and does not reach the systemic circulation in efficient amounts. This view is supported by the fact that the arterial concentration of 6-keto-$PGF_{1\alpha}$, the main metabolite of PGI_2, did not increase during inhalation of prostacyclin. From our data, however, we cannot decide whether the selective pulmonary dilation was due to the low concentration of inhaled PGI_2 or whether it is exclusively the route of administration that prevents systemic effects of the drug.

A reduction in PAP and PVR has previously been reported upon inhalation of aerosolized arachidonic acid in dogs [34]. The pulmonary changes were, however, accompanied by systemic vasodilation and by bronchoconstriction indicating that prostaglandin precursors were absorbed and metabolized into different prostanoids in lung tissue. In contrast, prostacyclin administered as aerosol is neither transformed into an active form nor metabolized by the lung [26]. Although we did not measure dynamic compliance and airway resistance, we observed no change in total static compliance and in plateau airway pressure during PGI_2 inhalation. Hence, PGI_2

aerosolized at low concentrations appears to act as pulmonary vasodilator without causing side effects (e.g. bronchoconstriction) attributable to other arachidonic acid metabolites or to systemic absorption.

In this study, we compared PGI_2 aerosol to NO in order to assess the maximal dilation of pulmonary vessels that can be pharmacologically achieved during HPV without systemic side effects. We chose an inspiratory concentration of 50 ppm NO, because it has been demonstrated that a further increase of the inspiratory NO concentration produces virtually no additional vasodilatory effect [10]. In this study, the inhalation of NO at a concentration of 50 ppm produced a reduction of hypoxia-induced increases in PAP by 76% and in PVR by 73% accompanied by a slight decrease in arterial pressure (fig. 1) and no change in cardiac output. A complete reversal of hypoxic pulmonary vasoconstriction, as demonstrated in spontaneously breathing sheep at a NO concentration of 40 ppm [10], was not achieved in ventilated dogs in this study. The failure of NO to reverse HPV completely may be partially explained by species differences or by positive pressure ventilation producing a more heterogeneous distribution of pulmonary ventilation and blood flow. The slight systemic vasodilation observed was not reported in other studies [9, 10].

At the concentrations used, the magnitude of pulmonary vasodilation was more pronounced during NO than during PGI_2 inhalation. However, PGI_2 infused intravenously did not fully reverse HPV [5, 28]. Thus, inhaled NO may be the more potent pulmonary vasodilator when compared to prostacyclin at both routes of administration.

It must be noted that NO is a radical ($^{\cdot}N = O$) with the potential for cytotoxicity. Superoxide (O_2^-) and NO, which both are generated by endothelial cells, macrophages and neutrophils, combine to form peroxynitrite anion ($ONOO^-$). Peroxynitrite decomposition products are nitrogen dioxide and hydroxyl radical which exert cytotoxic effects by initiating lipid peroxidation of cell membranes [35]. In the presence of molecular oxygen, NO reacts in a concentration- and FiO_2-dependent manner to form nitrogen dioxide (NO_2), which is subsequently transformed to nitric and nitrous acid. Severe damage to the lung and airways may result. NO binds avidly to hemoglobin to form nitrosylhemoglobin (Hb-NO) which is subsequently oxidized to methemoglobin (Met-Hb). Reduction of Met-Hb occurs, however, much slower than Met-Hb formation from Hb-NO and the rate of Met-Hb reduction is decreased by increasing NO concentrations [36]. Although Met-Hb plasma concentrations at doses of NO inhaled for pulmonary vasodilation remain low (<1–2%), methemoglobinemia following inadvertent overdosage of NO may lead to critical reduction of oxygen transport. Oxidative cross-linking of membrane proteins of erythrocytes exposed to NO may impair their rheological properties [36]. Although there is little evidence for severe toxicity at low concentrations (<100 ppm) of NO inhaled for short time periods in different species, little is known about the toxicity of long-term breathing of NO at low concentrations and the effect of NO on lungs with pre-existing acute or chronic disease [10].

The feasibility of NO inhalation in clinical routine seems cumbersome: due to its potential toxicity, the application of gaseous NO requires specially designed respirators which permit exact adjustment and long-term constancy of the inspired NO concentration. Expensive chemiluminescence analyzers for continuous monitoring of the inspired NO concentration and for detection of NO oxidation products (NO_2) are mandatory and finally, NO gas has not been approved for medical purposes so far.

Thus, the clinical application of inhaled NO as a selective pulmonary vasodilator appears limited by the lack of data on long-term toxicity and effects on preinjured lungs, but also due to the necessity of complicated and expensive techniques to safely allow and control NO administration.

In contrast to the potential toxicity of NO, no severe toxic side effects of PGI_2 have been reported. Furthermore, PGI_2 can be administered as aerosol using a standard jet nebulizer and no sophisticated system to monitor the inspired PGI_2 concentration is required. In further studies the dose-response relationship for inhaled PGI_2 has to be established, the pulmonary vasodilatory effects have to be assessed in different models of pulmonary hypertension and the effects of long-term inhalation have to be investigated. Finally, due to the different mechanisms of action, the combination of inhaled NO and aerosolized PGI_2 may have additive pulmonary vasodilatory effects. Upon clarification of these open questions, PGI_2 aerosol may become an attractive alternative to the inhalation of NO in the treatment of acute pulmonary hypertension.

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