Combined inhaled nitric oxide and inhaled prostacyclin during experimental chronic pulmonary hypertension

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Hill, Laureen, L., and Ronald G. Pearl. Combined inhaled nitric oxide and inhaled prostacyclin during experimental chronic pulmonary hypertension. J. Appl. Physiol. 86(4): 1160–1164, 1999.—Inhaled nitric oxide (NO) and inhaled prostacyclin (PGI2) produce selective reductions in pulmonary vascular resistance (PVR) through differing mechanisms. NO decreases PVR via cGMP, and PGI2 produces pulmonary vasodilation via cAMP. As a general pharmacological principle, two drugs that produce similar effects via different mechanisms should have additive or synergistic effects when combined. We designed this study to investigate whether combined inhaled NO and PGI2 therapy results in additive effects during chronic pulmonary hypertension in the rat. Monocrotaline injected 4 wk before study produced pulmonary hypertension in all animals. Inhaled NO (20 parts/million) reversibly and selectively decreased pulmonary artery pressure (Ppa) with a mean reduction of 18%. Four concentrations of PGI2 were administered via inhalation (5, 10, 20, and 80 µg/ml), both alone and combined with inhaled NO. Inhaled PGI2 alone decreased Ppa in a dose-dependent manner with no change in mean systemic arterial pressure. Combined inhaled NO and PGI2 selectively and significantly decreased Ppa more did than either drug alone. The effects were additive at the lower concentrations of PGI2 (5, 10, and 20 µg/ml). The combination of inhaled NO and inhaled PGI2 may be useful in the management of pulmonary hypertension.

PULMONARY HYPERTENSION is a serious clinical problem with significant morbidity and mortality. Elevated pulmonary vascular resistance (PVR) may occur in patients with primary pulmonary hypertension, acute respiratory failure, chronic lung disease, and cardiac disease; after cardiopulmonary bypass; and in neonates. Pulmonary hypertension may produce right heart failure and death. Therapy is directed at reducing PVR. Intravenous vasodilators have been used to decrease PVR, but they also decrease systemic vascular resistance (SVR) and produce systemic hypotension, limiting the usefulness of these drugs. One strategy to selectively affect the pulmonary circulation is to deliver the drugs via inhalation. Inhaled agents with a short biological half-life will decrease pulmonary vascular tone but will be metabolized or inactivated before reaching the systemic circulation. Additionally, inhaled drugs will selectively dilate blood vessels in well-ventilated lung segments, thereby improving ventilation-perfusion relationships.

In 1987, nitric oxide (NO) was identified as endothelium-derived relaxing factor, an endogenous modulator of vascular tone. NO is produced in endothelial cells from L-arginine by NO synthase and is released in response to a variety of stimuli, including increased shear stress. It diffuses from endothelium to vascular smooth muscle where it stimulates guanylyl cyclase to generate cGMP, producing vasorelaxation. Inhaled NO selectively dilates the pulmonary circulation because any NO that diffuses into blood is rapidly bound to hemoglobin and inactivated.

Prostacyclin (PGI2) is an arachidonic acid metabolite that produces vasodilation via cAMP. It binds to cell-surface receptors, stimulating adenyl cyclase to produce cAMP. cAMP activates protein kinase A, leading to decreases in intracellular calcium and vasorelaxation. PGI2 also stimulates endothelial cell release of NO. PGI2 is released in response to mechanical, immunological, and chemical stimuli. Unlike other prostaglandins, PGI2 is not inactivated by the lung (16). It undergoes spontaneous hydrolysis at physiological pH and has a half-life of ~3 min, limiting systemic effects when delivered via inhalation.

As a general pharmacological principle, two drugs that produce similar effects via different mechanisms should have additive or synergistic effects. This principle has been observed in patients with pulmonary hypertension undergoing combined systemic drug therapy (13, 14). Synergistic activity of NO donors and PGI2 in vitro has been reported in porcine coronary arteries (31). The present study was designed to determine whether combined inhaled NO and inhaled PGI2 produces additive effects in an experimental model of chronic pulmonary hypertension. Monocrotaline provides a reproducible model of pulmonary hypertension in rats in which to test potential therapeutic agents (20, 21).

MATERIALS AND METHODS

Ten male Sprague-Dawley rats weighing 300–400 g were treated with monocrotaline (60 mg/kg sc) 3–4 wk before the study to produce pulmonary hypertension. On the day of study, rats were anesthetized with pentobarbital sodium (80 mg/kg sc), ketamine (40 mg/im), and atropine (0.28 mg/kg ip). Anesthesia was maintained with subcutaneous pentobarbital sodium (10 mg·kg·h–1·sc). A carotid artery catheter and two internal jugular venous catheters were inserted via bilateral neck cutdown. A pulmonary artery catheter created from heat-coiled microbe Tygon tubing was inserted via the jugular vein by using pressure waveforms for guidance. Hydroxyethyl starch 6% (10 ml/kg iv) was administered before pulmonary artery catheter placement as replacement...
for surgical blood loss, and a 2-ml bolus was administered intravenously every 2 h thereafter as maintenance fluid. After tracheostomy with a 16-gauge catheter, rats were mechanically ventilated by using a Sechrist pediatric pressure-cycled ventilator with gas flows of 2 l/min, 40% inspired O₂ fraction, 2 cmH₂O positive end-expiratory pressure, 12 cmH₂O peak inspiratory pressure (PIP), and a rate that was adjusted between 30 and 35 breaths/min to maintain arterial Pco₂ of 36–40 Torr. After baseline systemic (Psa) and pulmonary arterial (Ppa) pressure measurements, inhaled NO was added to the inspiratory limb of the circuit to produce an inspired concentration of 20 parts/million (ppm). Levels of NO were confirmed by chemiluminescence (Ecophysics). Psa and Ppa were recorded after 5 min of NO delivery and after a 10-min recovery after discontinuation of NO. PGI₂ solution (1 ml) was administered by jet nebulization (Hudson RCI, Temecula, CA) with oxygen (8 l/min) into the inspiratory limb of the circuit over a 1-min period while a PIP of 12 cmH₂O was maintained. This oxygen flow rate was chosen to produce a greater fraction of small particles (<2 µm), which are more likely to be delivered to the alveoli. Psa and Ppa were recorded at 5 min after the start of drug delivery, which corresponded to the maximal pulmonary vasodilation response. After a recovery period of 20 min, hemodynamic measurements were repeated to establish that values had returned to baseline. Inhaled NO alone for 10 min, and then with inhaled PGI₂ nebulized over 1 min, was administered, and Psa and Ppa were recorded as before. Four concentrations of PGI₂ (5, 10, 20, and 80 µg/ml) were studied with and without NO in randomized order in each animal. The order of administration of NO and PGI₂ was alternated in each animal.

Statistical analysis was performed by using two-way repeated measures ANOVA and the Newman-Keuls test for multiple comparisons. Statistical significance was considered P < 0.05.

RESULTS

Monocrotaline produced pulmonary hypertension in all rats (Fig. 1). Normal Ppa values in our laboratory are 11–14 mmHg (1). Inhaled NO decreased Ppa in all animals studied, with a mean decrease of 18% (Figs. 1 and 2). This effect was reversible with discontinuation of inhaled NO. Psa did not change with inhaled NO.

Fig. 1. Pulmonary artery pressure (Ppa) before and during nebulization of increasing concentrations of prostacyclin (PGI₂) with and without inhaled nitric oxide (NO). Values are means ± SE. Inhaled NO and all doses of prostacyclin significantly decreased Ppa with no significant interaction by ANOVA. *P < 0.05 compared with corresponding group.

Inhaled PGI₂ decreased Ppa in a dose-dependent manner (Fig. 1) with no change in Psa, and the magnitude of the decrease in Ppa at the lower doses of PGI₂ (5, 10, and 20 µg/ml) was similar to that achieved with inhaled NO alone (Fig. 2). The highest dose of PGI₂ (80 µg/ml) decreased Ppa significantly more than did inhaled NO alone. Combined inhaled NO and PGI₂ decreased Ppa...
and did not affect Psa. At all doses of PGI2, the effect of combined inhaled NO + PGI2 was statistically greater than with either agent alone (Fig. 2). There was no significant interaction by ANOVA between the effects of inhaled NO and inhaled PGI2.

**DISCUSSION**

In the present study, monocrotaline produced stable pulmonary hypertension in all animals. Monocrotaline is a pyrrolizidine alkaloid compound extracted from Crotalaria spectabilis with known toxicity in various organs, including the liver and lungs (18). It is metabolized in the liver to produce reactive pyrroles. These substances bind to pulmonary endothelial cells, causing vascular injury, inflammation, pulmonary edema, hemorrhage, and interstitial fibrosis (19). Medial thickening and muscularization of pulmonary arteries and arterioles result in pulmonary hypertension and right heart enlargement. Monocrotaline-induced pulmonary injury in rats has been advocated as a reproducible model of pulmonary hypertension in which to test potential therapeutic interventions (11, 19, 21).

Inhaled NO has been shown to decrease PVR and/or improve ventilation-perfusion matching in experimental pulmonary hypertension produced by hypoxia (25), thromboxane analog administration (9), endotoxin (36), and monocrotaline (16). In humans, the selective pulmonary vasodilation and improved oxygenation effects of inhaled NO make it useful in treating primary pulmonary hypertension and pulmonary hypertension associated with underlying lung injury or after cardiopulmonary bypass (2, 24, 27–29, 38).

Studies evaluating effective doses of inhaled NO in rats have produced varying results. In their study of monocrotaline-induced pulmonary hypertension, Katakama et al. (16) demonstrated a maximal decrease in elevated Ppa by using inhaled NO at 60 ppm. Dose-response studies in other animal models of pulmonary hypertension have demonstrated maximal response at inhaled NO concentrations as low as 5–10 ppm (8, 33), whereas Emil et al. have shown increasing responses to higher doses of NO related to the degree of hypoxia present (6, 7). Earlier dose-response studies in our laboratory in rats with monocrotaline-induced pulmonary hypertension have demonstrated maximum pulmonary vasodilation with use of inhaled NO concentrations of 5 ppm (unpublished observations). We selected a concentration of 20 ppm in this study to ensure maximal NO response. This dose produced a consistent and reversible reduction in Ppa.

PGI2 when administered intravenously produces significant reduction in Ppa, but its use is limited by systemic hypotension and an increase in intrapulmonary shunting (26, 30, 39). Animal studies investigating hypoxic pulmonary vasoconstriction and thromboxane-induced pulmonary hypertension have demonstrated that inhaled PGI2 produces a dose-dependent and selective reduction in Ppa (3, 37, 39). In their study comparing the effects of inhaled PGI2 and inhaled NO in sheep, Welte et al. (37) delivered PGI2 aerosol at a rate of 0.87 ± 0.26 ng·kg⁻¹·min⁻¹ to decrease hypoxia-induced elevated Ppa. Zobel et al. (39) delivered 30–60 ng·kg⁻¹·min⁻¹ nebulized PGI2 to piglets with acute respiratory failure and pulmonary hypertension to produce selective pulmonary vasodilation. Clinical studies have shown that PGI2 aerosol is effective in selectively reducing Ppa in acute respiratory distress syndrome, severe pulmonary hypertension, and after cardiopulmonary bypass (12, 23, 35). Clinical trials evaluating inhaled PGI2 suggest that the doses needed to produce substantial, selective pulmonary vasodilation range from 1 to 50 ng·kg⁻¹·min⁻¹ (23, 34, 40). The doses of inhaled PGI2 used to produce pulmonary vasodilation have been variable and difficult to measure accurately. The variability and uncertainty in PGI2 dosing is due to different methods of delivery (ultrasonic vs. jet nebulization) and the inability to accurately measure the amount of inhaled PGI2 reaching the alveolar space. Estimates of aerosol fraction deposited in the alveolar space are small, generally <5–10% (10, 18, 32). In this study, we nebulized a 1-ml PGI2 solution. The large majority of the drug was never inhaled, because we were delivering 8 l/min gas flow while the animal's minute ventilation was ~40 ml/min. In addition, we cannot determine the exact amount of inhaled drug reaching the alveolar space; however, we were able to demonstrate greater effects on Ppa with increasing PGI2 concentrations.

Our results and analyses are based on changes in Ppa without measurements of pulmonary blood flow. Although we did not measure cardiac output, neither inhaled NO nor PGI2 has depressant effects on cardiac output. In fact, cardiac output may have increased with therapy due to decreased PVR with both agents and decreased SVR with PGI2. The observed decreases in Ppa should therefore have represented at least as large a decrease in PVR.

Clinical experience with inhaled NO demonstrates a ceiling effect, suggesting that the vascular smooth muscle relaxation produced by guanylyl cyclase activation is limited. Studies have demonstrated that drugs acting via cGMP, such as nitroglycerin or sodium nitroprusside, do not produce additional vasodilation when administered with inhaled NO (1, 33). Drugs that act via different mechanisms would be expected to have additive or synergistic effects when administered together. Shimokawa and colleagues (31) observed potentiation of the vasodilatory effects of PGI2 in the presence of endothelium-derived relaxing factor in isolated porcine coronary arteries. In their study evaluating microcirculatory effects in hamsters, de Wit et al. (5) demonstrated that drugs that increase cAM P produce greater vasodilation when combined with drugs that increase cGMP. This was also shown in clinical studies where oral beraprost, a PGI2 analog, combined with inhaled NO produced greater pulmonary vasodilation than did either drug alone in children with pulmonary hypertension (13, 14). In our study, we observed that combined therapy with inhaled NO and inhaled PGI2 produced a greater decrease in Ppa than did either drug administered alone. We did not demonstrate a ceiling effect with the doses of PGI2 studied, and it is possible that increased doses of PGI2 could achieve a greater
reduction in PPa than that observed in this study, but we know from other published studies that PGI₂ loses its pulmonary selectivity at higher doses (39). The obvious advantage of combination therapy is the ability to produce increased desired effects while minimizing potential dose-related side effects or toxicities such as methemoglobinemia or systemic hypotension.

The results of our study suggest that combined inhaled NO and inhaled PGI₂ may be a useful therapy in the management of patients with pulmonary hypertension. We evaluated the acute effects of combined drug therapy, but it may also be effective as an extended treatment modality. Bigatello et al. (2) studied the effects of long-term therapy with low-dose inhaled NO (2–20 ppm) in patients with pulmonary hypertension secondary to acute respiratory distress syndrome and observed no evidence of tachyphylaxis or toxicity in patients treated for up to 27 days. Channick et al. (4) have demonstrated the efficacy of chronic inhaled NO in patients with primary pulmonary hypertension. Desensitization of the PG1₂ receptor has been described after prolonged exposure, but daily inhaled administration in a patient with severe pulmonary hypertension associated with CREST syndrome demonstrated sustained responsiveness of the pulmonary vasculature over a 1-yr period (23). Clinical trials to evaluate the efficacy of combined inhaled NO and inhaled PGI₂ therapy in patients with pulmonary hypertension, to determine optimal drug dosing and delivery strategies, and to study the responsiveness and potential side effects of long-term therapy should be considered.

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