Potent effects of aerosol compared with intravenous treprostinil on the pulmonary circulation

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Submitted 24 January 2005; accepted in final form 20 July 2005

Inhalation delivery of pulmonary vasodilators has potential advantages over systemic delivery. Aerosols only reach ventilated areas of the lung, and local vasodilation in those areas should improve ventilation-perfusion matching and oxygenation, thereby complementing the effects of hypoxic vasoconstriction. Limited experience in patients with acute lung injury has shown that aerosolized prostacyclin can improve shunt fraction and oxygenation and reduce pulmonary vascular resistance (30, 31). If the biological effects are limited to the lung, then systemic side effects should be avoided.

Iloprost is a carbacyclin analog of prostacyclin that is currently used in Europe. It has been administered by aerosol and intravenously to children with pulmonary hypertension due to congenital heart disease. Delivery by either route decreased mean pulmonary arterial pressure and pulmonary vascular resistance. Given intravenously, the drug caused a significant decrease in systemic blood pressure that was not observed with aerosol (9). In a study of 35 patients with primary pulmonary hypertension, inhaled iloprost reduced mean pulmonary arterial pressure and pulmonary vascular resistance significantly more than inhaled nitric oxide (10). Prostacyclin delivered by aerosol to patients with primary pulmonary hypertension or scleroderma-associated pulmonary hypertension reduced both pulmonary vascular resistance and pulmonary arterial pressure significantly, but it also decreased systemic vascular resistance and increased cardiac output (20).

Another prostacyclin analog, treprostinil (Remodulin, United Therapeutics), has been recently introduced for treatment of pulmonary hypertension. The drug is delivered by subcutaneous infusion, eliminating the need for an indwelling catheter; however, pain at the injection site is a significant problem. Treprostinil also has a longer half-life than prostacyclin and iloprost, leading to less rebound if the medication is abruptly discontinued.

Because of the potential advantages of aerosol delivery and the encouraging clinical studies with prostacyclin and other analogs, we conducted studies to compare the vasodilatory potential effects of aerosol compared with intravenous treprostinil on the pulmonary circulation. J Appl Physiol 99: 2363–2368, 2005. First published September 1, 2005; doi:10.1152/japplphysiol.00083.2005. — Inhaled vasodilator therapy for pulmonary hypertension may decrease the systemic side effects commonly observed with systemic administration. Inhaled medications only reach ventilated areas of the lung, so local vasodilation may improve ventilation-perfusion matching and oxygenation. We compared the effects of intravenous vs. aerosolized treprostinil on pulmonary and systemic hemodynamics in an unanesthetized sheep model of sustained acute pulmonary hypertension. Acute, stable pulmonary hypertension was induced in instrumented unanesthetized sheep by infusing a PGH2 analog, U-44069. The sheep were then administered identical doses of treprostinil either intravenously or by aerosol. Systemic and pulmonary hemodynamics were recorded during each administration. Both intravenous and aerosol delivery of treprostinil reduced pulmonary vascular resistance and pulmonary arterial pressure, but the effect was significantly greater with aerosol delivery ($P < 0.05$). Aerosol delivery of treprostinil had minimal effects on systemic hemodynamics, whereas intravenous delivery increased heart rate and cardiac output and decreased left atrial pressure and systemic blood pressure. Aerosol delivery of the prostacyclin analog treprostinil has a greater vasodilatory effect in the lung with minimal alterations in systemic hemodynamics compared with intravenous delivery of the drug. We speculate that this may result from treprostinil stimulated production of vasodilatory mediators from pulmonary epithelium.

PULMONARY ARTERIAL HYPERTENSION is commonly thought to be a consequence of long-standing vascular remodeling characterized by proliferation of vascular smooth muscle cells, endothelial cells and extracellular matrix (2, 11, 13). However, it can also occur abruptly as is seen in acute lung injury. In such setting, it is believed to be because of active vasoconstriction rather than remodeling. Local vasoconstriction resulting from alveolar hypoxia acts to improve ventilation-perfusion matching. Global pulmonary vasoconstriction may result from an imbalance between endogenous vasoconstricting and vasodilating mediators (17).

Intravenous prostacyclin has improved survival and exercise tolerance in the chronic forms of pulmonary hypertension and has been the cornerstone of treatment for the last several years (1). However, administration requires a central venous catheter, and the short half-life of the drug requires continuous infusion. Systemic administration in acute lung injury has been shown to cause increasing shunt fraction with worsening oxygenation (24). Systemic effects of prostacyclin include hypotension, alterations in cardiac output, nausea and vomiting, headache, and rash.

Inhaled delivery of pulmonary vasodilators has potential advantages over systemic delivery. Aerosols only reach ventilated areas of the lung, and local vasodilation in those areas should improve ventilation-perfusion matching and oxygenation, thereby complementing the effects of hypoxic vasoconstriction. Limited experience in patients with acute lung injury has shown that aerosolized prostacyclin can improve shunt fraction and oxygenation and reduce pulmonary vascular resistance (30, 31). If the biological effects are limited to the lung, then systemic side effects should be avoided.

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Because of the potential advantages of aerosol delivery and the encouraging clinical studies with prostacyclin and other analogs, we conducted studies to compare the vasodilatory
activity of aerosol vs. intravenous treprostinil. We developed a model of stable acute pulmonary hypertension in chronically instrumented unanesthetized sheep and determined effects of identical doses of treprostinil delivered either by aerosol or intravenously.

METHODS

Surgical preparation. Six yearling sheep (3 males, 3 females; 21–37 kg) were fasted for 18–24 h then sedated with thiopental to allow for intubation. Surgical procedures were performed with the sheep receiving 1.5–2.5% halothane. A left thoracotomy was performed, and a Transonic blood flow probe (Transonic Bloodflow Meter, Ithaca, NY) was placed around the main pulmonary artery, and Silastic catheters were placed in the main pulmonary artery and left atrium. Sheep were allowed to recover for 7 days. Subsequently, the sheep were reanesthetized and a catheter was inserted into the left carotid artery, a Cordis Introducer Sheath was inserted in the left jugular vein, and a tracheostomy was performed. The sheep were allowed to recover for an additional 3–5 days before experimentation. This instrumentation was used to measure pulmonary arterial pressure, left atrial pressure, central venous pressure, systemic arterial pressure, heart rate, and cardiac output. Cardiac output was measured on a Transonic Systems T101 Ultrasonic Bloodflow Meter. Pressures were monitored with Hewlett-Packard transducers (model 1290A), recorded on Astromed MT-9500 Stripchart Recorder, and digitally recorded with Easy Data Acquisition Software. Drug aerosolization was performed with a Healthline Medical AM-601 Medicator Aerosol Delivery System. Intravenous infusions were via a Manostat Casette Pump. Sheep procurement, housing, surgical procedures and experimental protocols were approved by the Vanderbilt University Animal Care Committee and overseen by the Vanderbilt Division of Animal Care.

Induction of pulmonary hypertension. Acute pulmonary hypertension was induced with an infusion of the PGH2 analog, U-44069 (9,11-dideoxy-9α,11α-epoxymethanoprostaglandin F2α). This substance is similar to endogenously formed thromboxane A2 and can be titrated to induce the desired degree of pulmonary vasoconstriction. U-44069 was mixed with sterile normal saline and was protected from light by wrapping the saline bag with aluminum foil. Previous experiments had determined that U-44069 infused at 1,000 ng·kg−1·min−1 elevated the pulmonary vascular resistance to approximately four times baseline. Pulmonary vascular resistance was calculated as (mean pulmonary arterial pressure − left atrial pressure)/cardiac output. After a 30-min period of baseline hemodynamic measurements, four sheep received U-44069 at 1,000 ng·kg−1·min−1 for 180 min to demonstrate its ability to maintain a steady-state increase in pulmonary vascular resistance.

Experimental protocol. Each sheep underwent 30 min of baseline measurements followed by a U-44069 infusion at 1,000 ng·kg−1·min−1. After each sheep was allowed to reach steady state for 30–60 min, treprostinil was infused at 250, 500, and 1,000 ng·kg−1·min−1. Each infusion lasted 30–60 min. The experiment was repeated with the same dose of U-44069 but with the treprostinil delivered via aerosol at 0.28 ml/min in escalating doses of 250, 500, and 1,000 ng·kg−1·min−1. Pulmonary and systemic hemodynamic measurements were recorded at each dose for each route of administration.

To evaluate the duration of action of vasodilator aerosols, we delivered treprostinil for 30 min at 1,000 ng·kg−1·min−1 after reaching a steady-state elevation in pulmonary vascular resistance with U-44069. At the end of 30 min, the treprostinil was stopped, and the U-44069 infusion was continued for an additional 30 min to estimate the duration of action of the medication by following the return toward the steady-state pulmonary vascular resistance. As a comparison, this experiment was repeated using aerosol epoprostenol at 1,000 ng·kg−1·min−1. In addition, arterial blood gases were drawn to follow changes in oxygenation.

Statistical analysis. One-way repeated-measures ANOVA and Dunnett’s method were used to test for statistical significance during the U-44069 steady-state experiment. Two-way repeated-measures ANOVA and the Student-Newman-Keuls test were used to compare data for the remaining experiments. Significance was assumed for values of P < 0.05 for all experiments.

RESULTS

As shown in Fig. 1, infusion of the thromboxane analog, U-44069, at 1,000 ng·kg−1·min−1 produced a stable increase in pulmonary vascular resistance to almost four times the baseline value; the steady-state pulmonary hypertension remained constant throughout the 180-min infusion. Similarly, U-44069 caused a significant increase in pulmonary arterial pressure as illustrated in Fig. 2B. The U-44069 also had significant effects on systemic hemodynamics. As shown in Fig. 3A there was a significant drop in cardiac output during the infusion of U-44069 before administration of the medication. Similarly, there was, on average, a decrease in heart rate during U-44069 that did not reach statistical significance (Fig. 3B). With the decreased cardiac output and heart rate there was, on average, an increase in the directly measured left atrial pressure that did not reach significance (Fig. 3C). The vasoconstricting properties of intravenously administered U-44069 also increased the average systemic arterial blood pressure, but this did not reach statistical significance (Fig. 3D).

During a stable period of pulmonary hypertension produced by infusing the thromboxane analog, sheep received treprostinil either intravenously or by aerosol. The same doses of the drug were delivered by either route, although the actual amount of drug delivered to the lungs with aerosol was considerably less than that delivered intravenously because of the inefficiency of aerosol delivery systems. Figures 2 and 3 summarize the hemodynamic effects of treprostinil.

Intravenous delivery of treprostinil caused a dose-related decrease in both pulmonary vascular resistance and pulmonary arterial pressure in a dose-dependent manner (Fig. 2, open symbols). Effects were seen even at the lowest dose (250 ng·kg−1·min−1) infused, and further vasodilation occurred with increasing doses. However, even at the highest dose
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Fig. 2. A: U-44069 infusion causes a significant increase in PVR. Intravenous (IV) treprostinil caused a dose-dependent decrease in PVR that remained significantly elevated above baseline (*P < 0.05). Aerosol treprostinil caused a dose-dependent decrease in PVR that was significantly lower compared with intravenous delivery (*P < 0.05). B: U-44069 caused a significant increase in mean pulmonary arterial pressure. Intravenous treprostinil caused a dose-dependent decrease in pulmonary arterial pressure that remained significantly elevated above baseline (*P < 0.05). Aerosol delivery caused a dose-dependent decrease in pulmonary arterial pressure that was significantly lower compared with intravenous delivery (*P < 0.05). Values are means ± SE; n = 6 animals.

(1,000 ng·kg⁻¹·min⁻¹), neither pulmonary vascular resistance nor pulmonary arterial pressure returned to baseline levels. Effects of intravenous treprostinil on systemic hemodynamics are summarized in Fig. 3 (open symbols). Infusion of the drug caused a dose-related increase in cardiac output and heart rate and a dose-related decrease in left atrial pressure and systemic arterial pressure. At doses that were necessary to cause substantial pulmonary vasodilation, there were significant alterations in systemic hemodynamics.

The effectiveness of treprostinil as a pulmonary vasodilator was much greater when the drug was delivered by aerosol than when it was delivered intravenously. As shown in Fig. 2 (solid symbols), aerosol treprostinil reduced both pulmonary vascular resistance and pulmonary arterial pressure significantly even at a dose of 250 ng·kg⁻¹·min⁻¹. At the highest dose (1,000 ng·kg⁻¹·min⁻¹), aerosol delivery of the drug returned both pulmonary vascular resistance and pulmonary artery pressure to baseline levels even though infusion of the vasoconstrictor continued. This marked pulmonary vasodilation was achieved with minimal effects on systemic hemodynamics. As shown in Fig. 3 (solid symbols), aerosol delivery of the drug caused no significant changes in cardiac output or heart rate even at the highest dose. Systemic arterial pressure also did not change significantly even at the highest dose of the drug. The only significant hemodynamic effect that we observed was a small increase in left atrial pressure that occurred at the lowest dose and did not change further with higher doses of the drug.

The duration of action of aerosol treprostinil was much greater than that observed with aerosol epoprostenol as seen in Fig. 4. Within 10 min of stopping the epoprostenol, the pulmonary vascular resistance was almost back to steady state. However, 30 min after stopping treprostinil, the pulmonary vascular resistance remained less than steady state. The slope of the off-transient indicates that the duration of effect of treprostinil was about three times that of epoprostenol. Arterial blood-gas data showed that the oxygen saturation remained above 90% throughout the experiments.

DISCUSSION

Although, at least in its later stages when usually diagnosed, pulmonary hypertension is characterized by extensive remodeling of the pulmonary vascular bed. The hypertension is sometimes partially reversible by administration of vasodilators like nitric oxide and iloprost by inhalation or prostacyclin by either intravenous infusion or inhalation (1, 10, 20). In the primary form of the disease, urinary concentrations of prostanoids show increased production of the pulmonary vasoconstrictor thromboxane relative to the vasodilator prostacyclin, implicating this prostanoid imbalance as a possible cause of some degree of persistent pulmonary vasoconstriction (3). Improved survival from chronic administration of prostacyclin in patients with primary pulmonary hypertension suggests effects on the progressive remodeling process. Although not demonstrated in humans with pulmonary hypertension after prolonged treatment, in vitro studies suggest that prostacyclin can alter smooth muscle proliferation (4). Whether the mechanism of the effects of chronic prostacyclin administration is similar to that for acute vasodilation is unknown.

In acute lung injury, in which pulmonary hypertension contributes to hypoxemia, inhaled vasodilators have shown significant improvements in pulmonary vascular resistance, shunt fraction, and oxygenation (30, 31). In this setting, pulmonary hypertension is initially caused by hypoxic vasoconstriction and an imbalance of endogenous vasoactive substances. However, in the later stages of acute lung injury vascular, remodeling occurs and is characterized by concentric deposition of fibrin, hyperplasia of endothelial cells, and medial hypertrophy. This has been shown to occur in the small muscular arteries, veins, and lymphatics (29).

To test the acute vasodilatory effects of treprostinil, a prostacyclin analog that is in clinical use, we produced stable pulmonary vasoconstriction in chronically instrumented unanesthetized sheep by infusing an analog of thromboxane, U-44069 (23). Constant infusion of this drug produces a stable increase in pulmonary vascular resistance and pulmonary arterial pressure that is directly related to the infusion rate of the drug, permitting testing of vasodilator responses in the precon-
stricted pulmonary vascular bed. Such data may be relevant to acute lung injury-associated pulmonary hypertension in humans because an imbalance between endogenous vasoconstrictors such as thromboxane and vasodilators such as nitric oxide may facilitate the elevation in pulmonary arterial pressures. However, this acute model does not reproduce the structural alterations in the pulmonary vascular bed typical of pulmonary arterial hypertension. This approach is similar to that others have used to evaluate vasodilator potency (12, 15, 26).

In our studies, treprostinil delivered either by aerosol or intravenously caused a dose-dependent decrease in pulmonary arterial pressure and systemic arterial pressure. A: intravenous treprostinil caused a dose-dependent increase in cardiac output, whereas there was no change from aerosol treprostinil. B: intravenous treprostinil caused a dose-dependent increase in heart rate that was significantly elevated above baseline at the highest dose (*P < 0.05), whereas aerosol delivery caused no change. C: intravenous treprostinil caused a significant decrease in left atrial pressure relative to U-44069 and baseline at the higher doses (*P < 0.05). Aerosol delivery caused an increase in left atrial pressure that was significantly above U-44069 and baseline (*P < 0.05) at the lower doses. D: intravenous and aerosol treprostinil caused a nonsignificant dose-dependent decrease in systemic arterial pressure. Values are means ± SE; n = 6 animals. bpm, Beats/min.

Fig. 3. U-44069 caused a decrease in cardiac output (*P < 0.05) and heart rate (not significant), whereas it caused nonsignificant increases in left atrial pressure and systemic arterial pressure. A: intravenous treprostinil caused a dose-dependent increase in cardiac output, whereas there was no change from aerosol treprostinil. B: intravenous treprostinil caused a dose-dependent increase in heart rate that was significantly elevated above baseline at the highest dose (*P < 0.05), whereas aerosol delivery caused no change. C: intravenous treprostinil caused a significant decrease in left atrial pressure relative to U-44069 (##P < 0.05) and baseline (##P < 0.05) at the higher doses. D: intravenous and aerosol treprostinil caused a nonsignificant dose-dependent decrease in systemic arterial pressure. Values are means ± SE; n = 6 animals. bpm, Beats/min.

Fig. 4. U-44069 was infused at 1,000 ng·kg⁻¹·min⁻¹ to achieve a steady-state elevation of PVR for up to 70 min. The sheep received either epoprostenol or treprostinil aerosol at 1,000 ng·kg⁻¹·min⁻¹ for a period of 30 min. Within 10 min, the PVR had almost returned to baseline with epoprostenol. After 30 min, the PVR had increased but not returned to steady state after treprostinil administration.
Airway epithelial cells can produce "relaxant factors" that have been studied mostly in relation to airway rather than vascular function (7). Whether prostacyclin stimulated lung epithelial cells to produce vasodilatory mediators that amplify the direct effects of the drug has not been investigated. However, in response to hydrostatic pressure, prostacyclin produced in bone cells activates the transcription factor activator protein-1, and the prostacyclin analog iloprost caused a similar response in cultured bone cells (8). The activator protein-1 family of transcription factors is involved in numerous processes in the lung, including inflammation, apoptosis, and cell proliferation (21, 28). Activator protein-1 also increases expression of inducible nitric oxide synthase that could enhance vasodilation (15). Activator protein-1 has been found to be involved in the signal transduction of bone morphogenetic protein (14), and mutations of a bone morphogenetic protein receptor (BMPR2) is causally implicated in a familial form of primary pulmonary hypertension (18). We have preliminary data indicating that prostacyclin increases expression of activator protein-1 and several activator protein-1-regulated genes in human airway epithelial cells in culture (25).

Our studies and what other data are available indicate that prostacyclin analogs are more potent pulmonary vasodilators when delivered by aerosol than when given intravenously. Systemic hemodynamic effects are also minimized by aerosol administration of the drug, making this approach especially appealing. Duration of action of prostacyclin is short, requiring an unrealistic frequency of administration for clinical use (6, 33), but development of analogs (e.g., treprostinil) or formulations that are long acting could make this approach feasible. Also given the high cost of these medications, delivery via aerosol may provide a monetary benefit if less total drug can be given on a daily basis by using intermittent inhalation compared with continuous infusion. The reason for an enhanced pulmonary vasodilatory effect with aerosol administration is not yet clear, but we speculate that the effect may be mediated by effects of prostacyclin on epithelial cells, possibly a consequence of activation of the transcription factor activator protein-1.

DISCLOSURES

This work was supported by a grant from United Therapeutics Corporation to Vanderbilt University and was conducted in the Division of Allergy, Pulmonary, and Critical Care Medicine, Department of Medicine, Vanderbilt University School of Medicine.

REFERENCES


