Pulmonary Vascular Effects of Vasodilators

Potent Effects of Aerosol Compared to Intravenous Treprostinil on the Pulmonary Circulation

Brett L. Sandifer¹,³, Kenneth L. Brigham¹,²,³, E. Clinton Lawrence¹,³, David Mottola⁴, Chris Cuppels¹,² and Richard E. Parker¹,²

¹Division of Pulmonary, Allergy and Critical Care
²Center for Translational Research in the Lung
³McKelvey Center for Lung Transplantation
Emory University School of Medicine
Atlanta, Georgia
and
⁴United Therapeutics, Corp.
Research Triangle Park, NC

Address requests for reprints and all correspondence to: (after publication)

Richard E. Parker, PhD, 615 Michael Street, Suite 250, Whitehead Research Building, Atlanta GA 30322, phone 404-712-2550, fax 404-727-2336, email reparke@emory.edu

Address all correspondence to: (before publication)

Brett L. Sandifer, MD, 615 Michael Street, Suite 205, Whitehead Research Building, Atlanta, GA 30322, phone 404-712-8228, fax 404-712-2974, email blsandifer@yahoo.com
Abstract

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 versus 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, U44069. 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 artery pressure but the effect was significantly greater with aerosol delivery (p<0.05). Aerosol delivery of treprostinil had minimal effects on systemic hemodynamics while 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 to intravenous delivery of the drug. We speculate that this may result from treprostinil stimulated production of vasodilatory mediators from pulmonary epithelium.

Key Words – pulmonary hypertension, aerosols, transcription factor AP-1, epoprostenol
Introduction

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 \((2,10,12)\). However it can also occur abruptly as is seen in acute lung injury. In such setting it is felt to be due to active vasoconstriction rather than remodeling. Local vasoconstriction resulting from alveolar hypoxia acts to improve V/Q matching. Global pulmonary vasoconstriction may result from an imbalance between endogenous vasoconstricting and vasodilating mediators \((16)\).

Intravenous prostacyclin \((\text{PGI}_2)\) 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 \((22)\). 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 (27,28). If the biologic effects are limited to the lung, then systemic side effects should be avoided.

Iloprost is a carbacyclin analogue of PG\textsubscript{I\textsubscript{2}} 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 artery pressure (mPAP) and pulmonary vascular resistance (PVR). Given intravenously, the drug caused a significant decrease in systemic blood pressure that was not observed with aerosol (8). In a study of 35 patients with primary pulmonary hypertension inhaled iloprost reduced mPAP and PVR significantly more than inhaled nitric oxide (iNO) (9). Prostacyclin delivered by aerosol to patients with primary pulmonary hypertension or scleroderma-associated pulmonary hypertension reduced both PVR and PAP significantly, but also decreased systemic vascular resistance and increased cardiac output (19).

Another prostacyclin analog, treprostinil (Remodulin, United Therapeutics, Corp.) 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 activity of aerosol versus 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 hours then sedated with thiopental to allow for intubation. Surgical procedures were performed with the sheep receiving halothane 1.5-2.5%. 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 re-anesthetized and a catheter was inserted into the left carotid artery, a Cordis Introducer Sheath inserted in the left jugular vein and a tracheostomy was performed. The sheep were allowed to recover for an additional 3-5 days prior to experimentation. This instrumentation was used to measure pulmonary artery pressure (PAP), left atrial pressure (PLA), central venous pressure (CVP), systemic arterial pressure (PSA), heart rate (HR) and cardiac output (CO). 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 Cassette 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 PGH$_2$ analog, U44069 (9,11-dideoxy-9α, 11α-epoxymethanoprostaglandin F$_{2α}$). This substance is similar to endogenously formed thromboxane A2 and can be titrated to induce the desired degree of pulmonary vasoconstriction. U44069 was mixed with sterile normal saline and was protected from light by wrapping the saline bag with aluminum foil. Previous experiments had determined that U44069 infused at 1000 ng/kg/min elevated the pulmonary vascular resistance (PVR) to approximately four times baseline. Pulmonary vascular resistance was calculated as (mean PAP-PLA)/CO. After a 30 minute period of baseline hemodynamic measurements, four sheep received U44069 at 1000 ng/kg/min for 180 minutes to demonstrate its ability to maintain a steady state increase in PVR.

Experimental Protocol

Each sheep underwent 30 minutes of baseline measurements followed by a U44069 infusion at 1000 ng/kg/min. After allowing each sheep to reach steady state for 30 to 60 minutes, treprostinil was infused at 250, 500 and 1000 ng/kg/min. Each infusion lasted 30 to 60 minutes. The experiment was repeated with the same dose of U44069 but with the treprostinil delivered via aerosol at 0.28 cc/min in escalating doses of 250, 500 and 1000 ng/kg/min. Pulmonary and systemic hemodynamic measurements were recorded at each dose for each route of administration.
In order to evaluate the duration of action of vasodilator aerosols, we delivered treprostinil for 30 minutes at 1000 ng/kg/min after reaching a steady state elevation in PVR with U44069. At the end of 30 minutes the treprostinil was stopped and the U44069 infusion was continued for an additional 30 minutes to estimate the duration of action of the medication by following the return toward the steady state PVR. As a comparison this experiment was repeated using aerosol epoprostenol at 1000 ng/kg/min. 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 U44069 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 figure 1, infusion of the thromboxane analog, U44069, at 1000 ng/kg/min produced a stable increase in pulmonary vascular resistance to almost 4 times the baseline value; the steady state pulmonary hypertension remained constant throughout the 180 minute infusion. Similarly U44069 caused a significant increase in pulmonary artery pressure as illustrated in figure 2B. The U44069 also had significant effects on systemic hemodynamics as shown in figure 3A there was a significant drop in cardiac output during the infusion of U44069 prior to administration of the medication. Likewise there was, on average, a decrease in heart rate during U44069 that did not reach statistical significance (figure 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 (figure 3C). The vasoconstricting properties of intravenously administered U44069 also increased the average systemic arterial blood pressure, but this did not reach statistical significance (figure 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 artery pressure in a dose dependant manner (figure 2, open symbols). Effects were seen even at the lowest dose (250 ng/kg/min) infused and further vasodilation occurred with increasing doses. However, even at the highest dose (1000 ng/kg/min) neither pulmonary vascular resistance nor pulmonary artery pressure returned to baseline levels. Effects of intravenous treprostinil on systemic hemodynamics are summarized in figure 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 the closed symbols in figure 2, aerosol treprostinil reduced both pulmonary vascular resistance and pulmonary artery pressure significantly even at a dose of 250 ng/kg/min. At the highest dose (1000 ng/kg/min) aerosol delivery of the drug returned both PVR 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 figure 3 (closed 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 Figure 4. Within 10 minutes of stopping the epoprostenol, the PVR was almost back to steady state. However, 30 minutes after stopping treprostinil, the PVR 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 (1,9,19). 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. Though 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 PVR, shunt fraction and oxygenation (27,28). 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 (26).

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, U44069 (21). Constant infusion of this drug produces a stable increase in pulmonary vascular resistance and pulmonary artery pressure that is directly related to the infusion rate of the drug, permitting testing of vasodilator responses in the preconstricted pulmonary vascular bed. Such data may be relevant to acute lung injury associated pulmonary hypertension in humans since an imbalance between endogenous vasoconstrictors such as thromboxane and vasodilators such as nitric oxide may facilitate the elevation in pulmonary artery 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 (11,14,23).

In our studies, treprostinil delivered either by aerosol or intravenously caused a dose dependant decrease in both pulmonary artery pressure and pulmonary vascular resistance in the preconstricted pulmonary vascular bed. Surprisingly, aerosol delivery of the drug had a much greater vasodilatory effect than intravenous delivery. This difference is especially striking in light of the fact that
we delivered the same doses of drug by either route. Continuous aerosol delivery is notoriously inefficient, delivering 0 to 42% of the nebulized dose to the lower respiratory tract (5).

Several studies document that aerosol delivery of prostacyclin can effectively vasodilate the pulmonary vasculature (19,24), but few studies have compared aerosol and intravenous delivery of such drugs directly. In one such study, Hallioglu, et al (8) compared inhaled and intravenous iloprost in children with pulmonary hypertension secondary to congenital heart disease. They delivered the same dose of drug by either route and found similar decreases in pulmonary artery pressure and pulmonary vascular resistance. However, since aerosol delivery is inefficient, it is likely that the actual amount of drug delivered that way was less than when given intravenously, so that the potency of the drug delivered by aerosol may have been greater. The differences between our findings and theirs could also be a result of the fact that we used different prostacyclin analogs, although most data would indicate that the analogs have similar actions to the parent compound. It is also possible that sheep react differently than humans or that the existence of pulmonary hypertension due to congenital heart disease alters how the drug acts. In order to achieve an effect in sheep, it was necessary to administer doses of treprostinil that were much higher than those used in treating patients, regardless of the route of delivery. Whether this is due to differences in species or a requirement for higher doses of vasodilator to
overcome thromboxane induced vasoconstriction of the degree we produced experimentally is not clear.

We found that with aerosol delivery, even large doses of treprostinil had minimal effects on systemic hemodynamics. Intravenous drug caused a dose related increase in heart rate and cardiac output and decrease in left atrial pressure while aerosol delivery resulted only in a modest elevation in left atrial pressure that was unrelated to dose. With aerosol delivery, this was true even though the dose of the drug was sufficient to return pulmonary hemodynamics completely to normal. This is in contrast to data from studies in humans with chronic pulmonary hypertension. For example, alterations in systemic hemodynamics were seen in patients receiving intravenous epoprostenol for pulmonary hypertension in a randomized trial (1). Studies with aerosol delivery of prostacyclin in patients with pulmonary hypertension also reported altered systemic hemodynamics (19,24). In a recent study by Olschewski, et al, (18) the inhaled prostacyclin analogue iloprost, now available in the United States, was given to patients with pulmonary arterial hypertension. They not only demonstrated significant improvements in PVR but also noted significant changes in cardiac output, systemic arterial pressure, PCWP and arterial oxygen saturation. These patients were evaluated over 12 weeks of therapy so it is difficult to compare these results to our acute model of pulmonary hypertension with one time dosing of therapy in otherwise normal sheep with acute pulmonary vasoconstriction.
It is clear from this study and others that aerosolized delivery of prostacyclin analogs can reverse acute pulmonary vasoconstriction with minimal systemic side effects (8,9,19). Furthermore, when similar doses of intravenous and aerosolized medication have been used, the effects of aerosol are similar to or greater than systemically administered drug (8). Assuming that only a fraction of the aerosol reaches the distal airways and alveoli it appears the aerosol delivery is much more potent. If prostacyclin acts directly on lung resistance vessels, this finding is especially surprising since with intravenous delivery the drug directly accesses those vessels and with aerosol delivery, the drug would need to traverse epithelial and interstitial barriers to reach the vessels. It is possible that actual concentrations of drug reaching resistance vessels is greater with targeted delivery of the drug by aerosol, but given the inefficiency of aerosol delivery, that seems unlikely.

Airway epithelial cells can produce “relaxant factors” that have been studied mostly in relation to airway rather than vascular function (6). 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 (AP-1) and the prostacyclin analog iloprost caused a similar response in cultured bone cells (7). The AP-1 family of transcription factors is involved in numerous processes in the lung including inflammation, apoptosis and cell proliferation (20,25). Activator protein-1 also
increases expression of inducible nitric oxide synthase that could enhance
vassodilation (15). Activator protein-1 has been found to be involved in the signal
transduction of bone morphogenetic protein (13) and mutations of a BMP
receptor (BMPR2) is causally implicated in a familial form of primary pulmonary
hypertension (17). We have preliminary data indicating that prostacyclin
increases expression of AP-1 and several AP-1 regulated genes in human airway
epithelial cells in culture (Abstract, 47th Annual Thomas L. Petty Aspen Lung
Conference).

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 (29,30), 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 to 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
AP-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


29. Flolan (epoprostenol) Package Insert. GlaxoSmithKline, Research Triangle Park, NC.

30. Remodulin (treprostinil) Package Insert. United Therapeutics, Research Triangle Park, NC.
**Figure Legends**

Figure 1: U44069 infusion (1000 ng/kg/min) induces an increase in PVR to approximately four times baseline. The increase is maintained at this level throughout a 180 minute infusion. (n=4 animals)

Figure 2: (A) U44069 infusion causes a significant increase in PVR. Intravenous treprostinil caused a dose dependent decrease in PVR which remained significantly elevated above baseline (# p<0.05). Aerosol treprostinil caused a dose dependent decrease in PVR which was significantly lower compared to intravenous delivery (* p<0.05). (B) U44069 caused a significant increase in mean PAP. Intravenous treprostinil caused a dose dependent decrease in PAP which remained significantly elevated above baseline (# p<0.05). Aerosol delivery caused a dose dependent decrease in PAP which was significantly lower compared to intravenous delivery (* p<0.05). (n=6 animals)

Figure 3: U44069 caused a significant decline in CO (# p<0.05) and HR (NS) while causing non significant increases in PLA and PSA. (A) Intravenous treprostinil caused a dose dependent increase in CO while there was no change from aerosol treprostinil. (B) Intravenous treprostinil caused a dose dependent increase in HR that was significantly elevated above baseline at the highest dose (# p<0.05) while aerosol delivery caused no change. (C) Intravenous treprostinil caused a significant decrease in PLA relative to U44069 (& p<0.05) and baseline
at the higher doses (# p<0.05). Aerosol delivery caused an increase in PLA that was significantly above U44069 (& p<0.05) and baseline (# p<0.05) at the lower doses. (D) Intravenous and aerosol treprostinil caused a non significant dose dependent decrease in PSA. (n=6 animals)

Figure 4: U44069 was infused at 1000 ng/kg/min to achieve a steady state elevation of PVR for up to 70 minutes. The sheep received either epoprostenol or treprostinil aerosol at 1000 ng/kg/min for a period of 30 minutes. Within 10 minutes the PVR had almost returned to baseline with epoprostenol. After 30 minutes the PVR had increased but not returned to steady state after treprostinil administration.
Figure 1

[Graph showing pulmonary vascular resistance over time with U44069 1000 ng/kg/min]

Pulmonary Vascular Effects of Vasodilators

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Figure 2A

![Graph showing Pulmonary Vascular Resistance](image-url)
Figure 2B

[Graph showing pulmonary arterial pressure responses to different doses of U44069 and Treprostinil.]

- U44069 1000 ng/kg/min
- Treprostinil
Figure 3A

[Graph showing the effects of U44069 and Treprostinil on cardiac output.]
Figure 3B
Figure 3C

![Graph showing Pulmonary Vascular Effects of Vasodilators with data points and annotations.]
Figure 3D

[Graph showing systemic arterial pressure changes with different dosages of U44069 and treprostinil.]
Figure 4

![Graph showing PVR Off Transit with two drug treatments: Epoprostenol Aerosol and Treprostinil Aerosol. The graph includes a best fit line for each treatment with corresponding slopes. The steady state PVR is 16.63 cmH2O*min/L.](http://jap.physiology.org/)

- Epoprostenol Aerosol
- Treprostinil Aerosol

Drug stopped by 10.220.33.4 on November 11, 2017

Steady State PVR = 16.63

Best fit line slope = 0.578

Best fit line slope = 0.2026