Pharmacokinetics and Metabolism of Infused versus Inhaled Iloprost in Isolated Rabbit Lungs

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ABSTRACT

Iloprost is a potent prostacyclin analog, which has been shown to exert beneficial effects in several vascular disorders. Inhalation of aerosolized iloprost was found to cause selective pulmonary vasodilatation, and this approach is under current investigation for treatment of chronic pulmonary hypertension. The present study investigated pharmacokinetics and metabolism of aerosolized iloprost in isolated buffer-perfused rabbit lungs, compared with intravascular administration of the prostanoid. After buffer admixture of iloprost, a steady decline of perfusate concentrations of the intact prostanoid was noted (half-life ~3.5 h), mostly attributable to progressive metabolism to dinor- and tetranoriloprost. Inhaled iloprost rapidly entered the intravascular compartment, with peak buffer concentrations being noted after 30 min (bioavailability ~63%). Compared with infused iloprost, significantly more rapid metabolism to dinor- and tetranoriloprost was noted for iloprost administered via the inhalative route of application. However, the percentage of the nebulized agent that enters the intravascular space as intact iloprost displays the same clearance rate as directly perfusate-admixed prostanoid. We conclude that a high percentage of inhaled iloprost rapidly enters the intravascular compartment in intact rabbit lungs. The lung is capable of metabolizing iloprost via β-oxidation, and more rapid appearance of dinor- and tetranoriloprost is noted for the inhalative as compared with the intravascular route of iloprost administration.

Prostanoids are cyclooxygenase products of arachidonic acid, which exert multiple, partly opposing physiological actions in vascular functions and hemostasis. Among these compounds, prostacyclin (PGI2, epoprostenol) represents the most potent vasodilatory agent and inhibitor of platelet aggregation. Prostacyclin is the major cyclooxygenase product in macrovascular endothelium and mediates its biological effects through prostanoid receptors at the cell surface and downstream activation of the adenylate cyclase pathway. Iloprost is a chemically stable analog of prostacyclin and mimics its pharmacological properties, namely inhibition of platelet aggregation and vasodilatation, rendering this substance appropriate for therapeutic use. Clinical benefits of iloprost infusion were reported for patients with peripheral arterial occlusive disease, thromboangiitis obliterans, and Raynaud’s phenomenon (Fitscha et al., 1987; McHugh et al., 1988; Fiessinger and Schafer, 1990).

Recent clinical studies suggested the potential utility of aerosolized iloprost, administered via the inhalative route, for the management of severe pulmonary hypertension (Olschewski et al., 1996, 1999; Hoepf et al., 2000a,b; Olschewski et al., 2000; Gessler et al., 2001). Inhaled iloprost was found to cause selective pulmonary vasodilatation with pulmonary artery pressure decrease and increase in cardiac output, without affecting mean systemic arterial pressure. A large, randomized, controlled multicenter study of the long-term effects of daily repetitive iloprost inhalation in patients with severe primary and secondary pulmonary hypertension has just been completed, demonstrating significant clinical benefit of this new therapeutic approach (Olschewski et al., 2002). However, detailed pharmacokinetic data on iloprost being deposited in the bronchoalveolar compartment by aerosol administration are currently not available. The present study addresses this issue in the model of isolated perfused rabbit lungs. Comparison with intravascular administration of iloprost was undertaken in this model, and putative lung metabolism of this agent was investigated.

Materials and Methods

Isolated Lung Model

As described previously (Seeger et al., 1994), rabbits of either sex weighing between 2.6 and 2.9 kg were anticoagulated with heparin (1000 U/kg) and anesthetized with ketamine/xylazine via the ear arterial route.
vein. After tracheostomy, the animals were ventilated with room air (tidal volume, 9–13 ml/kg; frequency, 10 breaths/min). A positive end-expiratory pressure of 1 mm Hg was used throughout. After mid-sternal thoracotomy, catheters were placed into the pulmonary artery and the left atrium, and perfusion with Krebs-Henseleit buffer was started. The lungs were perfused with a flow of 120 ml/min in a recirculating system, setting left atrial pressure at 1.5 mm Hg. The overall volume of the perfusion fluid was 500 ml. In parallel with the onset of artificial perfusion, room air supplemented with 4% CO₂ was used for ventilation. Lungs were freely suspended from a force transducer for monitoring of organ weight. Pressure in the pulmonary artery and the left atrium was measured with fluid-filled catheters (zero referenced at the hilum).

**Aerosolization**

Iloprost was nebulized with an ultrasonic device (Pulmo Sonic 5500; DeVilbiss Medizinische Produkte GmbH, Langen, Germany), which was characterized by a mass median aerodynamic diameter of 4.5 μm and a geometric standard deviation of 2.6, as measured with a laser-diffractometer (HELOS; Sympatec, Clausthal-Zellerfeld, Germany). As described previously, the nebulizer was located between the ventilator and the lung in the inspiratory limb of the ventilation system (Schermuly et al., 2000). The total lung deposition of the iloprost aerosol in the lung was determined on-line by use of a mass spectrometer (Sympatec HELOS; Sympatec, Clausthal-Zellerfeld, Germany). As described previously, the nebulizer was located between the ventilator and the lung in the inspiratory limb of the ventilation system (Schermuly et al., 2000). The total lung deposition of the iloprost aerosol in the lung was determined on-line by use of a laser-diffractometer (HELOS; Sympatec, Clausthal-Zellerfeld, Germany). As described previously, the nebulizer was located between the ventilator and the lung in the inspiratory limb of the ventilation system (Schermuly et al., 2000). The total lung deposition of the iloprost aerosol in the lung was determined on-line by use of a laser-diffractometer (HELOS; Sympatec, Clausthal-Zellerfeld, Germany). As described previously, the nebulizer was located between the ventilator and the lung in the inspiratory limb of the ventilation system (Schermuly et al., 2000).

**Bronchoalveolar Lavage**

After termination of perfusion, the entire bronchoalveolar space was lavaged with 150 ml of saline in three fractions, each fraction being injected and reaspirated three times. The total recovery of lavage fluid was ~95%. The lavage fluid was immediately cooled and spun at 300g for 10 min (5°C) to remove cells.

**Lung Homogenate**

After lavage, the lungs were weighed and homogenized with 4 volumes of isotonic saline using a Polytron homogenizer. Ten-milliliter samples of the homogenate were used for further measurements. pH was adjusted to 2.0 with 1 N HCl, and the solution was centrifuged (2100 g, 15 min). After extraction with 20 ml of diethyl ether, the supernatant was dried with nitrogen and resuspended with acentonitrile/water/acetic acid (20%/79.9%/0.1%), and radioactivity counting was undertaken.

**Determination of Iloprost in the Recirculating Buffer**

Perfusate levels of iloprost were determined by radioimmunoassay as previously described (Hildebrand et al., 1990). Briefly, the corresponding antibody (Rb 65005; Schering AG Berlin, Biochemical Pharmacology) was obtained by immunization of rabbits with iloprost-9-butylnloxy-bovine serum albumin. The crossreactivity against dinor- and tetranoriloprost was 1.5% and 0.02%, respectively. ³H-Iloprostmethylster (specific activity 2475 GBq/mmol) was used as tracer. Perfusate samples (0.2 ml) were mixed with tracer solution and diluted antibody solution and incubated overnight (16–18 h) at 4°C. Next, 0.2 ml of a cold dextran-coated charcoal suspension was added. The mixture was incubated for 30 min at 4°C and the phases were separated by centrifugation. The supernatant containing the antibody-bound iloprost was decanted. After addition of 4.2 ml of scintillation cocktail (Atomlight; PerkinElmer Life Sciences, Boston, MA), the samples were subjected to radiometric analysis.

**Determination of Iloprost Metabolites in the Recirculating Buffer by HPLC-Radiochromatography**

³H-Iloprost was used for analyzing metabolism of this agent in the isolated rabbit lung. Iloprost metabolites in recirculating buffer were determined by reversed phase high-performance liquid chromatography (HPLC) as described (Hildebrand, 1992). Briefly, 100 μl of buffer fluid were applied to the column (Spherisorb ODS 2.5 μm; 250 × 4.6 mm) with a convex gradient of acetonitrile and water. The radioactivity of eluting fractions (after addition of 5 ml of Atomlight) was determined by radiometric analysis. Samples having a radioactivity level of less than or equal to double background were considered below the limit of quantitation. Radiochromatograms, which described the quantitative as well as the qualitative biotransformation of iloprost, were obtained (example depicted in Fig. 1).

**Experimental Protocols**

**Pharmacokinetics of Iloprost. Iloprost infusion (n = 6).** After obtaining steady-state conditions, 50 ng of iloprost was admixed to the perfusate reservoir (i.v.) and time was set at zero. Post-lung perfusate samples were taken at 0, 5, 10, 15, 30, 45, 60, 90, 120, 150, and 180 min.

**Iloprost inhalation (n = 6).** A solution of 150 ng of iloprost/ml was nebulized over a 10-min period, resulting in a lung deposition of 75 ng of iloprost. As in the preceding group, perfusate samples were taken at 0, 5, 10, 15, 30, 45, 60, 90, 120, 150, and 180 min.

**Metabolism of Iloprost. Iloprost infusion (n = 6).** After steady state, 50 ng of ³H-iloprost was applied intravascularly and time was set at zero. Perfusate samples were taken at 0, 5, 10, 15, 30, 45, 60, 90, 120, 150, and 180 min. Then perfusion was stopped, bronchoalveolar lavage was performed, and lungs were homogenized. Iloprost inhalation (n = 6). A solution of 150 ng of ³H-iloprost/ml was nebulized over a 10-min period, resulting in a lung deposition of 75 ng. Perfusate sampling, lavage, and homogenization were undertaken correspondingly.

**Calculations and Statistics**

Multiexponential equations were used to describe the pharmacokinetics. The area under the perfusion concentration-time curve (AUC) was calculated by using the linear trapezoidal rule and extrapolating to infinity by dividing the last buffer concentration by the slope of the terminal phase. Clearance was calculated by standard formula employing the overall dose and the AUC. Computer analysis was performed with TOPFIT, Version 2.0 (Tanswell et al., 1995), applying a non-linear least squares algorithm.

**Fig. 1. Chromatogram of iloprost and its metabolites. The chromatogram shows iloprost and its metabolites tetranor- and dinoriloprost in the perfusate medium 180 min after inhalation of 75 ng of iloprost in isolated rabbit lungs. Iloprost displays the characteristic double peak, due to its diastereomeric properties, with retention times between 46.5 and 48.5 min. The retention times of the intermediate dinoriloprost ranges between 27.0 and 28.5 min. Tetranoriloprost, the final metabolite, appears between 20.0 and 24.5 min. Tetranoriloprost is a diastereomer with a pair of enantiomers characterized by four signals.**
model-independent approach. All values are given as mean ± S.E.M. For analyzing statistical difference \((p < 0.05)\), two-tailed Student’s \(t\) test for unpaired samples was performed.

Results

Baseline Conditions

After termination of the steady-state period, all lungs displayed pulmonary artery pressure values in the range between 6 and 8 mm Hg. No significant increase in weight gain was measured over the entire observation period (ranging consistently <0.3 g/h).

Pharmacokinetics of iloprost

Iloprost Infusion. Buffer admixture of 50 ng of iloprost did not affect pulmonary artery pressure or lung weight gain. As detailed in Fig. 2, a peak perfusate level of 143 pg/ml was obtained, with subsequent slow decrease of the iloprost concentration in the recirculating buffer medium. The terminal half-life was calculated as \(220 ± 18\) min (Table 1), resulting in an AUC of \(44,899 ± 4,504\) pg · min/ml.

Iloprost Inhalation. The aerosolization of 150 ng/ml iloprost resulted in a lung deposition of \(75 ± 5\) ng, as assessed by the laserphotometric technique. Immediately after commencing nebulization, the level of iloprost in the recirculating buffer started to increase (Fig. 2), with a maximum of \(114 ± 24\) pg/ml 20 min after termination of nebulization. As in the preceding group, the perfusate level of iloprost subsequently decreased slowly. No effect on pulmonary artery pressure was noted, whereas weight gain increased by \(0.4\) g, reflecting the total fluid volume being deposited during the aerosolization maneuver. The terminal half-life of iloprost was calculated as \(170 ± 50\) min (Table 1), and AUC was \(42,623 ± 16,301\) pg · min/ml. The bioavailability was 63% for inhaled iloprost, when calculating the percentage of agent reaching the intravascular compartment as compared with the amount of substance totally being deposited within the lung. Per definition, bioavailability was set at 100% for direct buffer admixture of iloprost.

![Fig. 2.](image-url) Perfusion levels of iloprost after nebulization or infusion in the recirculating buffer fluid. Mean ± S.E.M. of six independent experiments each are given. Maneuvers are indicated by the arrow and the horizontal bar.

![Fig. 3.](image-url) Counts of iloprost and its metabolites in the recirculating perfusate after admixture of the labeled iloprost to the buffer fluid. Mean ± S.E.M. of six independent experiments each are given. The bolus application of 50 ng of \(^3\)H-iloprost is indicated by the arrow.

<table>
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<tr>
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<th>Half-life</th>
<th>AUC</th>
<th>Bioavailability</th>
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<tr>
<td>Iloprost i.v.</td>
<td>(220 ± 18)</td>
<td>(44,899 ± 4,504)</td>
<td>100</td>
</tr>
<tr>
<td>Iloprost aerosol</td>
<td>(170 ± 50)</td>
<td>(42,623 ± 16,301)</td>
<td>63 ± 4</td>
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Metabolism of iloprost

Iloprost Infusion. Perfusate admixture of 50 ng of \(^3\)H-iloprost resulted in initial buffer counts attributable to the iloprost fraction of 110,000, as shown in Fig. 3. Subsequently, the iloprost counts decreased to 18,000 dpm within 180 min. In parallel, the quantity of the metabolites dinoriloprost and tetrnoriloprost progressively increased. At 45 min after infusion of iloprost, 4.9% of total radioactivity was attributable to dinor- and 11.5% to tetrnoriloprost (Fig. 4). These metabolites increased to 22.9% and 42.5%, respectively, at the end of the 180-min perfusion period.

The overall distribution of radioactivity within the different compartments of the lung was analyzed after termination of the 180-min perfusion period (Table 2). At that time, 98% of total radioactivity was recovered, mostly contained in the perfusate. The lavage fluid was found to contain <1% of radioactivity at that time. Counts in the lung tissue (being corrected for remaining perfusate and remaining lavage fluid “trapped” within the lung homogenate) amounted to ~18% of total radioactivity.

Iloprost Nebulization. Within a 10-min nebulization period, 75 ng of \(^3\)H-iloprost were deposited in the bronchoalveolar space of the isolated lungs. Already during aerosolization, iloprost and its metabolites began to be detectable in the buffer fluid (Figs. 5 and 6). In analogy to the experiments with buffer admixture of iloprost, initial predominance of iloprost was noted, which subsequently declined, accompanied by the progressive appearance of dinoriloprost and tetrnoriloprost. When referenced to intact iloprost, these me-
tabolites appeared even more rapidly in the inhalation experiments compared with the studies with intravascular administration of iloprost. When assessing the overall distribution of radioactivity in the different lung compartments after 180 min, 94% of total radioactivity was recovered. The bulk of tracer was detected in the perfusate medium, with minor percentages being found in the lavage fluid and in the homogenized lung tissue (Table 2).

**Discussion**

Isolated lung models have repeatedly been employed for evaluating pharmacokinetic and pharmacodynamic parameters of agents of interest, mostly after admixture to the perfusion medium (Aislaitner et al., 1997; Valodia and Syce, 2000). In the current study, we focused on iloprost, a stable prostacyclin analog, and compared the inhalative to the intravascular route of administration. After admixture to the recirculating buffer fluid, a progressive decrease of the iloprost buffer concentrations was noted, with a half-life of perfusate clearance of 3.5 h. The disappearance of iloprost from the intravascular compartment was only partly attributable to some redistribution of this prostanoid into other compartments: at the end of the 180-min perfusion experiments employing labeled iloprost, only 18% of radioactivity was detected in the lung tissue, and 1% was accessible by bronchoalveolar lavage. The main reason for the decline of iloprost buffer concentrations was its metabolism to dinor- and tetranoriloprost, which, due to the specificity of the antibody employed, are not detected by the iloprost radioimmunoassay. This finding is of interest, as lung metabolism of iloprost has hitherto not been described. After intravenous administration in intact animals and humans, the liver is assumed to be the predominant site of iloprost metabolism (Krause and Krais, 1986). In a previous study in rabbits, the infusion of 300 ng of iloprost/kg/min over 30 min resulted in a more rapid disappearance of the prostanoid from the intravascular compartment (terminal half-life ~30 min) than in the currently investigated isolated lungs; however, the arising metabolites (dinor- and tetranoriloprost) corresponded to those currently detected by HPLC. Thus, β-oxidation represents the predominant mechanism of iloprost metabolism both in the lung and in the liver.

After onset of iloprost nebulization, rapid entry of the
prostanoid into the intravascular compartment was noted: significant iloprost levels began to be detectable within 5 min of the 10-min aerosolization maneuver, and maximum perfusate iloprost concentrations were measured after 30 min. The absolute bioavailability of aerosolized iloprost, defined as percentage of totally applied substance appearing in the intravascular compartment as intact agent at the time of peak buffer concentration, was calculated to range at ~63%. The gap between this percentage and a 100% bioavailability is explained by two independent phenomena. First, a significant retention of inhaled iloprost in the bronchoalveolar space and/or lung tissue may be assumed for the initial postaerosolization period. At the end of the 180-min lung perfusion period, however, only very minor percentages of radioactivity were found to be retained in these nonvascular compartments. Second, inhaled iloprost underwent a more rapid metabolism to dinor- and tetranoriloprost as compared with the intravascularly administered prostanoid. Already 5 min after commencement of nebulization, these β-oxidation products appeared in the buffer fluid and amounted to ~20% of total radioactivity at that time. In contrast, neither dinor- nor tetranoriloprost was detectable within 5 min after intravascular administration of iloprost. Similarly, the percentages of dinor- plus tetranoriloprost in relation to intact iloprost were higher for the inhalative route of application as compared with intravascular administration both 45 and 90 min after prostanoïd application. This observation suggests rapid access of aerosolized iloprost to cells capable of β-oxidation. Such cell types include epithelial cells (Alpert and Walenga, 1993), smooth muscle cells (Lacape et al., 1992), endothelial cells (Fang et al., 1999), and (alveolar) macrophages (Mathur et al., 1990). It is well conceivable that nebulized iloprost comes into close contact to these cells during its passage from the alveolar surface to the intravascular space, thereby enhancing its rate of β-oxidation. Having reached the intravascular compartment, the further metabolism of nebulized iloprost largely resembles that of iloprost directly admixed to the buffer fluid, as suggested by the parallel curves of iloprost decline from 30 min to the end of experiments, and by the fact that the calculated half-lives range in the same magnitude.

In conclusion, inhaled iloprost rapidly enters the intravascular compartment in intact rabbit lungs. Both intravascularly administered and nebulized iloprost are metabolized via β-oxidation in lung cells; however, more rapid metabolism to dinor- and tetranoriloprost is noted for the inhalative route of application. Once intact iloprost has entered the vascular lumen, the subsequent decline in perfusate concentrations resembles that of intravascularly administered iloprost.

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References


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