Inhalation of the ET<sub>A</sub> receptor antagonist LU-135252 selectively attenuates hypoxic pulmonary vasoconstriction

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PULMONARY ARTERIAL HYPERTENSION (PAH) can appear in a chronic, progressive idiopathic form, or as a consequence of acute cardiopulmonary decompensation, such as pulmonary embolism. In addition, PAH occurs together with chronic obstructive lung disease or chronic high-altitude exposure (23). A main pathophysiological mechanism for PAH consists of an imbalance between endogenously produced vasodilating and constricting mediators (14). In particular, increased concentrations of the potent constricting peptide endothelin (ET)-1 (11) have been identified as a major characteristic for PAH. The effects of ET<sub>1</sub> to increase pulmonary vascular tone are mediated by ET<sub>A</sub> and ET<sub>B</sub> receptors on smooth muscle cells, while ET<sub>B</sub> receptors at the pulmonary endothelium cause vasodilation (2). Consequently, blockade of pulmonary ET<sub>A</sub> receptors is an important option for the treatment of PAH, and the use of the dual ET<sub>A</sub>/ET<sub>B</sub> receptor antagonist bosentan has been proven to induce clinically relevant improvements with respect to pulmonary hemodynamics and exercise capacity (22). Currently, the selective ET<sub>A</sub> receptor antagonists sitaxsentan and ambrisentan are evaluated for the treatment of PAH (1, 10).

A serious disadvantage of any orally or intravenously applied vasodilator in PAH consists of the systemic vasodilation, which parallels the beneficial pulmonary effects. This may not only reduce exercise capacity, but will become deleterious in hemodynamically unstable patients and has to be avoided by dose titration. To overcome these difficulties, it is necessary to achieve a direct delivery of drugs to the pulmonary vasculature. This has prompted the concept of selective pulmonary vasodilation with inhaled nitric oxide (iNO) for the treatment of PAH (20). Hereby, iNO affects exclusively vascular smooth muscle cells in the ventilated lung areas and induces vasodilation by an increase in cyclic guanosine monophosphate; there are no relevant systemic hemodynamic effects of iNO, since most of the gas is inactivated by immediate reaction with hemoglobin (16). Similarly, inhalation of the prostacyclin analog iloprost significantly improved cardiopulmonary hemodynamics in patients with PAH, whereas systemic side effects were minimized using a low dose (19).

In analogy to the benefits of iNO and inhaled iloprost, we hypothesized that inhalation of an ET<sub>A</sub> receptor antagonist in pulmonary hypertension might restrict vasodilation predominantly to the pulmonary circulation without relevant systemic effects. Our hypothesis was tested in an animal model of hypoxic pulmonary vasoconstriction (HPV).

METHODS

General experimental procedure. This study was approved by the Berlin Animal Protection Committee in accordance with the German Animal Protection Law, and conforms to the Guide for the Care and Use of Laboratory Animals (Department of Health and Human Services, Public Health Service, National Institutes of Health Publication no. 85–23). Sixteen piglets with a body weight of 25 ± 1 kg were studied. After intramuscular premedication with azaperone (5 mg/kg) and atropine (0.05 mg/kg), anesthesia was induced with an intravenous bolus of thiopental (10 mg/kg) and fentanyl (5 μg/kg), followed by a continuous infusion of thiopental (0.13 mg·kg<sup>–1</sup>·min<sup>–1</sup>) and fentanyl (0.05–0.08 μg·kg<sup>–1</sup>·min<sup>–1</sup>). The animals were orally intubated (inner diameter 6.5 mm), and muscle relaxation was obtained with pancuronium bromide (a bolus of 0.15 mg/kg followed by 2.5

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were taken at hourly intervals. In addition, blood samples for later analysis of ET-1 plasma levels were obtained with Student’s t-test for unpaired samples (2-tailed). A P value <0.05 was considered to be significant.

RESULTS

The groups were comparable with regard to body weight and pre-study conditions. Averaged over all animals, induction of hypoxia decreased arterial PO2 (PaO2) from 151 ± 5 to 60 ± 2 Torr and reduced the arterial oxyhemoglobin fraction from 96 ± 0.1 to 89 ± 1% (P < 0.05; values for single groups are presented in Table 1). MPAP increased from 23 ± 1 to 32 ± 1% (P < 0.05; Table 1). Systemic arterial blood pressure and CO remained stable at 94 ± 3 mmHg and 4.8 ± 0.3 l/min, respectively. In all animals, ET-1 plasma levels increased significantly from mild hypoxia to hypoxia baseline (0.37 ± 0.04 vs. 0.52 ± 0.04 fmol/ml, P < 0.05; Table 1).

Inhalation of the ETα receptor antagonist LU-135252 at a dose of 0.3 mg/kg for 20 min induced a substantial and sustained improvement in pulmonary hemodynamics during hypoxia. In the LU group, MPAP decreased from 33 ± 2 mmHg at hypoxia baseline following short-term inhalation of LU-135252 after 1 h to 25 ± 1 mmHg and remained low at 27 ± 1 mmHg after 3 h. These values were significantly different from those of the control group, in which MPAP continued to stay at a high level of 32 ± 1 mmHg at 3 h after hypoxia baseline (Fig. 1A). Concomitantly, systemic mean pressure decreased by 10.2 ± 0.3 mmHg after 3 h. These values were significantly different from those of the control group, in which MPAP continued to stay at a high level of 32 ± 1 mmHg at 3 h after hypoxia baseline (Fig. 1A).

Table 1. Effects of hypoxia on gas exchange, hemodynamics, and ET-1 plasma levels

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Mild Hyperoxia (FrO2 0.3)</th>
<th>Hypoxia Baseline (FrO2 0.15)</th>
</tr>
</thead>
<tbody>
<tr>
<td>PaO2 Torr</td>
<td>150 ± 7</td>
<td>59 ± 3*</td>
</tr>
<tr>
<td>Controls</td>
<td>151 ± 8</td>
<td>62 ± 2*</td>
</tr>
<tr>
<td>PaCO₂ Torr</td>
<td>36 ± 1</td>
<td>30 ± 1*</td>
</tr>
<tr>
<td>LU group</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Controls</td>
<td>37 ± 1</td>
<td>29 ± 1*</td>
</tr>
<tr>
<td>HF, beats/min</td>
<td>101 ± 6</td>
<td>106 ± 5</td>
</tr>
<tr>
<td>LU group</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Controls</td>
<td>93 ± 4</td>
<td>97 ± 4</td>
</tr>
<tr>
<td>MAP, mmHg</td>
<td>101 ± 4</td>
<td>94 ± 4</td>
</tr>
<tr>
<td>LU group</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Controls</td>
<td>90 ± 5</td>
<td>93 ± 5</td>
</tr>
<tr>
<td>MPAP, mmHg</td>
<td>24 ± 1</td>
<td>33 ± 2*</td>
</tr>
<tr>
<td>LU group</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Controls</td>
<td>22 ± 1</td>
<td>30 ± 1*</td>
</tr>
<tr>
<td>CVP, mmHg</td>
<td>11 ± 1</td>
<td>13 ± 1</td>
</tr>
<tr>
<td>LU group</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Controls</td>
<td>10 ± 1</td>
<td>11 ± 1</td>
</tr>
<tr>
<td>PCWP, mmHg</td>
<td>12 ± 1</td>
<td>11 ± 1</td>
</tr>
<tr>
<td>LU group</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Controls</td>
<td>11 ± 1</td>
<td>11 ± 1</td>
</tr>
<tr>
<td>CO₂ l/min</td>
<td>5.7 ± 0.5</td>
<td>5.3 ± 0.4</td>
</tr>
<tr>
<td>LU group</td>
<td>4.6 ± 0.2</td>
<td>4.3 ± 0.2</td>
</tr>
<tr>
<td>Plasma ET-1, fmol/ml</td>
<td>0.37 ± 0.07</td>
<td>0.54 ± 0.04*</td>
</tr>
<tr>
<td>LU group</td>
<td>0.36 ± 0.06</td>
<td>0.50 ± 0.06*</td>
</tr>
</tbody>
</table>

Values are means ± SE. FrO₂, inspiratory oxygen fraction; hypoxia baseline, measurement point after 1 h of hypoxia; LU, LU-135252; PaO₂, partial pressure of oxygen in arterial blood; PaCO₂, partial pressure of carbon dioxide in arterial blood; HF, heart frequency; MAP, mean systemic arterial blood pressure; MPAP, mean pulmonary arterial pressure; CVP, central venous pressure; PCWP, pulmonary capillary wedge pressure; CO, cardiac output; plasma ET-1, plasma concentration of endothelin-1. *P < 0.05 vs. mild hypoxia.
arterial pressure remained stable during hypoxia, and measured values were not significantly different between groups (Fig. 1B). CVP and PCWP were not different between groups at hypoxia baseline (Table 1) and remained stable after 3 h of hypoxia [CVP: 12 ± 1 vs. 11 ± 1 mmHg, not significant (NS); PCWP: 11 ± 1 vs. 10 ± 1 mmHg, NS; LU group vs. controls].

CO tended to be lower in controls than the LU group already at mild hyperoxia and at hypoxia baseline (Table 1), but the difference was mainly unchanged throughout the protocol and did not reach statistical significance (LU group: 5.3 ± 0.4 l/min; controls: 4.1 ± 0.2 l/min; values 3 h after hypoxia baseline; NS). Accordingly, there were no significant relative changes in CO from hypoxia baseline during the protocol in both groups (Fig. 2). Inhalation of LU-135252 induced a significant and sustained decrease in MPAP, whereas systemic MAP remained unchanged. *P < 0.05 vs. controls.

Inhalation of LU-135252 at a dose of 0.3 mg/kg for 20 min immediately after hypoxia baseline induced a significant decrease in MPAP, whereas systemic MAP remained unchanged. *P < 0.05 vs. controls.

Fig. 1. Mean pulmonary arterial pressure (MPAP; A) and mean systemic arterial blood pressure (MAP; B) during hypoxia in animals receiving aerosolized LU-135252 (LU group, ●, n = 8) compared with controls (○, n = 8). Inhalation of LU-135252 at a dose of 0.3 mg/kg for 20 min immediately after hypoxia baseline induced a significant and sustained decrease in MPAP, whereas systemic MAP remained unchanged. *P < 0.05 vs. controls.

Fig. 2. Relative change in cardiac output (CO) from hypoxia baseline in LU animals (●, n = 8) compared with controls (○, n = 8). Inhalation of LU-135252 at a dose of 0.3 mg/kg for 20 min immediately after hypoxia baseline had no significant influence on CO.

value of 0.62 fmol/ml was measured in both groups (Fig. 4). Mean airway pressure of volume-controlled mechanical ventilation was not influenced by inhalation of LU-135252, and measured values remained stable in both groups (LU group: change in mean airway pressure 0.3 ± 0.1%; controls 0.4 ± 0.2%; relative changes from hypoxia baseline after 3 h).

Fig. 3. Relative changes in pulmonary vascular resistance (PVR; A) and in systemic vascular resistance (SVR; B) among LU animals (●, n = 8) compared with controls (○, n = 8). Inhalation of LU-135252 at a dose of 0.3 mg/kg for 20 min immediately after hypoxia baseline induced a significant and sustained decrease in PVR, while SVR remained unchanged, thus demonstrating pulmonary selectivity of vasodilation. *P < 0.05 vs. controls.
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during hypoxia only by ~40%. The attenuation of HPV in our study is in line with the results of Holm and colleagues (13), who applied the ETA receptor antagonist BMS-182874 in pigs at a dose of 30 mg/kg intravenously during hypoxia at FIO₂ = 0.1 and reported a decrease in MPAP from 38 ± 4 to 30 ± 5 mmHg, a reduction similar in magnitude as what we found. In contrast to the inhaled treatment in our study, intravenous injection of BMS-182874 was, however, accompanied by a significant decrease in systemic blood pressure by 20% (13). Although the effects of the inhaled treatment are expected to be dose dependent, the incomplete inhibition of HPV might also indicate that the measured increase in ET-1 during hypoxia represents a modulating factor rather than a causal effect of HPV. It is widely accepted that HPV is mainly evoked by low alveolar oxygen partial pressures, which increase Ca²⁺ concentration in pulmonary arterial smooth muscle cells via a membrane depolarization (18).

Our measurements of ET-1 plasma levels revealed a steady increase over 4 h of hypoxia, with the main change of 80% during the first 2 h, and the timing of the inhalation has most likely influenced the response to the ETA receptor antagonist. This is paralleled by the finding in humans that a short-term hypoxic challenge of 5–10 min in which MPAP appears to approach a plateau is followed by a slower second phase of HPV that reaches a maximum at 2 h of isocapnic hypoxia (9). Both investigations reveal the necessity for sufficient duration of study protocols when aiming to investigate effects of ETA blockade at hypoxia, which could be otherwise underestimated.

The findings of our present study may be compared with results being reported from previous applications of inhaled LU-135252 in an experimental model of acute lung injury. In these studies, inhalation of LU-135252 at doses of 0.3 and 3 mg/kg induced similar effects as iNO; however, MPAP was prevented to increase rather than being reduced, compared with untreated controls (6, 7, 15). The main effect of inhaled LU-135252 in experimental acute lung injury was a significant increase in PaO₂-to-FIO₂ ratio due to a redistribution of blood flow from unventilated shunt areas toward ventilated lung regions, which was analogous to the action of iNO reported in patients with acute respiratory distress syndrome (21). Application of the inhaled ETA receptor antagonist during hypoxia in the present study did not improve gas exchange, most likely because the largely homogenous distribution of HPV without a relevant shunt fraction offers only minor occasion to redirect blood flow.

The dose of 0.3 mg/kg that we used in our study was chosen, since it induced an improvement in gas exchange and pulmonary hemodynamics without systemic vasodilation following inhalation in experimental acute lung injury (6). Interestingly, inhalation of a 10-fold higher dose in experimental acute lung injury tended to increase systemic vascular resistance and to decrease CO rather than cause systemic vasodilation (7); these effects were possibly a consequence of increased ET-1 plasma levels due to a partial ETA blockade at the higher concentrations of LU-135252. Whether this may also be associated with the inhalation of LU-135252 at doses >0.3 mg/kg during hypoxia remains to be investigated. Additionally, it might be argued that pulmonary selectivity could be attenuated by a spillover of inhaled LU-135252 into the pulmonary circulation. Supplementary measurements using normal phase high-pressure liquid chromatography with ultraviolet detection in plasma samples of three animals having received nebulized LU-135252 in our study revealed LU-135252 plasma concentrations <0.9 μmol/l. These low levels, which are further attenuated by plasma binding, are not expected to influence systemic hemodynamics. Reporting measurements in dogs, Chernacek and colleagues (5) found a significant systemic hypotensive effect of LU-135252 only at plasma concentrations of 200 and 400 μmol/ml, but not at values between 50 and 100 μmol/ml. This implies that LU-135252 plasma levels of the intervention group in our study were insufficient to affect the systemic circulation.

Besides contributing to pulmonary vasoconstriction, ET-1 causes bronchoconstriction in intact airways, and ET receptor antagonism should induce vasodilation. However, the vast majority of the binding sites for ET-1 on bronchial smooth muscle cells consists of ETB receptors. Consequently, in humans and guinea pigs, the ETA receptor antagonist BQ-123 antagonized ET-1-induced contraction of the pulmonary artery, but had no effect on bronchoconstriction, which was, on the other hand, markedly enhanced by application of the ETB agonist sarafotoxin S6c (12). In line with these findings, there was no bronchodilating effect due to inhaled LU-135252 in our study; moreover, mean airway pressure of volume-controlled mechanical ventilation remained unchanged in both groups.

There is no reason why the pulmonary selective effect of inhaled LU-135252, as demonstrated in our study, should be restricted to hypoxia; moreover, a similar result can be expected in other forms of pulmonary vasoconstriction, which are accompanied by increased ET levels. Therefore, inhaled ETA receptor antagonists offer a new therapeutic option for PAH, adding to iNO and the inhaled prostacyclin analog iloprost (19), while acting on a different pharmacological pathway (14). Since the inhalation of ETA receptor antagonists targets the pulmonary vasculature directly, it allows the reduction of the...
effective dose compared with intravenous or oral application. This way, inhaled treatment is also expected to reduce side effects like headache, nausea, nasal congestion, increased amino transferases, peripheral edema, and anemia, as have been reported following prolonged oral application of bosentan (8), or of the novel ETA receptor antagonist sitaxsentan (1, 24). In particular, when bosentan has been orally applied in mountaineers at high altitude to prevent acute mountain sickness, the beneficial effects on the pulmonary arterial pressure were opposed by an increase in fluid retention, which might enhance the risk for pulmonary edema formation (17).

Perspectives and Significance

We demonstrated that inhaled LU-1352525 induced a significant and sustained reduction in pulmonary arterial pressure during hypoxia without influencing systemic hemodynamics. Our results suggest the potential of inhaled ETA receptor antagonists as a future option for the selective treatment of pulmonary hypertension that may be already effective at lower doses and thus might contribute to reduce side effects of an alternative oral application.

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REFERENCES


