The inhaled ET\textsubscript{A} receptor antagonist LU-135252 acts as a selective pulmonary vasodilator

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ABSTRACT

To investigate the hypothesis that the inhaled ET\textsubscript{A} receptor antagonist LU-135252 acts as selective pulmonary vasodilator, we compared inhaled LU-135252 and inhaled nitric oxide (iNO) in an experimental model of acute lung injury (ALI), in a prospective, randomized, controlled animal study. A total of 30 anaesthetized, tracheotomized and mechanically ventilated pigs underwent induction of ALI by repeated saline washout of surfactant. The animals were then randomly assigned to receive the nebulized ETA receptor antagonist LU-135252 (0.3 mg \textper kg, inhaled over 20 min; ETA-A group; \(n=10\)), inhaled NO (30 p.p.m. continuously; iNO group; \(n=10\)) or nebulized saline buffer (5 ml inhaled over 20 min; control group; \(n=10\)). Measurements of pulmonary gas exchange and haemodynamics were performed hourly over a 4 h period after induction of ALI. In the ETA-A group, the arterial oxygen tension (\(P_{aO_2}\)) increased from 58\(\pm\)3 to 377\(\pm\)39 mmHg at 4 h after intervention, while the intrapulmonary shunt (\(Q_S/Q_T\)) decreased from 53\(\pm\)4\% to 18\(\pm\)2\% (\(P!0.01\) compared with controls). In the iNO group, \(P_{aO_2}\) increased from 62\(\pm\)4 to 224\(\pm\)48 mmHg, and \(Q_S/Q_T\) decreased from 47\(\pm\)2\% to 27\(\pm\)5\%, at 4 h after induction of ALI (\(P!0.05\) compared with controls). In the ETA-A and iNO groups, the increase in mean pulmonary artery pressure was significantly attenuated compared with controls (ETA-A group, 14\(\pm\)4\%; iNO group, 6\(\pm\)4\%; values at 4 h; \(P!0.01\) compared with controls). In contrast, there were no significant differences in changes of mean arterial pressure and cardiac output between groups. Thus, in this experimental model of ALI, both inhaled LU-135252 and iNO significantly improved gas exchange and prevented an increase in mean pulmonary artery pressure, without significant systemic effects, when compared with controls. Our results indicate the occurrence of selective pulmonary vasodilation in both treatment groups.

INTRODUCTION

Acute lung injury (ALI) and the acute respiratory distress syndrome (ARDS) are characterized by the presence of alveolar collapse, a significant mismatch of ventilation to perfusion, increased intrapulmonary right-to-left shunt and acute pulmonary arterial hypertension, resulting in a deterioration of arterial oxygenation [1]. In patients with ARDS, marked increases in plasma endothelin levels have been reported [2,3]. However, the role of these increased endothelin concentrations remains to be determined. Besides having other effects, endothelin-1 (ET-1), a major endothelin subtype, is a potent vasoconstrictor and a mitogen that induces pulmonary hypertension and
bronchoconstriction [4], and is possibly involved in pulmonary inflammatory responses [4,5]. The effects of endothelins are mediated by two receptor subtypes: ET$_A$ and ET$_B$ receptors [4]. While endothelial ET$_B$ receptors induce the release of vasodilators (e.g. NO and prostacyclin), the stimulation of pulmonary ET$_A$ receptors was shown to induce airway and vascular smooth muscle contraction. This reveals the ability of ET$_A$ receptor antagonists to act as vasodilators [4].

In patients with ARDS, inhaled nitric oxide (iNO) has been demonstrated to improve matching of ventilation to perfusion via selective vasodilation of pulmonary vessels in ventilated lung regions, thereby improving gas exchange and reducing pulmonary hypertension, without effects on the systemic circulation [6,7]. We hypothesized that inhaled ET$_A$ receptor antagonists may induce selective pulmonary vasodilation in a similar fashion. Therefore the present study was designed to compare the effects of the inhaled ET$_A$ receptor antagonist LU-135252 and iNO on gas exchange and haemodynamics in an experimental model of ALI.

**METHODS**

This study was approved by the Berlin Animal Protection Committee in accordance with the German Animal Protection Law, and conforms with the Guide for the Care and Use of Laboratory Animals (DHHS, PHS, NIH publication no. 85–23).

**General experimental procedures**

A total of 30 piglets (body weight 24 ± 2 kg) were studied. After premedication with azaperone (5 mg · kg$^{-1}$, intramuscular) and atropine (0.05 mg · kg$^{-1}$, intramuscular), anaesthesia was induced using thiopental (10 mg · kg$^{-1}$, intravenous) and fentanyl (5 µg · kg$^{-1}$), followed by a continuous infusion of thiopental (0.13 mg · kg$^{-1}$ · min$^{-1}$) and fentanyl (0.05–0.08 µg · kg$^{-1}$ · min$^{-1}$). Muscle relaxation was obtained with pancuronium bromide (0.15 mg · kg$^{-1}$ intravenous bolus, followed by a continuous infusion of 2.5 µg · kg$^{-1}$ · min$^{-1}$). Immediately after induction, the piglets were tracheotomized and intubated with a tracheal tube (inner diameter 8.0 mm), fitted with a heat moisture exchanger.

During baseline and induction of ALI, animals were placed in a supine position and ventilated in a volume-controlled mode (tidal volume 12 ± 2 ml · kg$^{-1}$; respiratory rate 16 min$^{-1}$; fraction of inspired O$_2$ (FiO$_2$) 1.0; inspiratory/expiratory ratio 1:1; positive end-expiratory pressure 5 cmH$_2$O) using a Servo 300 A/NO ventilator (Siemens-Elema, Solna, Sweden). The core temperature of the animals was maintained within ±0.5 °C of the pre-study value using a heating pad. Throughout the experiments, no cardiotonic or vasoactive drugs were administered.

In each pig, a pulmonary artery catheter (model 93A-431–7.5Fr; Baxter Healthcare Corp., Irvine, CA, U.S.A.) was inserted via the femoral vein, and an arterial line (18 G; Vygon, Ecouen, France) was placed into the femoral artery. These catheters served for blood sampling and haemodynamic measurements. Heart rate, mean arterial pressure and mean pulmonary artery pressure (MPAP) were recorded using a Hewlett-Packard monitoring system (Model 66 S; Hewlett-Packard, Böblingen, Germany). Measurements were taken with the pigs in the supine position with the zero reference level at the midaxilla level. Cardiac output was determined using the thermodilution technique, and is expressed as the mean of four measurements during different phases of the respiratory cycle. The intrapulmonary shunt ($Q_s/Q_T$) was calculated using a standard formula.

Blood samples for blood gas analysis were collected anaerobically, and analysed immediately (ABL 520; Radiometer, Copenhagen, Denmark). Arterial oxygen saturation and mixed venous oxygen saturation were measured by spectrophotometry, with the analyser calibrated for pig blood (OSM 3 Hemoximeter; Radiometer).

**Induction of ALI**

Repeated lung lavage with isotonic saline was performed to wash out lung surfactant, as reported and described in detail elsewhere [8]. An arterial oxygen tension ($P_{aO_2}$)/FiO$_2$ ratio persistently below 100 mmHg for 1 h was considered to indicate ALI.

**Experimental protocol**

After induction of ALI, animals were randomly assigned to receive the nebulized ET$_A$ receptor antagonist LU-135252 (Knoll AG, Ludwigshafen, Germany) at a dosage of 0.3 mg · kg$^{-1}$ inhaled for 20 min after induction of ALI (ET$_A$-A group; $n = 10$), or continuous inhalation of 30 p.p.m. NO (iNO group; $n = 10$), or a nebulized saline buffer (5 ml over 20 min; control group; $n = 10$). For the ET$_A$-A group, 10 mg of LU-135252 was dissolved in 5–10 ml of buffer and titrated to a pH of 7.4 with approx. 100 µl of 0.1 M HCl, followed by nebulization of 0.3 mg · kg$^{-1}$ over 20 min, using an ultrasonic nebulizer (Servo Ultra Nebulizer 345; Siemens-Elema). The nebulizer was placed between the tracheal tube and the inspiratory limb of the ventilator tubing. Of the aerosol produced by the nebulizer, 80% consists of particles with diameters between 0.5 and 5.0 µm. For the iNO group, 30 p.p.m. NO (AGA, Bottrop, Germany) was mixed into the inspired gas using a commercially available ventilator (Servo 300 A/NO; Siemens-Elema). A rapidly responding chemiluminescence analyser (CLD 700 AL;
ECO Physics, Duernten, Switzerland) was used to measure NO concentrations in the inspiratory gas.

**Statistical analysis**

Results are expressed as means ± S.E.M. The data were obtained at baseline (pre-lavage), immediately after the induction of ALI (post-lavage), and at hourly intervals over a 4 h period. Statistical analysis was performed using SPSS for Windows 8.0 (SPSS Inc., Chicago, IL, U.S.A.). Differences between groups were evaluated by Kruskal–Wallis ANOVA followed by post hoc comparisons using the Mann–Whitney U test with Bonferroni’s correction. Intra-group comparisons were performed using Friedman’s test with post hoc comparisons according Wilcoxon and Wilcox. Statistical significance was assumed at P < 0.05.

**RESULTS**

**Gas exchange**

The animals were comparable with regard to body weight and pre-study conditions. Induction of ALI decreased PaO₂ from 564 ± 9 mmHg (pre-lavage) to 56 ± 2 mmHg in all animals. Both inhalation of LU-135252 and iNO induced substantial and sustained improvements in gas exchange (Table 1). In the ET₄-A group, PaO₂ had increased from 58 ± 3 mmHg after induction of ALI to 377 ± 39 mmHg at 4 h (P < 0.01 compared with controls), while iNO induced an improvement in PaO₂ from 62 ± 4 mmHg to 224 ± 48 mmHg at 4 h of treatment (P < 0.05 compared with controls). In the ET₄-A group, Qs/QT was significantly reduced compared with controls (from 53 ± 4 % after induction of ALI to 18 ± 2 % at 4 h; P < 0.01 compared with controls). In iNO-treated animals, Qs/QT decreased from 47 ± 2 % after induction of ALI to 27 ± 5 % at 4 h of treatment (P < 0.05 compared with controls). There were qualitative, albeit not statistically significant, differences in the time course of gas exchange parameters between the two treatment groups. iNO induced a maximum effect on arterial oxygenation within 1 h, and this improvement was sustained for the rest of the experiment. In animals treated with LU-135252, however, PaO₂ increased steadily, and had reached a maximum value at the end of study period.

**Haemodynamics**

During the experiments, no significant differences between groups were measured for heart rate or cardiac output (Table 1). Induction of ALI increased MPAP in all animals from 23 ± 1 to 28 ± 1 mmHg. In controls, MPAP continued to increase over the subsequent 4 h by 42 ± 7 %. Differences in MPAP between controls and the iNO group or the ET₄-A group were significant at 3 h and at 4 h respectively. Treatment with LU-135252 or iNO attenuated the percentage increase in MPAP significantly (ET₄-A group, 14 ± 4 %; iNO group, 6 ± 4 %; values at 4 h; each P < 0.01 compared with controls).

**TABLE 1** Parameters of haemodynamics and gas exchange

Groups were as follows: ET₄-A group, inhalation of 0.3 mg · kg⁻¹ LU-135252 for 20 min after onset of ALI; iNO group, continuous inhalation of 30 p.p.m. NO after onset of ALI; controls, inhalation of saline solution for 20 min after onset of ALI. ALI onset is defined as the time point before intervention. ΔMAP, relative change in mean arterial pressure compared with onset of ALI; ΔMPAP, relative change in MPAP compared with onset of ALI. Significance of differences:* P < 0.05, ** P < 0.01 compared with controls; † P < 0.05 compared with ALI-onset values.

<table>
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<th>Parameter</th>
<th>Protocol</th>
<th>ALI onset</th>
<th>1 h</th>
<th>2 h</th>
<th>3 h</th>
<th>4 h</th>
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<td>Controls</td>
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<td>85 ± 7</td>
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<td>75 ± 5</td>
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<td>Cardiac output (L/min)</td>
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<td>4.9 ± 0.4</td>
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<td>PaO₂ (mmHg)</td>
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<td>284 ± 29**</td>
<td>328 ± 40**</td>
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<td>228 ± 33**</td>
<td>205 ± 43**</td>
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Mean arterial pressure remained stable, and relative changes were not significantly different between groups.

**DISCUSSION**

Using an experimental model of ALI, we found that inhalation of the ET<sub>A</sub> receptor antagonist LU-135252 at a dosage of 0.3 mg·kg<sup>−1</sup> significantly improved gas exchange and haemodynamics, similar to the effects of 30 p.p.m. iNO, without systemic vasodilation. The effects of LU-135252 were sustained for more than 3 h after termination of the inhalation.

Since iNO is rapidly bound and inactivated by haemoglobin in the circulating blood within the lung, its action is limited to ventilated lung regions. Therefore iNO is considered to be the most selective pulmonary vasodilator known to date [7]. Due to the similarity of the effects of iNO and LU-135252, it appears likely that improvements in gas exchange following the inhalation of the ET<sub>A</sub> receptor antagonist are due mainly to selective vasodilation in ventilated lung regions, leading to redirection of blood flow towards these regions. This notion is supported further by the vasodilatory properties of ET<sub>A</sub> receptor antagonists [3], and by the fact that ET<sub>A</sub> receptor blockade stimulates release of NO and prostacyclin from the endothelium [9,10].

Whether the effects observed can be attributed to ET<sub>A</sub> receptor blockade alone is not entirely clear, because high local concentrations of LU-135252 will result in additional blocking of ET<sub>B</sub> receptors (the affinity of LU-135252 for the ET<sub>A</sub> receptor is about 130 times higher than for the ET<sub>B</sub> receptor) [11]. The pulmonary ET<sub>B</sub> receptors, however, are considered as clearance receptors for the endothelins, and blockade of these may increase the plasma concentration of ET-1 [12]. This may consecutively augment pulmonary vasoconstriction in the non-ventilated areas, whereas vasodilation will prevail in the ventilated areas due to the inhaled ET<sub>A</sub> receptor antagonist. A balance between these two opposite effects may explain why pulmonary artery pressure did not decrease after inhalation of the ET<sub>A</sub> antagonist, but was found to be stable at or slightly above the levels present after induction of ALI.

The different time course of arterial oxygenation in the two treatment groups may indicate the influence of additional mechanisms in animals treated with inhaled LU-135252. It has been found that ET<sub>A</sub> receptor antagonists inhibited antigen-induced lung inflammation in mice [13]. A possible anti-inflammatory effect could contribute to further reduce intrapulmonary right-to-left shunt and additionally improve gas exchange.

In conclusion, our results strongly support the hypothesis that the inhaled ET<sub>A</sub> receptor antagonist LU-135252, at a dosage of 0.3 mg·kg<sup>−1</sup>, acts as a selective pulmonary vasodilator. Further investigations should focus on: (1) the minimal dose of nebulized LU-135252 that is still effective in improving arterial oxygenation, (2) the effects of LU-135252 and iNO on tissue and plasma endothelin concentrations, (3) the efficacy of LU-135252 in reducing the progression of lung tissue damage, and (4) the determination of anti-inflammatory and/or anti-oedematous effects of ET<sub>A</sub> receptor antagonists in the injured lung.

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