Effects of inhaled nitric oxide on oxygenation and haemodynamic performance during one-lung ventilation in pigs

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Abstract
Objective: To study the effects of 20 ppm inhaled nitric oxide (iNO) on haemodynamics and systemic oxygenation during one-lung ventilation (OLV) in vivo. Design: Prospective animal study with a cross-over design. Setting: Animal laboratory of a university hospital. Subjects: Eight female pigs. Outcome measures: The pigs were anaesthetized, tracheally intubated, and mechanically ventilated. Following placement of femoral arterial and pulmonary artery catheters, a left-sided double-lumen tube (DLT) was placed via tracheotomy. After DLT placement, in each animal OLV was performed during intravenous anaesthesia in a cross-over design with and without iNO (20ppm) in 100% oxygen. After haemodynamic stabilization, haemodynamic measurements and blood gas analyses were made, in addition we measured differential lung perfusion with colored microspheres in three animals. Results and Conclusions: iNO did not improve oxygenation nor did iNO reduce pulmonary arterial pressure in our animal model. Mixed venous \( \text{PvO}_2 \) and cardiac output were comparable during the study periods.

Key words: Thoracic anaesthesia; One-lung ventilation; Nitric oxide; Oxygenation

Introduction
One-lung ventilation (OLV) is frequently required for lung, mediastinal, esophageal or aortic surgery. Although OLV is not always mandatory for such procedures, it improves access to the operative field and expedites the surgery. The most important problem that occurs during OLV is hypoxemia. The incidence of hypoxaemia during OLV is 5-10%, even if 100% oxygen is used for ventilation.1,2 The reason for this is that the non-ventilated lung remains perfused during the procedure, increasing the transpulmonary shunt fraction. Increasing the perfusion of the ventilated lung by drugs may be a possible strategy to improve oxygenation during OLV without inflating the non-dependent lung. One possible agent for this purpose is the endothelial dependent vasorelaxing factor nitric oxide (NO). In theory inhaled nitric oxide (iNO) could increase oxygenation by selectively decreasing pulmonary resistance and increasing blood flow to the ventilated lung. To investigate the effects of iNO on oxygenation and haemodynamics during OLV in vivo, we performed an animal study in pigs.

Methods
With approval of the local animal protection committee, eight female pigs (German land race, 32±1 kg) were studied. The animals were premedicated with ketamine (500mg intramuscularly) to allow placement of an intravenous catheter in an auricular vein, and to initiate ECG and pulse oximetry monitoring. Anaesthesia was induced with propofol (2-3mg/kg intravenously), muscle relaxation was achieved with pancuronium (0.2mg/kg intravenously), and the trachea of the animal was orally intubated with a 7.5ID tracheal tube. Ventilation was adjusted to maintain arterial CO\(_2\) tension (\(\text{PaCO}_2\)) at approximately 35-40mmHg. During preparation
anaesthesia was maintained with a 1:1 mixture of nitrous oxide (N₂O) and oxygen (O₂) and a continuous infusion of propofol (20–35mg·kg⁻¹·h⁻¹), remifentanil (10-20µg·kg⁻¹·h⁻¹) and pancuronium (0.15–0.2mg·kg⁻¹·h⁻¹). Using a sterile technique, an arterial catheter was placed in the left femoral artery and a central venous catheter was placed via the right internal jugular vein, to measure arterial and central venous blood pressure, respectively. A flow-directed thermodilution pulmonary artery catheter was passed through the right external jugular vein to measure cardiac output and pulmonary artery pressure. We positioned the tip of the pulmonary artery catheter just beyond the pulmonary valve to ensure placement in the main pulmonary artery (i.e. the catheter was not advanced to the wedge-position). The catheter was connected to a cardiac output (CO) device. CO measurements were performed in triplicate, using an injectate of 10ml cold saline (1-5°C), and averaged for each time point.

Tracheostomy was performed and the orotracheal tube replaced using Fibreoptic control by a left-sidet, specially designed 39Ch double-lumen tube. This DLT ensured that the right upper bronchus (which, according to the pig’s anatomy originates from the trachea) could also be ventilated or acceseasured through the tracheal limb. After DLT placement, the animals were positioned in the right lateral decubitus position, and an 8.5F tracheal tube was passed through a left-sidet mini-thoracotomy into the left pleural space. Ventilation to the left lung was then discontinued and lung collapse was verified by Fibreoptic observation of the left pleural space. OLV of the right lung was maintained during the whole study and correct DLT placement was verified by continuous dual capnography, Fibreoptic bronchoscopy, and by thoracoscopy at the end of both study periods.5

After these measures, remifentanil and N₂O were discontinued, and intravenous anaesthesia was continued with propofol without changing the infusion rate throughout the experiment.

FIO₂ was adjusted at 1.0. We set the ventilation pressure at 25cmH₂O, expiratory pressure (PEEP) at 5cmH₂O, and varied the respiratory frequency to achieve an end-tidal CO₂ at 33-38mmHg.

We used a modified ICU respirator including a NO delivery device (Evita 2 NODOMO). The NODOMO adjusts NO flow proportionally to the flow of the respirator to maintain a stable NO concentration. In addition it reliably measures NO concentrations in the inspiratory limb of the respiratory circuit. Each animal underwent in randomized order, two study periods, i.e. OLV with and without iNO 20ppm. Data were collected in each study period after equilibration times of at least 30 minutes with variations of blood pressure, heart rate and end-tidal CO₂ of no more than 10%. We noted cardiorespiratory parameters and obtained blood gas analysis. In three animals colored microspheres (see below) were administered through a central venous line for measurement of pulmonary perfusion in each study phase.

Microsphere technique: Application and methodological consideration of microsphere measurements have been presented in detail elsewhere.6 In summary, for measurements of regional pulmonary perfusion, 1.2-10⁶ colored microspheres with a nominal diameter of 15µm were injected slowly over 2min via the central venous catheter into the superior vena cava. Microsphere injections were performed at the end of the two experimental phases, using different colored microspheres in random sequence. At the end of the experiment the right and the left lungs were removed, dissected, and digested separately by placing them in a 4N concentrated solution of KOH. To obtain the microspheres, the digested samples were filtered through 9µm pore polyester membranes filters. The colored microspheres were quantified by their dye content. The dye was removed from the microspheres by adding 150µl dimethylformamide as a solvent. The photometric absorption of each dye solution was determined using a spectrophotometer at wave lengths 190-820nm. The number of microspheres was calculated using the specific absorbance value of the different dyes. Percentage of the right lung perfusion was calculated as the proportion of the microsphere number obtained from right lung on total number of microspheres.

Throughout the experiment the animals were kept in the right lateral decubitus position. The pigs received 10 ml/kg of body-warm balanced electrolyte solutions during the study period. At the end of the experiment the pigs were euthanized with a lethal dose of potassium chloride.

The data was statistically analyzed with the computing program SPSS, using Wilcoxon’s signed rank test. A p value of <0.05 was considered statistically significant. No statistical test was used to compare lung perfusion data because these data were only obtained in three animals.

Results
Oxygenation during OLV with application of 20ppm nitric iNO was not improved as compared with oxygenation during OLV without iNO. In addition, all haemodynamic parameters in both study periods, including pulmonary arterial pressure, were comparable with and without the application of NO (Table 1). Perfusion of the ventilated lung seemed to remain unchanged during application of iNO (Perfusion of the right lung during OLV measured in three animals with the microsphere technique: 89±14% with iNO vs. 88±11% without iNO).

<table>
<thead>
<tr>
<th>Table 1: The effects of nitric oxide on haemodynamic variables and oxygenation during one-lung ventilation</th>
<th>OLV with iNO</th>
<th>OLV without iNO</th>
</tr>
</thead>
<tbody>
<tr>
<td>PaO₂ (mm Hg)</td>
<td>347±124</td>
<td>353±110</td>
</tr>
<tr>
<td>PaCO₂ (mm Hg)</td>
<td>43±9</td>
<td>44±10</td>
</tr>
<tr>
<td>etCO₂ (mm Hg)</td>
<td>40±7</td>
<td>38±6</td>
</tr>
<tr>
<td>PVO₂ (mm Hg)</td>
<td>48±6</td>
<td>50±6</td>
</tr>
<tr>
<td>SvO₂ (%)</td>
<td>82±4</td>
<td>82±5</td>
</tr>
<tr>
<td>SpO₂ (%)</td>
<td>99±1</td>
<td>99±1</td>
</tr>
<tr>
<td>HR (min)</td>
<td>88±18</td>
<td>88±19</td>
</tr>
<tr>
<td>PAP (mm Hg)</td>
<td>20±4</td>
<td>21±4</td>
</tr>
<tr>
<td>MAP (mm Hg)</td>
<td>91±21</td>
<td>81±18</td>
</tr>
<tr>
<td>CVP (mm Hg)</td>
<td>8±2</td>
<td>9±2</td>
</tr>
<tr>
<td>CO (L/min)</td>
<td>3.8±1</td>
<td>3.8±2</td>
</tr>
<tr>
<td>Vt (ml)</td>
<td>345±80</td>
<td>357±75</td>
</tr>
<tr>
<td>RR (min)</td>
<td>22±7</td>
<td>20±7</td>
</tr>
<tr>
<td>PAW (cm H₂O)</td>
<td>25±1</td>
<td>25±1</td>
</tr>
</tbody>
</table>

Means ± SD; No significant differences between OLV with and without iNO for all variables. PAP = mean pulmonary artery pressure, MAP = mean systemic arterial pressure, CVP = central venous pressure, CO = cardiac output, Vt = tidal volume, RR = Respiratory rate, etCO₂ = endtidal CO₂, PAW = peak airway pressure.
Discussion

The main result of our animal study is in accordance with the results of most clinical studies during OLV in patients, where oxygenation was not improved by using iNO in concentrations of 20-40ppm. 3-9 Only Rocca demonstrated in a clinical study in 30 patients a small benefit of iNO in high-risk patients with a severe ventilation-perfusion mismatch. 10

In 2002 Sticher published data of an animal study in pigs, using different concentrations of iNO (4, 8, 16, 32ppm) during one-lung ventilation. 11 Oxygenation during OLV with iNO was improved in a dose-dependent manner, in the course of which iNO at 4ppm improved oxygenation to a greater extent than 8, 16 or 32ppm. The improvement in oxygenation was due to a reduction in intrapulmonary shunt flow, measured with the multiple inert gas elimination technique. The authors hypothesized that iNO at larger doses may spill over into the systemic circulation, thus attenuating the hypoxic pulmonary vasoconstriction effect of the upper lung which decreases PaO₂.

The improvement of oxygenation during OLV with iNO in the study of Sticher may be as a result of the fact that Sticher ventilated the smaller left lung of the animal using a FiO₂ of 0.8 compared with the setting in our study (ventilation of the larger right lung with a FiO₂ of 1.0). One may assume that the positive effect of iNO could be seen more easily during the use of a lower PaO₂. One reason why iNO could not divert blood from the non-ventilated to the ventilated lung in our experiment could be that the blood flow to the ventilated right lung in our experiment was already very high (88% of the total blood flow) without application of iNO, just as a result of the lateral decubitus position and of the maximum effect of hypoxic pulmonary vasoconstriction, which is not impaired by propofol. 12 In this situation, a further dilatation of the pulmonary vessels in the ventilated lung is probably not possible.

Conclusion

20ppm inhaled nitric oxide failed to improve oxygenation in our study in pigs during OLV in the right lateral decubitus position with total intravenous anaesthesia.

References