Effect of position, nitric oxide, and almitrine on lung perfusion in a porcine model of acute lung injury

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RECENT EXPERIMENTAL STUDIES investigating the effects of prone position, nitric oxide, and almitrine on lung perfusion in a porcine model of acute lung injury. J Appl Physiol 93: 2181–2191, 2002. First published August 30, 2002; 10.1152/japplphysiol.00313.2002.—In a porcine model of oleic acid-induced lung injury, the effects of inhaled nitric oxide (iNO) and intravenous almitrine bismesylate (ivALM), which enhances the hypoxic pulmonary vasoconstriction on the distribution of regional pulmonary blood flow (PBF), were assessed. After injection of 0.12 ml/kg oleic acid, 20 anesthetized and mechanically ventilated piglets [weight of 25 ± 2.6 (SD) kg] were randomly divided into four groups: supine position, prone position, and 10 ppm iNO for 40 min followed by 4 μg·kg⁻¹·min⁻¹ ivALM for 40 min in supine position and in prone position. PBF was measured with positron emission tomography and H215O. The redistribution of PBF was studied on a pixel-by-pixel basis. Positron emission tomography scans were performed before and then 120, 160, and 200 min after injury. With prone position alone, although PBF remained prevalent in the dorsal regions it was significantly redistributed toward the ventral regions (P < 0.001). A ventral redistribution of PBF was also obtained with iNO regardless of the position (P = 0.043). Adjunction of ivALM had no further effect on PBF redistribution. PP and iNO have an additive effect on ventral redistribution of PBF.

This context, it has been shown that putting OA-injured canine lungs in the prone position enhanced the effect of a recruitment maneuver on oxygenation (4). In addition, it has been reported that the resulting, more homogenous, ventilation-perfusion matching throughout the lungs was the principal explanation of the prone position-induced improvement of oxygenation (12). Exogenously administered inhaled nitric oxide (iNO) promotes pulmonary vasodilatation in well-aerated areas of the lung without any systemic hemodynamic effects (18, 46). However, the response to iNO in terms of gas exchange or pulmonary artery pressure is heterogeneous and unpredictable (41). One explanation for this finding may be that the pre-iNO pattern of pulmonary blood flow distribution strongly influences the response of iNO (10). A redistribution of pulmonary blood flow can alternatively be obtained with a selective pulmonary vasoconstrictor, such as intravenous almitrine bis- mesylate (ivALM). This agent promotes a selective vasoconstriction of shunt areas and redistributes pulmonary blood flow toward lung units with normal ventilation (29). In patients with acute respiratory distress syndrome, the combination of iNO and ivALM dramatically increased oxygenation (6). The above results were obtained during mechanical ventilation in the supine position. According to the effects of prone position on both pulmonary blood flow and ventilation distribution, iNO and ivALM could be expected to influence regional pulmonary blood flow and, hence, arterial oxygenation in a different way, depending on whether the animals are in a supine position or a prone position. Because prone position promotes alveolar recruitment and preservation of pulmonary blood flow in the dorsal lung regions, iNO should redirect a greater amount of blood toward the dorsal lung regions in the prone position compared with that in the supine position. The combination of iNO and ivALM should enhance the effect of iNO alone. We designed the present study to test these hypotheses.

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MATERIALS AND METHODS

Animal Preparation

This study protocol was approved by the University of Lyon animal research committee and performed in 20 male pigs [weight of 25 ± 2.6 (SD) kg]. They were premedicated with an intramuscular injection of xylazine (20 mg), ketamine (70 mg), and droperidol (5 mg) and then anesthetized with 100 mg iv propofol followed by a continuous intravenous infusion of 350 mg/h. Analgesia was performed with repeated injections of 100 μg of fentanyl every 30 min. Finally, a continuous intravenous infusion of pancuronium bromide (3 mg/h) was used to obtain complete muscle relaxation.

Instrumentation was performed with animals in the supine position. After tracheotomy, all pigs were mechanically ventilated in a volume-controlled mode with a squared inflation flow (Cesar II ventilator, Taema, Antony, France), inspired O₂ fraction (FIO₂) of 21%, and tidal volume (VT) of 10 ml/kg, and the respiratory rate was adjusted to achieve normocapnia (mean respiratory rate ± SD = 17.6 ± 2.4 breaths/min) on zero end-expiratory pressure (ZEEP). The body temperature was kept normal with a heating pad. The carotid artery was cannulated with a 8.5-Fr catheter. A 7.5-Fr pulmonary artery catheter (Swan-Ganz CCOMbo, Edwards Lifesciences LLC, Irvine, CA) was inserted via the left internal jugular vein. A 8.5-Fr three-lumen central venous catheter was then inserted into the right internal jugular vein.

Physiological Measurements

Pressure transducers (Baxter Healthcare, Deerfield, MI) were calibrated at the midstest level and connected to a NIDAQ 32-bit A/D card (National Instruments, Austin, TX). Systemic and pulmonary arterial pressures, airway pressure, and airflow were continuously recorded, sampled at 200 Hz, and analyzed with Biobench software (National Instruments). The value for VT was obtained from numerical integration of the airflow signal. The analog outputs of airway pressure and airflow located at the back of the ventilator were connected to the A/D card so that respiratory and hemodynamic parameters were acquired simultaneously. A complete set of physiological measurements was recorded after each assessment of pulmonary blood flow as follows.

Blood-gas measurements. Arterial blood was continuously monitored with a blood-gas analyzer (TrendCare, Agilent Technologies) and sampled every 10 s to obtain systemic arterial blood gas measurements. Mixed venous blood-gas measurements were obtained by analyzing blood taken from the distal port of the Swan-Ganz catheter (Ciba Corning 278).

Hemodynamic measurements. A complete set of measurements of mean pulmonary artery, mean systemic artery, pulmonary capillary wedge, and right atrial pressures was then obtained. Cardiac output was measured with a thermodilution technique in triplicate (Edwards Critical Care Explorer, Baxter Healthcare). Regardless of the respiratory cycle, 5 ml of a 0.9% saline solution, at room temperature, were injected into the proximal site of the Swan-Ganz catheter. The three values were then averaged.

Respiratory mechanics. During a baseline respiratory cycle, airways were occluded at the end of expiration for 3 s to assess the total positive end-expiratory pressure (PEEP) and at the end of the immediate inspiration for 4 s to obtain the end-inspiratory elastic recoil pressure of the respiratory system (Pst,rs). Static respiratory system compliance (Cst,rs) was obtained by dividing VT by (Pst,rs − PEEP).

Pulmonary Blood Flow Assessment With Positron Emission Tomography

We used an ECAT EXACT HR+ 3D positron emission tomography (PET) scanner (Siemens, Knoxville, TN), whose design and performance characteristics have already been reported (2, 19). PET tissue activity measurements were decay corrected back to the time of isotope administration. Fifteen centimeters were scanned from the top to the most caudal parts of the lung, i.e., 2–3 cm below the dome of the diaphragm, as confirmed by a 3-min transmission scan. A 10-min transmission scan was then performed to correct the subsequent emission data for attenuation. Next, 10 mCi of H₂¹⁵O were injected over 60 s. Data were collected for 10 min, 10 s from the start of the injection as follows: 10 images of 4-s duration each, 3 images of 10-s duration, 2 images of 30-s duration, 1 image of 2-min duration, and 1 equilibrium image of 6-min duration.

The methods used to measure pulmonary blood flow have been previously described (17, 31, 34). In general, PET is used to measure H₂¹⁵O blood and tissue concentrations. The lung activity measured with PET, when combined with blood activity (used as a reference) and analyzed with an appropriate compartmental model, yields tomographic images representative of pulmonary blood flow.

For each scan, the 63 original planes were summed in threes to obtain 21 planes. The five contiguous planes with the largest amount of lung tissue were selected for image analysis, starting from 1 to 2 cm above the diaphragm dome. Regions of interest (ROI) from the right and left lungs were defined on each scan, including all the lung elements of each side of the thorax, and excluded the hila, chest wall, and diaphragm (Fig. 1). ROIs were stored in the computer, and the radioactivity of each pixel in each region was measured. To normalize the regional pulmonary blood flow data for differences in cardiac output, the pulmonary blood flow in each pixel was expressed as a fraction of the total blood flow in the ROI.

Study Design

Allocation of treatments. Once the animal preparation was completed, the FIO₂ was increased to 100%. Experimental lung injury was obtained by injecting 0.12 ml/kg of OA into the right atrium (32) over 20 min, with the animals in supine position. During the 90-min period required to achieve stable lung injury, PEEP was set to 5 cmH₂O. The animals were then randomly divided into four experimental groups of five pigs each (Fig. 2). In the supine position group, animals were kept in the supine position until the end of the experiment. In the prone position group, animals were turned to the prone position until the end of the experiment. In the two iNO ivALM groups, the animals were first treated with 10 ppm of iNO for 40 min and then with the combination of 10 ppm iNO and 4 μg·kg⁻¹·min⁻¹ ivALM for 40 min, either in supine position (SP iNO ivALM group) or in prone position (PP iNO ivALM group).

iNO was administered from a tank containing NO in nitrogen at a concentration of 450 ppm (Air Liquide, Meudon, France) and sequentially delivered by using Opti-NO (TAEMA, Antony, France), attached at the inspiratory limb of the ventilator. With this device, iNO can be inhaled only during the inspiratory concentration (10 ppm) was achieved by setting the NO output for a given minute volume and inspiration-expiration ratio, using a slide ruler provided by the manufacturers. Almitrine (Vectorion, Servier, Neuilly sur Seine, France) was infused continuously as a solution of 15 mg almitrine by
bismesylate dissolved in 5 ml of malonic acid and diluted in 45 ml of isotonic glucose solution immediately before use. Because of the long plasma half-life of almitrine (31 h in humans), the sequence of treatments was not randomized to avoid a residual effect of ivALM.

**Timing of measurements.** Pulmonary blood flow determination and a complete set of hemodynamic and respiratory measurements were performed before lung injury (T0), at the end of the 90-min stabilization period after OA injection (T120), and 40 (T160) and 80 (T200) min later in each group (Fig. 2).

**Image Analysis**

The isogravitational ventral-to-dorsal distribution of pulmonary blood flow was assessed by using the method described by Schuster et al. (36). Briefly, the x- and y-coordinates for each pixel, along with the respective fractional pulmonary blood flow values for each pixel, were recorded. The pixel data were then sorted, first by their y-coordinate along the ventral-to-dorsal axis and then by their x-coordinate along an axis perpendicular to the y-axis. This provided a list of the pixels by location, beginning in the most ventral-medial portion of the region and ending with the most dorsal-lateral portion of the region. Each region contained 1,311 ± 292 pixels. Arbitrarily, the data were divided into 20 bins stacked vertically in the ventral-to-dorsal direction, so that each bin contained ~66 pixels, which could then be averaged. By keeping the number of bins per region and the number of tomographic planes per pig constant, the bin values could be averaged across the pigs, allowing comparisons between experimental groups.

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**Fig. 2.** Study protocol. SP, supine position; PP, prone position; SP iNO ivALM, inhaled NO and intravenous almitrine in SP; PP iNO ivALM, inhaled NO and intravenous almitrine in PP; OA, oleic acid. PET, positron emission tomography.
Measurement of Lung Density

To estimate regional lung density with PET, the camera was calibrated according to a method previously described (35). Both the measurement and distribution of the lung density were performed and expressed in the same way as for the pulmonary blood flow assessment (see above).

Statistical and Data Analysis

Continuous variables are expressed as means ± SD. The values of arterial blood gases continuously recorded during the H218O injection of each experimental condition were averaged. The values of pulmonary blood flow were expressed as milliliters per minute per 100 ml of lung volume. The groups were compared at T0 and T120 with one-way ANOVA. Relative variations were computed as the ratio of the difference in the mean between T160 or T200 and T120 and between T200 and T160 in each group. To quantify the pulmonary blood flow redistribution in each group, the differences in fractional pulmonary blood flow from T120 to T160 or T200 and from T160 to T200 were first determined over all the bins. Next, the differences in those bins in which the pulmonary blood flow increased from T120 to T160 or T200 and from T120 to T200 were summed (36, 40). These latter and the relative variations of physiological variables were compared with two-way ANOVA to test for interaction between and the respective effect of a two-level position factor (supine position and prone position) and of the respective effect of a two-level pharmacological factor. Oxygenation at T160 was markedly increased by prone position relative to supine position, irrespective of the presence or absence of iNO. Oxygenation at T160 was also significantly improved by iNO irrespective of the position. Oxygenation was not further improved by the addition of ivALM in either position. However, PaO2 was still higher at T200 than at T160 due to the continuing beneficial effect of prone position or iNO. Whereas mean arterial pressure was significantly increased in prone position, it remained unaffected by iNO at T160. At T200, mean arterial pressure did not change with prone position but increased with the addition of ivALM to iNO. By contrast, mean pulmonary artery pressure was significantly reduced by iNO but unchanged with position at T160. At T200, the mean values of mean pulmonary artery pressure returned to their levels at T120, due to the adjunction of ivALM, which had a similar quantitative effect in both positions. Slight relative changes were observed for pulmonary capillary wedge pressure and cardiac output.

Pulmonary Blood Flow Distribution

The pattern of ventral-to-dorsal distribution of fractional pulmonary blood flow in supine position was not different between the groups before (data not shown) and after lung injury (T120) (Fig. 4). The time course of this pattern was, however, markedly different between the four experimental groups (Fig. 5). In the supine position group, pulmonary blood flow distributions ob-

Table 1. Blood-gas, hemodynamic, and respiratory mechanics before and after acute lung injury

<table>
<thead>
<tr>
<th>Time of Measurement</th>
<th>SP</th>
<th>SP iNO ivALM</th>
<th>PP</th>
<th>PP iNO ivALM</th>
</tr>
</thead>
<tbody>
<tr>
<td>pH</td>
<td>T0</td>
<td>7.48 ± 0.03</td>
<td>7.48 ± 0.04</td>
<td>7.51 ± 0.03</td>
</tr>
<tr>
<td></td>
<td>T120</td>
<td>7.41 ± 0.11</td>
<td>7.36 ± 0.09</td>
<td>7.40 ± 0.09</td>
</tr>
<tr>
<td>PaO2, Torr</td>
<td>T0</td>
<td>515 ± 65</td>
<td>562 ± 60</td>
<td>599 ± 73</td>
</tr>
<tr>
<td></td>
<td>T120</td>
<td>393 ± 198</td>
<td>352 ± 185</td>
<td>418 ± 144</td>
</tr>
<tr>
<td>PaCO2, Torr</td>
<td>T0</td>
<td>41 ± 1</td>
<td>41 ± 1</td>
<td>40 ± 4</td>
</tr>
<tr>
<td></td>
<td>T120</td>
<td>47 ± 7</td>
<td>51 ± 7</td>
<td>48 ± 6</td>
</tr>
<tr>
<td>PvO2, Torr</td>
<td>T0</td>
<td>58 ± 4</td>
<td>56 ± 9</td>
<td>55 ± 5</td>
</tr>
<tr>
<td></td>
<td>T120</td>
<td>42 ± 3</td>
<td>43 ± 7</td>
<td>44 ± 6</td>
</tr>
<tr>
<td></td>
<td>T0</td>
<td>97 ± 5</td>
<td>100 ± 15</td>
<td>94 ± 11</td>
</tr>
<tr>
<td></td>
<td>T120</td>
<td>88 ± 10</td>
<td>95 ± 19</td>
<td>82 ± 14</td>
</tr>
<tr>
<td></td>
<td>T0</td>
<td>17 ± 2</td>
<td>20 ± 2</td>
<td>17 ± 2</td>
</tr>
<tr>
<td></td>
<td>T120</td>
<td>32 ± 7</td>
<td>40 ± 2</td>
<td>34 ± 7</td>
</tr>
<tr>
<td></td>
<td>T0</td>
<td>6 ± 3</td>
<td>9 ± 3</td>
<td>7 ± 1</td>
</tr>
<tr>
<td></td>
<td>T120</td>
<td>8 ± 2</td>
<td>13 ± 5</td>
<td>10 ± 2</td>
</tr>
<tr>
<td></td>
<td>T0</td>
<td>3 ± 0.7</td>
<td>3.0 ± 0.6</td>
<td>2.9 ± 0.5</td>
</tr>
<tr>
<td></td>
<td>T120</td>
<td>1.4 ± 0.3</td>
<td>1.6 ± 0.6</td>
<td>1.5 ± 0.7</td>
</tr>
<tr>
<td></td>
<td>T0</td>
<td>279 ± 38</td>
<td>283 ± 81</td>
<td>271 ± 48</td>
</tr>
<tr>
<td></td>
<td>T120</td>
<td>1461 ± 448</td>
<td>1521 ± 740</td>
<td>1626 ± 970</td>
</tr>
<tr>
<td></td>
<td>T0</td>
<td>23 ± 3</td>
<td>28 ± 4</td>
<td>28 ± 4</td>
</tr>
<tr>
<td></td>
<td>T120</td>
<td>16 ± 2</td>
<td>19 ± 2</td>
<td>19 ± 2</td>
</tr>
</tbody>
</table>

Values are mean ± SD. T0, before acute lung injury; T120, 120 min after the initiation of acute lung injury; SP, supine position; PP, prone position; iNO, inhaled nitric oxide; ivALM, intravenous almitrine; PaO2, arterial oxygen pressure; PaCO2, arterial carbon dioxide pressure; PvO2, mixed venous blood oxygen pressure; MAP, mean arterial pressure; MPAP, mean pulmonary artery pressure; PCWP, pulmonary capillary wedge pressure; CO, cardiac output; PVR, pulmonary vascular resistance; Cst.rs, static compliance of the respiratory system.

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The main findings of this study are that in OA-induced lung injury in pigs 1) iNO redistributed pulmonary blood flow toward the ventral regions; 2) although this effect was observed in both positions, it was markedly and significantly increased by prone position with or without the adjunction of iNO (P < 0.001; Fig. 7A). Inhalation of NO also increased the ventral pulmonary blood flow redistribution irrespective of the position (P = 0.043; Fig. 7B). No interaction was found between posture and iNO. The adjunction of ivALM had no detectable effect on pulmonary blood flow distribution irrespective of the position (Figs. 5C, 5D, 6B, and 7B).

**DISCUSSION**

The main findings of this study are that in OA-induced lung injury in pigs 1) iNO redistributed pulmonary blood flow toward the ventral regions; 2) although this effect was observed in both positions, it was markedly and significantly increased by prone position with or without the adjunction of iNO (P < 0.001; Fig. 7A). Inhalation of NO also increased the ventral pulmonary blood flow redistribution irrespective of the position (P = 0.043; Fig. 7B). No interaction was found between posture and iNO. The adjunction of ivALM had no detectable effect on pulmonary blood flow distribution irrespective of the position (Figs. 5C, 5D, 6B, and 7B).

Table 2. Relative changes in PaO2 and hemodynamic measurements

<table>
<thead>
<tr>
<th>Relative Variation</th>
<th>SP</th>
<th>SP iNO ivALM</th>
<th>PP</th>
<th>PP iNO ivALM</th>
<th>Posture Effect</th>
<th>Pharmacological Effect</th>
</tr>
</thead>
<tbody>
<tr>
<td>∆(T160 – T120)</td>
<td>12 ± 7</td>
<td>43 ± 36</td>
<td>40 ± 36</td>
<td>97 ± 64</td>
<td>P = 0.041*</td>
<td>P = 0.031*</td>
</tr>
<tr>
<td>∆(T200 – T120)</td>
<td>10 ± 23</td>
<td>41 ± 36</td>
<td>43 ± 34</td>
<td>108 ± 49</td>
<td>P = 0.008*</td>
<td>P = 0.011*</td>
</tr>
<tr>
<td>∆(T200 – T160)</td>
<td>-2 ± 9</td>
<td>-2 ± 4</td>
<td>3 ± 2</td>
<td>11 ± 17</td>
<td>P = 0.040*</td>
<td>P = 0.040*</td>
</tr>
</tbody>
</table>

MAP

| ∆(T160 – T120)    | -1 ± 9 | 9 ± 11  | 15 ± 8  | 17 ± 13  | P = 0.018* | P = 0.240 |
| ∆(T200 – T120)    | -5 ± 16 | 30 ± 16 | 20 ± 16 | 24 ± 20  | P = 0.271  | P = 0.027* |
| ∆(T200 – T160)    | -4 ± 9  | 21 ± 9  | 5 ± 11  | 7 ± 17   | P = 0.660  | P = 0.029* |

MPAP

| ∆(T160 – T120)    | -2 ± 3 | -22 ± 9 | -2 ± 5  | -35 ± 17 | P = 0.129  | P < 0.001* |
| ∆(T200 – T120)    | 0 ± 8  | -1 ± 9  | -10 ± 12 | -16 ± 30 | P = 0.134  | P = 0.686  |
| ∆(T200 – T160)    | 2 ± 6 | 21 ± 13 | -8 ± 10 | 19 ± 23  | P = 0.391  | P = 0.004* |

PCWP

| ∆(T160 – T120)    | -3 ± 7 | -9 ± 12 | -8 ± 26 | -13 ± 39 | P = 0.726  | P = 0.665  |
| ∆(T200 – T120)    | -5 ± 13 | -12 ± 18 | 0 ± 8  | 2 ± 35  | P = 0.384  | P = 0.810  |
| ∆(T200 – T160)    | -2 ± 12 | -3 ± 18 | 10 ± 25 | 14 ± 25  | P = 0.163  | P = 0.883  |

CO

| ∆(T160 – T120)    | -11 ± 10 | -5 ± 22 | 15 ± 20 | 3 ± 19  | P = 0.054  | P = 0.773  |
| ∆(T200 – T120)    | -17 ± 23 | 1 ± 27  | 12 ± 16 | 12 ± 9  | P = 0.045* | P = 0.337  |
| ∆(T200 – T160)    | -6 ± 14 | 6 ± 29  | 4 ± 23 | 9 ± 13  | P = 0.775  | P = 0.217  |

Values are means ± SD. For further details, see METHODS. ∆(T160 – T120), relative variation between T160 and T120; ∆(T200 – T120), relative variation between T200 and T120; ∆(T200 – T160), relative variation between T200 and T160. *P < 0.05.
was more marked in prone position, so that iNO and prone position had an additive effect on ventral pulmonary blood flow redistribution; 3) adding ivALM to iNO had no effect on pulmonary blood flow redistribution irrespective of the position. To our knowledge, this is the first study that systematically investigated the respective effect of position and iNO on pulmonary blood flow distribution.

Methodological Considerations

PET study. The assessment of pulmonary blood flow with PET has been validated vs. radioactive microspheres (17, 30). In the present study, pulmonary blood flow has been normalized to take into account the changes of cardiac output between animals and between experimental conditions by using the bin method already reported (36). In the particular context of the present study, one may argue that some spatial uncertainty is present between regions investigated in supine and in prone positions. This possibility is rather unlikely because, first, we have explored five contiguous planes, always located 1–2 cm above the diaphragm dome, corresponding to ~4 cm of lung height; second, the sensitivity of the camera is optimal for the slices located in the center of the volume sampled; and third, both size and shape of the lung are similar in the slices selected. Hence, a same amount of error due to volume-averaging effect between slices is expected. Finally, there was no significant difference in ventral-to-dorsal distribution of pulmonary blood flow in adjacent slices (data not shown).

The lack of direct assessment of alveolar ventilation in our study prevents full interpretation of our results. To try to circumvent this limitation, we measured the lung density from the transmission scan, an approach that has been found to be valid (35). In vivo, respiratory movement and high-density structure surrounding the lungs are responsible for a partial volume effect, which tends to overestimate true lung density. This overestimation has been found between 0.017 and 0.035 g/cm³ (average = 0.026 g/cm³) (3), which is <10% of the average density measured in this study and much lower than the density redistribution between experimental conditions. Furthermore, this experimental error is expected to be minimized by the increase in sensitivity of new generation PET camera as...
Therefore, we think that this method is suitable to detect relevant modifications of pulmonary density in lung-injured animals. However, lung density by itself cannot allow partitioning between lung tissue, blood, and edema fluid. This is also the case with computed tomography scan (27, 28). Therefore, although lung density is negatively correlated with alveolar gas, a given amount of regional alveolar volume may be associated with different levels of regional alveolar ventilation.

**Lung injury.** The OA model is a well-characterized and predictable model of acute lung injury (32). It has also been frequently used to investigate the effects of iNO in experimental acute lung injury (25, 37, 38). The dose of OA we have used is in the range of that found in the literature. Nevertheless, the degree of lung injury and the relative contribution of injury and atelectasis in our study deserve further discussion.

**Degree of lung injury in our study.** Judging from oxygenation and lung compliance, the degree of lung injury in our study may have been less than in recently reported investigations (4, 15, 16). Differences in animal species, method of OA administration, ventilatory settings, timing, and level of PEEP application and fluid administration may account for part of the suspected difference in the intensity of lung injury. In the 20 animals of our study, however, the mean values of PaO₂ on ZEEP between T0 and nadir after OA injection were 559 ± 91 and 218 ± 128 Torr, respectively (P < 0.001), suggesting that, on average, significant lung injury had been done. It should be noted that our values of Cst,rs (computed with a plateau pressure after a 4-s end-inspiratory pause) were overestimated relative to those obtained in the study of Cakar et al. (4) who used a 2-s end-inspiratory pause. Finally, our study suffered from the lack of a direct measurement of lung injury as histological examination. Nevertheless, although injury was rather less than that obtained in other reports, the acute lung injury was, in our investigation, similar between experimental conditions. In addition, it should be noted that we have found important hemodynamic derangements. Specifically, mean pulmonary arterial pressure and cardiac output changed by at least 100% relative to baseline in our study. These hemodynamic changes could be the expression of major vascular damage in the pulmonary circulation whose importance and relevance have recently been recalled (24). In the perspective of our study...
present work focusing on pulmonary circulation, this finding is, per se, as important as oxygenation data.

Relative contribution of lung injury and atelectasis in our study. The question of how much injury and how much atelectasis is contributing to gas-exchange abnormalities is important to address in this model. Indeed, OA caused significantly more atelectasis than lavage- or endotoxin-induced lung injury in pigs, as assessed from computed tomography scan (23). However, in assessing directly regional lung expansion during OA-induced lung injury in pigs, others have found that the lungs were not collapsed but derecruited (15, 16). The contribution of high FiO₂ to denitrogenation atelectasis was prevented in our study by performing animal preparation with 21% FiO₂. To separate the respective influence of injury and atelectasis in our work, an additional experiment was done as a posteriori as follows. Five additional pigs who remained in supine position underwent preparation and lung injury with OA similar to that in the previous 20 animals. At T120, aggressive recruitment maneuver was performed as a sustained inflation of 50 cmH₂O for 30 s. Arterial blood gases and Cst,rs were serially measured before and every 5 min for 30 min after injury. The results of this additional experiment are shown in Fig. 8. The recruitment maneuver failed to significantly improve both PaO₂ and Cst,rs relative to the pre-recruitment maneuver condition. This finding suggests that in our study atelectasis slightly contributed to oxygenation abnormalities. It should be noted that the mean values of PaO₂ at T120 in this additional experiment on five pigs were close to those obtained during the original experiment on 20 pigs (379 ± 128 vs. 349 ± 179 Torr) for similar body position, FiO₂, and PEEP.

Effect of Prone Position

The change in ventral-to-dorsal distribution of pulmonary blood flow from supine position to prone position is the opposite of what is expected from the law of gravity. This phenomenon has also been repeatedly observed in normal animals of different species (8, 21, 43) and in injured animals (33), challenging the classical view of the gravity-dependent distribution of pulmonary blood flow. To explain this relative lack of gravity dependence, the fractal behavior of pulmonary blood flow has been evoked (7, 9). Hypoxic pulmonary vasoconstriction has been found to be probably intact in OA-induced acute lung injury (10) but impaired in endotoxemia (1, 11, 44). The significant improvement of oxygenation with prone position should, therefore, result from the better balance between pulmonary blood flow, which continues to be redirected toward dorsal areas, and ventilation, which should increase in these nondependent regions (15, 16).

Effect of iNO in Supine Position

To our knowledge, only one study has investigated the effects of iNO on pulmonary blood flow distribution assessed with PET (10). In dogs in supine position, Gust et al. (10) reported a significant reduction of relative pulmonary blood flow in the dorsal regions and no effect of iNO, either on pulmonary blood flow pattern or oxygenation after OA. Our results are different because a slight but significant ventral shift of pulmonary blood flow, together with a significant improvement of oxygenation, has been observed in our SP iNO ivALM group at T160. In the literature, conflicting results have been reported on the effect of iNO on oxygenation in OA-induced acute lung injury, with some studies showing a significant effect of iNO (25, 47, 48) and another no effect on oxygenation (38). These discrepant results may be explained partly by the presence or absence of PEEP (26), since the studies in which iNO had no effect on oxygenation were done on ZEEP. Under these conditions, lungs are likely to be massively derecruited so that the iNO cannot reach its site of action in the nonventilated areas. In our study, the lung density distribution was virtually unaffected by iNO (Fig. 9C), suggesting that the pattern of alveolar ventilation was unchanged with iNO. Under these conditions, the intrapulmonary outcome of iNO should follow the distribution of alveolar ventilation. The change in the pulmonary blood flow pattern with iNO should suggest that the ventral lung regions received most of the alveolar ventilation. This is in line with the results reported by Mure et al. (21) who showed that the ventilation of ventral regions was greater than that of dorsal regions in normal pigs. Hence, the improvement of oxygenation in the SP iNO ivALM group is due to a redirection of the pulmonary blood flow toward the ventral well-ventilated areas in our study. If, however, before OA-induced acute lung injury, hypoxic pulmonary vasoconstriction was inhibited by priming the animals with an E. coli endotoxin injection, iNO would have restored the ventral shift of pulmonary blood flow and resulted in a marked improvement in oxygenation (10). This finding has been explained by the reduction of the postcapillary vascular resistance of ventilated lung units by iNO, without precapillary vasodilatation (10). Our pulmonary blood flow results differ from
those of the OA-group in Gust et al.’s study (10) but are similar to those reported for the endotoxin + OA group in their study. This suggests that some endotoxinemia may have occurred during our experiments, although this is unlikely. First, great care was taken to avoid, as much as possible, this risk during experiments by using sterile devices and by performing surgical procedures under sterile conditions. Second, mean pulmonary artery pressure and pulmonary vascular resistance significantly increased between the baseline state and T120 in our study (Table 1) as it did in the OA group in Gust et al.’s study (10). In their study, mean pulmonary artery pressure did not change in the endotoxin + OA group, so the preserved pulmonary vascular response in our study strongly suggests the absence of endotoxinemia. The discrepancy of the pulmonary blood flow distribution between our study and that of Gust et al. might be explained, besides by interspecies differences, by more severe lung injury in our study leading to a greater degree of ventilation to perfusion mismatch and/or by a prevalent injury of the dorsal regions in our study resulting in iNO being preferentially distributed in the ventral regions.

Effects of iNO in Prone Position

To our knowledge, there has been no experimental report of the effect of iNO on pulmonary blood flow distribution in prone position to which we can compare our observations. We observed that the effects of iNO and prone position on regional pulmonary blood flow were additive. This finding can be simply explained by a better alveolar ventilation in the ventral region in prone position. We cannot confirm this hypothesis because we did not measure the alveolar ventilation directly. However, the pattern of lung density does not strongly support a better ventilation in ventral regions in prone position. The density of these ventral regions increased in prone position compared with supine position (Fig. 9, B and D), but it is not the density distribution that is important but the alveolar ventilation. This latter has been reported to be greater in the ventral regions in normal, mechanically ventilated animals in prone position (21, 42). Our present results might be explained by a greater alveolar ventilation in the ventral regions in agreement with the previous studies.

Effect of ivALM in Combination With iNO

Almitrine constricts the pulmonary arteries by a mechanism that mimics and competes with hypoxic pulmonary vasoconstriction. Even intravenously administered almitrine has a constricting effect limited to pulmonary arteries and does not affect pulmonary veins nor systemic vessels. When hypoxic pulmonary vasoconstriction is present, almitrine does not further constrict pulmonary arteries; when it is absent, almitrine restores a pulmonary arterial constriction similar to hypoxic pulmonary vasoconstriction. From an extensive Medline search, we found only one experimental investigation testing iNO in combination with ivALM in acute lung injury (5). The authors reported that, in pigs submitted to surfactant depletion induced by saline washing, 1 μg·kg⁻¹·min⁻¹ ivALM had an additive effect to that of 10 ppm iNO on oxygenation improvement. This result was explained by a redirection of pulmonary blood flow from nonventilated lung regions toward those with normal ventilation to perfusion ratios, but regional pulmonary blood flow was not mea-

Fig. 9. Average ventral-to-dorsal distribution of pulmonary densities in the 4 experimental groups from T120 to T200. A: SP group. B: PP group. C: SP iNO ivALM group. D: PP iNO ivALM group. Each symbol represents the mean value for all pigs in each group.
sured in this study. We observed that ivALM increased mean pulmonary artery pressure with no change in either oxygenation or pulmonary blood flow distribution. This observation supports a diffuse nonspecific vasoconstrictive effect of ivALM, as has already been reported in dogs with OA-induced lung injury (13, 14). The nature of the acute lung injury may influence the effect of ivALM on pulmonary circulation. In conditions of surfactant depletion, contrary to those of OA lung injury, ivALM has been reported to induce an improvement in oxygenation (5). In this model, there was a dose-dependent effect of ivALM up to 2 μg·kg⁻¹·min⁻¹, above which arterial oxygen worsened (39). This dose-dependent effect of ivALM apparently does not exist in OA-induced lung injury (13, 14).

Conclusions

Prone position and iNO have an additive effect on the redistribution of pulmonary blood flow toward the ventral regions. This finding supports the theory that alveolar ventilation is greater in the ventral regions in the prone position, thus allowing more iNO to reach these regions. The OA model does not seem to be suitable for studying the effect of ivALM given at 4 μg·kg⁻¹·min⁻¹ on regional pulmonary blood flow. Further studies, combining regional pulmonary blood flow and alveolar ventilation assessment, may better explain the mechanisms responsible for oxygenation improvement using prone position and iNO.

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