Oxygen Attenuates Atelectasis-induced Injury in the In Vivo Rat Lung

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Background: Atelectasis results in impaired compliance and gas exchange and, in extreme cases, increased microvascular permeability, pulmonary hypertension, and right ventricular dysfunction. It is not known whether such atelectasis-induced lung injury is due to the direct mechanical effects of lung volume reduction and alveolar collapse or due to the associated regional lung hypoxia. The authors hypothesized that addition of supplemental oxygen to an atelectasis-prone ventilation strategy would attenuate the pulmonary vascular effects and reduce the local levels of vasoconstrictor eicosanoids.

Methods: In series 1, anesthetized, atelectasis-prone mechanically ventilated rats were randomly assigned to one of six groups based on inspired oxygen concentration and ventilation without recruitment. Series 2 was performed to determine if the cardiac and pulmonary vascular effects of 21% versus 100% inspired oxygen. In series 3, a computed tomography scans were performed after ventilation with a recruitment strategy (21% O2) or no recruitment strategy (21% O2 or 100% O2). In series 4, functional residual capacity was measured in animals where the gas was 21% or 100% O2.

Results: The partial pressure of arterial oxygen increased with increasing inspired oxygen, but the alveolar–arterial oxygenation gradient was also greater with higher inspired oxygen. Ventilation with 21% O2 (but not with 100% O2) was associated with progressive pulmonary vascular impedance and increased pulmonary vascular permeability. Prostaglandin F2α was increased by mechanical ventilation, especially without supplemental oxygen. Computed tomography scans demonstrated no atelectasis in recruited lungs, and atelectasis in nonrecruited lungs that was greater with supplemental oxygen. Increased atelectasis with 100% O2 (ex. 21% O2) was demonstrated by measurement of functional residual capacity.

Conclusions: Although supplemental oxygen worsened atelectasis in this model, it prevented the pathologic effects of atelectasis, including microvascular leak and pulmonary hypertension. Atelectasis-induced lung injury seems to be mediated by hypoxia rather than by the direct mechanical effects of atelectasis.

ATELECTASIS, defined as loss of lung volume, has important adverse effects on intrapulmonary shunt, oxygenation, and pulmonary mechanics.1–4 Prevention or reversal of atelectasis through recruitment of lung volume reduces injury caused by multiple etiologies, including high tidal volume5 or vascular reperfusion.6 In addition, ventilation with high-frequency oscillation at low lung volumes results in significantly greater lung injury compared with maintenance of higher lung volumes.7 Furthermore, with low tidal volume ventilation in experimental acid aspiration, the absence of lung recruitment increased mortality,8 a finding that was not associated with increase of pulmonary or systemic cytokines.8 In fact, we have recently described how, in the absence of supplemental oxygen, atelectasis can, during low tidal volume ventilation, result in pulmonary microvascular leak, as well as right ventricular failure associated with increased pulmonary vascular resistance.9 Therefore, the vascular consequences of atelectasis, including altered permeability, endothelial disruption, and increased resistance to pulmonary blood flow,10 may all contribute to increased morbidity or mortality in laboratory models.8,9 Simply ventilating these animals with room air (21% O2) and without either positive end-expiratory pressure (PEEP) or recruitment maneuvers results in a significant mortality. Although such effects clearly do not occur with comparable magnitude in the clinical context, recent work suggests that the prevention or early reversal of atelectasis in high-risk patients is associated with improved outcome.10

In the presence of lower oxygen tension, pulmonary arterial tone increases (i.e., hypoxic pulmonary vasoconstriction), diverting blood flow from poorly ventilated and less oxygenated to better oxygenated lung regions, thereby improving systemic arterial oxygenation.11,12 As a consequence, hypoxic pulmonary vasoconstriction leads to an increase in pulmonary arterial blood pressure. In a previous investigation, we observed that formation of atelectasis impaired right ventricular function and increased microvascular permeability in association with hypoxemia and increased pulmonary vascular arterial pressure. Previous studies have shown that atelectasis is not necessarily injurious,13 but when accompanied by regional lung hypoxia, it has dramatic consequences.9 Because alveolar oxygen is most readily increased by increasing inspired oxygen, we questioned whether supplemental oxygen would attenuate the acute lung injury and cardiac dysfunction associated with the development of atelectasis. We used an experimental model (i.e., low tidal volume ventilation in the absence of recruitment or PEEP) that we previously demonstrated causes atelectasis, as confirmed by a directly measured reduc-
tion in the functional residual capacity. We hypothesized that supplemental oxygen would correct the regional lung hypoxia and, despite increasing the formation of atelectasis, would attenuate atelectasis-associated adverse pulmonary vascular effects.

**Materials and Methods**

Institutional ethics approval (conforming to the guidelines of the Canadian Council for Animal Care) was obtained for all experiments.

*Anesthesia and Surgical Preparation*

Male Sprague-Dawley rats (200–300 g) were used in all experiments. General anesthesia was induced with ketamine and xylazine and was maintained with intravenous ketamine. A surgical tracheotomy was performed. The following ventilation parameters were used: tidal volume ($V_t$), 8 ml/kg; frequency, 50–60 min$^{-1}$; PEEP, 1 cm H$_2$O; and 21% O$_2$, using a small-animal ventilator (model 683; Harvard Apparatus, MA). Increments of pancuronium were used for muscle relaxation, having confirmed no response to stimulation. Four series of experiments were performed.

**Series 1**

To determine the dose–response effects of supplemental oxygen, animals were randomly assigned to one of six groups based on the inspired oxygen concentration (%) as follows: group 1, 21%; group 2, 24%; group 3, 30%; group 4, 40%; group 5, 70%; and group 6, 100%. After randomization, PEEP was discontinued, the assigned inspired oxygen was initiated, and the remainder of the ventilator settings was unchanged for the next 150 min. No recruitment maneuvers or PEEP was used during the remainder of the experiment. Pulmonary alveolar–capillary permeability was assessed by measurement of Evans blue in bronchoalveolar lavage (BAL) fluid after intravenous administration. Evans blue (15 mg/kg) was administered intravenously 20 min after randomization. At the end of the experiment, the animal was killed, and the heart–lung block was dissected. The right lung was lavaged with a single aliquot of 3 ml (normal saline), which was injected and aspirated a total of three times. The resulting bronchoalveolar fluid was collected, and the volume was recorded. The concentration of Evans blue in the supernatant was determined spectrophotometrically by measurement of its absorbance at 620 nm. At completion of the experiments, in addition to BAL, plasma samples were also collected, and lung wet/dry weight ratio was calculated.

**Series 2**

To determine the cardiac and pulmonary vascular effects of supplemental oxygen, six pairs of animals were randomly assigned to ventilation (same parameters as in series 1) with either room air (21% O$_2$) or 100% O$_2$. The same experimental protocol was followed as for series 1 except that Evans blue was not administered. A baseline echocardiogram was performed before randomization and repeated, at 30-min intervals for 150 min, by an experienced echocardiographer. Each echocardiogram took approximately 3 min to complete. The echocardiogram was used to determine the following parameters: pulmonary arterial acceleration time, right ventricular end-diastolic area, and left ventricular end-diastolic area. Echocardiography was performed using a Hewlett-Packard (Andover, MA) prototype Sonos 5500 echocardiographic system (M2424A) with a 7.5-MHz transducer. The animals were examined in the supine position with the chest closed. The transducer was gently applied to the left parasternal border to obtain a short-axis view of the left (distal to the mitral valve leaflets) and right ventricles at a frame rate of 113 Hz and depth of 2 cm. Serial two-dimensional images were acquired using short-axis views at baseline and at 30-min intervals until completion of the experiment. From a left parasternal long-axis view, the pulsed Doppler gate (with an angle of insonation below 20°) was carefully placed at the tip of the mitral valve leaflets to obtain diastolic flow patterns (E and A waves) and velocities. Data were obtained at baseline and at 30-min intervals. All images acquired were stored electronically for subsequent analysis.

Bronchoalveolar lavage was performed at the end of the experiment on all animals, and prostanoid concentrations were measured. An additional group (unventilated controls, n = 6) was anesthetized and killed, and BAL was performed for control concentrations of prostanoids. Prostanoids were measured using a Sciex API 4000 tandem mass spectrometry$^{15}$ in the electrospray ionization negative ion mode with TurbolonSpray (MDS Sciex, Ontario, Canada). Samples were spiked with 1.0 ng of a mixture of deuterated analogs of the prostanoids to be measured (Cayman Chemical Co., Ann Arbor, MI), acidified to pH 4 with 1N HCl, and extracted with ethyl acetate. The organic phase was evaporated to dryness and transferred to siliconized mini vials for analysis by mass spectrometry. Quantitation was performed by comparing the deuterium/proton ratio of the individual prostanoids in the sample with standard lines generated from authentic prostanoids. An Agilent high-performance liquid chromatography 1100 was at the front end, equipped with a Zorbax SB-phenyl column (3.0 × 50 mm, 3.5 m spherical size; Chromatographic Specialties Inc., Brockville, Ontario, Canada). The tandem mass spectrometry source temperature was maintained at 500°C, and the ion source voltage at −4,500 V. Compounds were separated on high-performance liquid chromatography with a direct inlet into the mass spectrometry source. The high-performance liquid chroma-
tography solvent followed the program: water/acetonitrile 80/20 at sample injection and maintained for 2 min, 75/25 for 0.5 min, 50/50 by 5 min, 45/55 by 6.2 min, and 0/100 by 11 min, where this was maintained for 1.5 min. The solvent was then recycled to 80/20 for the next run. The flow rate was at 400 ml/min. High-performance liquid chromatography solvents contained 2 μl/l propionic acid.

**Series 3**

To illustrate that atelectasis was present in animals ventilated without recruitment, computed tomography scans were performed in animals randomly assigned to one of three groups: ventilation with recruitment (21% O₂) or ventilation without recruitment (21% O₂ or 100% O₂). Animals were imaged with use of a General Electric Lightspeed Ultra 8 slice scanner (Milwaukee, WI). The scan protocol included a 9.6-cm field of view, 120 KVP, 100 mAs, and a 512 × 512 matrix. Axial 2-s scans through the lungs were obtained at a slice thickness of 0.65 mm with edge reconstruction. Sections at the level of the division of the right mainstem bronchus were selected.

**Statistical Analysis**

Parametric data are expressed as mean ± SD, and nonparametric data are expressed as median ± interquartile range. Two-way analysis of variance was used for parametric statistical analysis, and analysis of variance on ranks (Kruskal-Wallis) was used for nonparametric statistical analysis. For serial measurements over time (i.e., echocardiographic data), two-way repeated-measures analysis of variance was used, and where data were nonparametric, log transformation was performed before analysis of variance. The Bonferroni correction was applied where multiple comparisons were made with post hoc tests. Regression analysis was used to determine dose-response relations. Significance was set at *P* < 0.05.

**Results**

**Series 1: Dose-Response Effects of Supplemental Oxygen**

Thirty animals were entered for series 1. One animal did not meet the randomization criteria and was excluded from the study. All other 29 animals were eligible. All baseline parameters (weight, hemoglobin, partial pressure of arterial oxygen [PaO₂], partial pressure of arterial carbon dioxide [PaCO₂], bicarbonate, base excess, airway pressure, and lung compliance) were comparable among the six groups (table 1). The survival with 21% O₂ was four out of seven (57%); all animals survived the experimental protocol in the other groups (*P* = 0.05; table 1 and fig. 1).

The comparisons of the respiratory and acid-base data are presented (table 1). Pulmonary microvascular leakage was maximal in the absence of supplemental oxygen, and the addition of supplemental oxygen resulted in a dose-dependent decrease in protein leakage (fig. 2A). The baseline values of PaO₂ were similar in all the groups during breathing of 21% O₂, before changing to the experimental inspired oxygen (table 1). Atelectasis resulted in a decrease in PaO₂ in the 21% O₂ group (final vs. baseline; *P* < 0.05). There was a dose-dependent increase in PaO₂ associated with increasing inspired oxygen (fig. 2B).

Increased inspired oxygen was associated with an increased alveolar-arterial oxygenation gradient (AAO₂) in a dose-dependent manner in each of the experimental groups. To overcome effects of increasing inspired oxygen on AAO₂ gradient associated with the nonlinear shape of the oxygen dissociation curve, we also calculated the delta AA DO₂ (final − baseline AAO₂) and demonstrated that increases in inspired O₂ are associated with progressive increase in delta AA DO₂ (*P* < 0.05; fig. 2C).

**Series 2: Cardiac and Pulmonary Vascular Effects of 21% versus 100% O₂**

Twelve animals were anesthetized and were allocated in a pairwise manner to either 21% or 100% O₂. All animals met the entry criteria and were eligible for the study. Two out of six animals in the 21% O₂ group did not survive the complete protocol, and the corresponding paired animals in the 100% O₂ group were terminated at the same time point. Furthermore, six additional animals were anesthetized and killed and were used as controls (not mechanically ventilated).

Pulmonary arterial acceleration time, which reflects impedance to right ventricular ejection, decreased progressively over time during ventilation with 21% O₂ but not with 100% O₂ (fig. 3A). The ratio of right ventricular versus left ventricular diastolic dimension was greater overall in ventilation with 21% versus 100% O₂ (fig. 3B). Normal control conditions of the left and right ventricle are presented (fig. 4A). An illustrative example of the impairment of right ventricular function, and underfilling of the left ventricle in the 21% O₂ group, is presented (figs. 4B and C).

The BAL concentrations of important prostanoid metabolites were measured (fig. 5 and table 2). The con-
centrations of thromboxane B₂ (reflecting the vasoconstrictor thromboxane A₂; fig. 5A) and prostaglandin E₂ (the vasodilatory prostanoid; fig. 5B) were increased in both 21% and 100% O₂ with control animals, but neither was different between 21% versus 100% O₂ (figs. 5A and B). The concentrations of stable prostanoid, prostaglandin F₂α, was significantly greater with 21% O₂ versus both 100% O₂ and controls (P < 0.05), and there was no difference in the concentrations between 100% versus controls (fig. 5C). There were no among-group differences in the levels of 8-isoprostane, 6-keto-prostacyclin F₁α, prostaglandin D₂, leukotriene B₄, 12-hydroxyeicosatetraenoic acid, or arachidonic acid (table 2).

**Series 3**

Computed tomography demonstrated that atelectasis was not present in animals ventilated using a recruitment strategy (figs. 6A and B). Atelectasis was evident in animals ventilated without recruitment and with 21% O₂ (figs. 6C and D); however, atelectasis was far more apparent after ventilation without recruitment maneuvers and with 100% O₂ (figs. 6E and F).

**Series 4**

Functional residual capacity was significantly lower in animals ventilated with 100% versus 21% O₂ (P < 0.05; fig. 7).

**Discussion**

This article demonstrates the dose-dependent effect of supplemental oxygen in reducing adverse effects of atelectasis-associated regional lung hypoxia, despite worsening the degree of atelectasis, in anesthetized rats. We have previously demonstrated that ventilation in this model without recruitment leads to atelectasis or a reduced functional residual capacity. The survival rate in the animals ventilated without recruitment with 21% inspired O₂ was 57%, which was similar to the survival rate of 59% in our previous study for animals ventilated with 21% O₂ in the absence of recruitment. We con-

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Table 1. Baseline and Final Pulmonary Parameters

<table>
<thead>
<tr>
<th></th>
<th>Group 1 (21% O₂)</th>
<th>Group 2 (24% O₂)</th>
<th>Group 3 (30% O₂)</th>
<th>Group 4 (40% O₂)</th>
<th>Group 5 (70% O₂)</th>
<th>Group 6 (100% O₂)</th>
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<td>Number</td>
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<td>4</td>
<td>6</td>
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<td>Weight, g</td>
<td>259 ± 22.7</td>
<td>264 ± 11.1</td>
<td>238 ± 13.3</td>
<td>260 ± 4.10</td>
<td>268 ± 23.6</td>
<td>260 ± 20.0</td>
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<td>Hemoglobin, g/dl</td>
<td>15.5 ± 1.1</td>
<td>14.6 ± 0.6</td>
<td>14.1 ± 1.5</td>
<td>16.0 ± 0.7</td>
<td>15.6 ± 1.0</td>
<td>15.5 ± 1.31</td>
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<tr>
<td>pH</td>
<td>7.36 ± 0.03</td>
<td>7.29 ± 0.03</td>
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<td>7.33 ± 0.02</td>
<td>7.41 ± 0.03</td>
<td>7.35 ± 0.01</td>
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<td>PaCO₂, mmHg</td>
<td>40.0 ± 3.1</td>
<td>39.7 ± 1.9</td>
<td>37.2 ± 5.9</td>
<td>40.9 ± 2.9</td>
<td>37.5 ± 2.7</td>
<td>40.2 ± 5.1</td>
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<tr>
<td>PaO₂, mmHg</td>
<td>77.5 ± 11.5</td>
<td>76.2 ± 4.5</td>
<td>78.9 ± 14.6</td>
<td>78.6 ± 11.7</td>
<td>90.3 ± 9.7</td>
<td>87.7 ± 13.8</td>
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<td>HCO₃⁻, mmol/L</td>
<td>22.3 ± 1.7</td>
<td>19.5 ± 1.5</td>
<td>19.3 ± 2.6</td>
<td>21.4 ± 2.1</td>
<td>23.5 ± 3.1</td>
<td>21.8 ± 2.3</td>
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<td>Base excess, mmol/L</td>
<td>-2.5 ± 1.9</td>
<td>-6.2 ± 2.1</td>
<td>-5.2 ± 2.3</td>
<td>-3.8 ± 2.2</td>
<td>-0.8 ± 3.2</td>
<td>-3.1 ± 2.02</td>
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<td>Compliance, ml/cm² H₂O</td>
<td>0.7 ± 0.1</td>
<td>0.6 ± 0.05</td>
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<td>0.8 ± 0.1</td>
<td>0.7 ± 0.07</td>
<td>0.6 ± 0.14</td>
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<td><strong>Final data</strong></td>
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<td>Survival, %</td>
<td>4/7 (57%)</td>
<td>4/4 (100%)</td>
<td>6/6 (100%)</td>
<td>4/4 (100%)</td>
<td>4/4 (100%)</td>
<td>4/4 (100%)</td>
</tr>
<tr>
<td>Hemoglobin, g/dl</td>
<td>17.4 ± 0.9</td>
<td>15.7 ± 0.6</td>
<td>16.8 ± 1.5</td>
<td>16.7 ± 1.0</td>
<td>15.4 ± 1.7</td>
<td>16.8 ± 0.9</td>
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<tr>
<td>pH</td>
<td>7.21 ± 0.15</td>
<td>7.28 ± 0.20</td>
<td>7.31 ± 0.03</td>
<td>7.30 ± 0.03</td>
<td>7.33 ± 0.04</td>
<td>7.32 ± 0.03</td>
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<tr>
<td>PaCO₂, mmHg</td>
<td>41.3 ± 8.8</td>
<td>45.0 ± 4.2</td>
<td>41.6 ± 5.2</td>
<td>44.2 ± 3.2</td>
<td>41.5 ± 3.7</td>
<td>44.4 ± 4.3</td>
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<tr>
<td>PaO₂, mmHg</td>
<td>47.9 ± 18.8</td>
<td>49.8 ± 10.8</td>
<td>99.0 ± 26.1</td>
<td>126 ± 21.4</td>
<td>246 ± 52.8</td>
<td>433 ± 74.5</td>
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<td>HCO₃⁻, mmol/L</td>
<td>16.1 ± 3.7</td>
<td>17.9 ± 6.4</td>
<td>20.6 ± 2.7</td>
<td>21.3 ± 2.6</td>
<td>21.5 ± 2.9</td>
<td>22.9 ± 1.3</td>
</tr>
<tr>
<td>Base excess, mmol/L</td>
<td>-10.2 ± 5.5</td>
<td>-9.3 ± 7.6</td>
<td>-5.0 ± 2.7</td>
<td>-4.3 ± 2.7</td>
<td>-3.7 ± 3.0</td>
<td>-2.4 ± 1.7</td>
</tr>
<tr>
<td>Compliance, ml/cm² H₂O</td>
<td>0.4 ± 0.09</td>
<td>0.4 ± 0.05</td>
<td>0.3 ± 0.05</td>
<td>0.4 ± 0.09</td>
<td>0.4 ± 0.05</td>
<td>0.4 ± 0.06</td>
</tr>
<tr>
<td>P₅₀, mmHg</td>
<td>8.5 ± 2.38</td>
<td>9.5 ± 0.6</td>
<td>9.2 ± 1.5</td>
<td>7.7 ± 1.5</td>
<td>8.5 ± 1.7</td>
<td>9.5 ± 1.3</td>
</tr>
<tr>
<td>Wet/dry lung weight</td>
<td>5.99 ± 0.64</td>
<td>5.80 ± 0.53</td>
<td>5.21 ± 0.31</td>
<td>6.05 ± 1.04</td>
<td>5.19 ± 0.29</td>
<td>5.70 ± 0.33</td>
</tr>
</tbody>
</table>

The following were significant effects: partial pressure of arterial oxygen (PaO₂; group, time, and group x time); bicarbonate (HCO₃⁻; group); hemoglobin, partial pressure of arterial carbon dioxide (PaCO₂), airway pressure (Paw; time); and PaO₂, base excess, lung compliance (group and time). P < 0.05, two-way analysis of variance in all cases. There were no among-group differences in animal weight or wet/dry lung weight ratio (one-way analysis of variance). Data are expressed as mean ± SD.

**Fig. 1.** Kaplan-Meier survival curves. Survival with 21% O₂ was 57% versus 100% in each of the other groups (* P = 0.05; □ = 21% O₂ ○ = all other groups, i.e., 24, 30, 40, 70, and 100% O₂).
firmed that atelectasis without supplemental oxygen leads to reduced survival, impaired oxygenation, and adverse vascular effects but also demonstrated an increase in pulmonary prostanoid concentrations. In the current study, despite worsening atelectasis, addition of supplemental oxygen eliminated mortality, improved systemic oxygen tension and cardiac function, and decreased pulmonary prostaglandin F2α concentrations.

Clearly, such dramatic results are not observed in patients who undergo general anesthesia without the use of either PEEP or recruitment maneuvers. Nonetheless, atelectasis is a common occurrence after general anesthesia, and recent work suggests that prevention or reversal of atelectasis may be associated with a reduced incidence of lung injury and multiorgan failure in high-risk postoperative patients. The latter report demonstrated that the early use of continuous positive airway pressure reduces the need for intubation in postoperative respiratory insufficiency and lessens the incidence of severe complications. Our results may be relevant to the clinical setting and prevention of atelectasis, and the addition of supplemental oxygen may improve outcome in high-risk patients.

**Atelectasis and Impaired Oxygenation**

Atelectasis leads to reduced local alveolar lung tissue oxygen tension. Therefore, impaired oxygenation may be a mediating influence of atelectasis-induced increases in lung permeability. Our previous study demonstrated ultrastructural evidence of microvascular endothelial disruption in nonrecruited animals as a cause for increased lung permeability. In this study, pulmonary microvas-
Circular protein leakage was maximal in the absence of supplemental oxygen.

We assessed pulmonary microvascular protein leakage using Evans blue concentration in BAL fluid. The use of this technique has been used extensively both in the ex vivo and in vivo lung. The exact passage and distribution of Evans blue dye in atelectatic regions in comparison with expanded lung is probably a complicated process. However, we use liquid for the BAL, which enters and expands collapsed units far more easily than gas. Although there was no effect of inspired oxygen on lung wet/dry ratio, other authors have found wet/dry ratio to be a less sensitive marker of lung injury than Evans blue dye leakage. Numerous errors may occur in gravimetric assessment of lung injury, including evaporative losses, impact of ventilation, and failure to reveal any regional heterogeneity.

Areas of the lung that become atelectatic comprise alveoli that are nonaerated as well as alveoli that are poorly aerated. Volumetric computed tomography...
expression29 or may induce lung inflammation through pulmonary vascular leak via the atelectasis. Vents the injury and corrects the hypoxia but worsens volume loss due to positive pressure.

There are a number of mechanisms that could explain the microvascular injury in the atelectatic lung. First, the mechanism of decreased blood flow to an atelectatic lobe of lung seems to be through hypoxic pulmonary vasoconstriction,33,34 regardless of whether the chest is open or closed or whether ventilation is spontaneous or due to positive pressure. Second, the amount of lung tissue subjected to intratidal opening and closing might have been higher in the nonrecruited group, despite lung collapse, because these animals were ventilated with a PEEP of 0 cm H2O, in comparison with a PEEP of 2 cm H2O in the “recruited” group.9 Third, the injury might be the consequence of pulmonary hypertension and the reduction of the vascular bed available for pulmonary circulation. In this context, more energy may be dissipated through increased stress and strain that mechanical ventilation provides to the remaining opened alveoli after atelectasis has formed.

Table 2. Eicosanoid Concentrations in Bronchoalveolar Lavage Fluid

<table>
<thead>
<tr>
<th>Eicosanoid</th>
<th>Control 21% O2</th>
<th>21% O2</th>
<th>100% O2</th>
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<tbody>
<tr>
<td>6-Keto PGF1-α</td>
<td>0.10 ± 0.03</td>
<td>0.14 ± 0.06</td>
<td>0.13 ± 0.05</td>
</tr>
<tr>
<td>8-Iso prostaglandin</td>
<td>0.48 ± 0.12</td>
<td>0.41 ± 0.06</td>
<td>0.40 ± 0.03</td>
</tr>
<tr>
<td>PGD2</td>
<td>0.34 ± 0.13</td>
<td>0.48 ± 0.30</td>
<td>0.26 ± 0.11</td>
</tr>
<tr>
<td>Leukotriene B4</td>
<td>0.00 ± 0.01</td>
<td>0.02 ± 0.01</td>
<td>0.03 ± 0.02</td>
</tr>
<tr>
<td>12-HETE</td>
<td>1.45 ± 0.45</td>
<td>2.44 ± 0.71</td>
<td>1.83 ± 0.89</td>
</tr>
<tr>
<td>Arachidonic acid</td>
<td>59.5 ± 20.3</td>
<td>67.5 ± 22.6</td>
<td>64.5 ± 23.0</td>
</tr>
</tbody>
</table>

Data are expressed as mean ± SD. There were no significant among-group differences in any of the eicosanoid concentrations in the table (one-way analysis of variance).

12-HETE = 12-hydroxyeicosatetraenoic acid; 6-keto-PGF1-α = 6-keto-prostaglandin F1-α; PGD2 = prostaglandin D2.

Fig. 6. Illustrative computed tomography scans shown with lung windows at the level of the division of the right mainstem bronchus. (A and B) Computed tomography scans of two rats ventilated with positive end-expiratory pressure and a recruitment strategy (21% O2), demonstrating no evidence of atelectasis. (C and D) Computed tomography scans of two rats ventilated with no positive end-expiratory pressure and no recruitment strategy (21% O2), demonstrating evidence of atelectasis demonstrating minimal atelectasis on the right and more extensive atelectasis within the left lower lobe. (E and F) Computed tomography scans of two rats ventilated with no positive end-expiratory pressure and no recruitment strategy (100% O2), demonstrating evidence of significant atelectasis bilaterally.

Recent data indicating that alveolar hypoxia may result in pulmonary vascular leak via decreased lung nephrilysin expression20 or may induce lung inflammation through macrophage recruitment30 support this contention. Atelectasis in combination with pulmonary artery occlusion depletes rabbit lung adenosine triphosphate,31 a finding that is consistent with a pathogenic role of local oxygen supply.

When alveolar oxygen tension is reduced in regions of the lung, local vasoconstriction of small pulmonary arteries is induced. This phenomenon, hypoxic pulmonary vasoconstriction, results in a dual response of increased pulmonary perfusion pressure and diversion of blood flow from hypoxic regions to normoxic regions.32 The mechanism of decreased blood flow to an atelectatic lobe of lung seems to be through hypoxic pulmonary vasoconstriction,33,34 regardless of whether the chest is open or closed or whether ventilation is spontaneous or due to positive pressure.
pulmonary blood flow in relatively restricted (i.e., with less capacitance, higher resistance, or both), thereby causing flow-induced injury. Fourth, the local hypoxia induced by atelectasis formation might induce a direct hypoxic injury on the endothelial wall. In the current context, the first and second mechanisms may be less likely because the increase in atelectasis per se did not increase injury. However, microvascular endothelial injury may be relevant as supplemental oxygen normalized right ventricular function, and we have previously demonstrated ultrastructural evidence of microvascular endothelial disruption in nonrecruited lungs.39 In addition, local hypoxia-induced injury may be important, and although previous studies demonstrating the effects of hypoxia refer to expanded lung, they may be relevant here.30,35 In fact, in terms of effects on pulmonary vascular tone, this is important, because Barer et al.36 have demonstrated, using an isolated in vivo feline lung lobe, that the alveolar and mixed venous oxygen tension, not the volume of the lobe per se, were the major determinants of the local pulmonary vascular resistance.

Atelectasis and Cardiac Function

In addition to impaired oxygenation, atelectasis increases pulmonary vascular resistance.37,38 We have previously demonstrated that right ventricular dysfunction and pulmonary hypertension occur in the absence of recruitment37 and have demonstrated that the cause of death in this model is cardiogenic shock on the basis of right ventricular failure. There are several mechanisms whereby atelectasis can compromise the pulmonary vasculature. For example, functional residual capacity is related to pulmonary vascular resistance through direct effects on the pulmonary vasculature. Studies in ex vivo lungs suggest that the relation of lung volume with pulmonary vascular resistance followed a U-shaped curve, with the pulmonary vascular resistance lowest at functional residual capacity.39,40 However, such concepts may not translate to the in vivo setting, and when examined in vivo, it has been found that local oxygen tension, not lung volume, is the key determinant of hypoxic pulmonary vasoconstriction.36 Therefore, as long as increasing inspired oxygen tension is observed to increase systemic oxygenation, that implies that the atelectasis is relative (i.e., not total), and the reduction in pulmonary vascular resistance observed is consistent with previous literature.36 Therefore, high levels of alveolar oxygenation seem to supersede accompanying absorption atelectasis in regulation of pulmonary vascular tone as well as vascular leak.

Vasoactive Prostanoids

Constriction of small pulmonary arteries and arterioles and focal vascular injury are features of pulmonary hypertension. Thromboxane A2 is derived from cyclic endoperoxides by the action of thromboxane synthetase. It is a biologically active metabolite of arachidonic acid produced in the phospholipids of cell membranes. Because thromboxane A2 is both a vasoconstrictor and a potent stimulus for platelet aggregation, it may be an important mediator of pulmonary hypertension. Its effects are antagonized by prostacyclin, which is released by vascular endothelial cells.41 Thromboxane receptor blockade prevents the pulmonary hypertension and the decline in oxygenation seen in experimental acute lung injury.42

Prostaglandin endoperoxide is the direct precursor of the prostaglandins and thromboxane. Thromboxane B2 is the stable metabolite of thromboxane A2 formed through thromboxane synthase. Similarly, 6-keto-prostaglandin F1a is a stable metabolite of prostacyclin, formed through prostacyclin synthase, whereas prostaglandin E2 and prostaglandin F2 alpha are the stable end products formed through an isomerase and a reductase, respectively. The current study demonstrated an increase in both thromboxane B2 and prostaglandin E2 in the BAL fluid of derecruited rat lungs regardless of the addition of supplemental oxygen. However, in the presence of 100% inspired oxygen, the concentrations of prostaglandin F2 alpha were significantly lower than with 21% oxygen. Prostaglandin F2 alpha is a cyclooxygenase metabolite of arachidonic acid involving the action of a reductase on prostaglandin endoperoxide, and it is also responsible for potent vasoconstriction.43 A few possibilities exist as to why prostaglandin F2 alpha is decreased in the presence of oxygen but thromboxane B2 and prostaglandin E2 are not. One hundred percent oxygen may divert the substrate prostaglandin endoperoxide (arachidonic acid is converted to prostaglandin G2, which then decomposes to prostaglandin H2) from the pathway leading to the production of prostaglandin F2 alpha, or it may inhibit the reductase enzyme responsible for the production of prostaglandin F2 alpha. Ventilation in the presence of either 21% or 100% O2 was associated with increased BAL concentrations of thromboxane B2, prostaglandin E2, and prostaglandin F2 alpha compared with nonventilated control animals.

Fig. 7. Functional residual capacity was significantly less after ventilation with 100% O2 versus ventilation with 21% O2 (* P < 0.05, t test).
There was no difference in the local pulmonary concentrations of the vasoactive prostanoids thromboxane B₂ (reflecting constrictor thromboxane A₂) or prostaglandin E₂ (a vasodilatory prostanooid) between 21% and 100% O₂ conditions. However, because prostaglandin F₂α was increased in the 21% oxygen group and significantly lower in the presence of 100% inspired O₂, this mediator may be important in atelectasis-induced pulmonary hypertension and injury and warrants further investigation in a future study.

Limitations of the Current Study

The current study has several limitations that restrict extrapolation to the clinical context. First, we did not have a control group to compare the effects of oxygen during mechanical ventilation with PEEP and recruitment maneuvers. However, we have previously shown that mechanical ventilation with 21% O₂ in the context of recruitment was not injurious. Second, the higher inspired oxygen led to an increased alveolar-arterial gradient through absorption atelectasis, which should ultimately increase, not decrease, hypoxic pulmonary vasconstriction. We terminated the experiment at 2.5 h and did not explore the longer-term consequences of the use of a higher inspired oxygen. Third, echocardiograms were performed at 30-min intervals in all animals in series 2. Although a special pediatric probe was used, additional pressure on the chest wall might alter responses. In addition, we did not directly measure pulmonary artery pressures as we have previously done, which may have provided additional insight. Finally, although prostaglandin F₂α was identified as a mediator in atelectasis-induced injury, we did not explore the effects of antagonizing this prostanoid or other mediators of hypoxia-induced lung injury, such as nitric oxide.

Summary

The current data confirm that mechanical ventilation without recruitment results in increased microvascular protein leakage, which is attenuated, in a dose-dependent manner, by addition of supplementary oxygen. In the absence of supplemental oxygen, the animals develop progressive right heart failure associated with increased pulmonary vascular resistance. The application of 100% oxygen prevents these effects but worsens the computed tomography evidence of atelectasis, suggesting that the microvascular injury is mediated by hypoxemia, not volume loss per se, and possibly by the hypoxia-induced alteration in pulmonary vascular tone. Finally, analysis of local stable metabolites suggests that vasoactive prostanoids do not mediate the pulmonary vascular effects associated with atelectasis in this model.

The authors wish to thank Guila Ben David and Nancy Parfield (Technologists, Department of Radiology, The Hospital for Sick Children, Toronto, Ontario, Canada) for their expertise in obtaining the computed tomography radiographs and Derek Stephens, Ph.D. (Biostatistician, Programme in Population Health Sciences, The Research Institute, The Hospital for Sick Children), for statistical advice.

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