Role of positive airway pressure on pulmonary acinar perfusion heterogeneity

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Departments of 1Anesthesiology, 2Physiology/Biophysics, and 3Pediatrics, Indiana University School of Medicine, Indianapolis, Indiana 46202-5120; and 4Department of Chest Medicine, Chiba University School of Medicine, Chiba 260, Japan.

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Tanabe, Nobuhiro, Thomas M. Todoran, Gerald M. Zenk, Jun Aono, Wiltz W. Wagner, Jr., and Robert G. Presson, Jr. Role of positive airway pressure on pulmonary acinar perfusion heterogeneity. J Appl Physiol 89: 1943–1948, 2000.—Perfusion of the pulmonary acinus has been shown to be generally homogeneous, but there is a significant component that is heterogeneous. To investigate the contribution of the alveolar septal capillary network to acinar perfusion heterogeneity, the passage of fluorescent dye boluses through the subpleural microcirculation of isolated dog lung lobes was videotaped using fluorescence microscopy. As the videotapes were replayed, dye-dilution curves were recorded from each of the tributary branches of Y-shaped venules that drained single acini. For each Y-shaped venule, the mean appearance time difference between the pair of tributary branches was calculated from the dye curves. When the complex septal capillary networks were derecruited by high positive airway pressure, venular perfusion became proportionally more homogeneous. This result shows that septal capillary resistance and pathlength differences are important contributors to intra-acinar perfusion heterogeneity.

METHODS

Animal preparation. These experiments were approved by the Animal Care Committee of the Indiana University School of Medicine. Healthy adult male mongrel dogs (21–27 kg, n = 9) were anesthetized by pentobarbital sodium (30–40 mg/kg iv), intubated, and mechanically ventilated with room air via a constant-volume respirator. After heparinization (1,000 U/kg), the animals were rapidly exsanguinated through a cannula (3-mm ID) placed in the left common carotid artery. With the lungs inflated to a constant airway pressure of 5 mmHg, a left thoracotomy was performed, and the left upper lobe was excised to provide access to the left lower lobe. The left lower lobe was then excised along with a Teflon fluorinated ethylene polypropylene cannula (6-mm ID), and the left lower lobe bronchus was clamped to maintain constant inflation. The left lower lobe was then excised along with a cuff of left atrium and placed on a microscope stand. The left atrial cuff was secured around another Teflon fluorinated ethylene polypropylene cannula (10-mm ID), and the left lower lobe bronchus was clamped to maintain constant inflation. The left lower lobe was then excised along with a cuff of left atrium and placed on a microscope stand. The left atrial cuff was secured around another Teflon fluorinated ethylene polypropylene cannula (10-mm ID), and the left lower lobe bronchus was clamped to maintain constant inflation.

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finally a dye injection loop before entering the lobe (Fig. 1). Venous blood drained passively from the lobe into a reservoir. The height of the tubing between the vein and the reservoir could be raised or lowered to change venous pressure. The lobe was ventilated with 6% CO₂-17% O₂-77% N₂ at a tidal volume of 100 ml. End-expiratory pressure was set to 5 mmHg by a water overflow on the expiratory limb of the ventilator. Arterial and venous pressures were measured continuously with two transducers (model P23 XL, Statham) zeroed at the level of the microcirculatory observations and connected to polyethylene (PE-200) tubing, the tips of which were located at the ends of the arterial and venous cannulas. Airway pressure was measured intermittently (Statham P23 XL transducer).

Data collection. The lobe was suspended by two small spring-backed paper clips attached to opposite edges of the lobe (18) and raised until the uppermost pleural surface (the diaphragmatic surface in this orientation) came into contact with a transparent window. A 1.3-cm² area on the surface of the lobe was observed through the window, which was surrounded by a vacuum ring to prevent lateral movement (14, 15). The subpleural microcirculation under the window was observed with a modified Olympus BH2 reflectance microscope coupled to a Leitz Ultropak illuminator and a ×11 objective. Bright-field illumination through the Ultropak illuminator was provided by a 200-W mercury arc lamp mounted on an optical bench. This light source was heavily filtered with a combination of dichroic infrared-reflecting filters, broad band-pass ultraviolet-absorbing filters to prevent tissue damage, and a narrow band-pass interference filter to illuminate the field only with the mercury green line (546 nm). This wavelength was absorbed by Hb, thereby increasing the contrast between the erythrocytes and surrounding tissue. Illumination for fluorescence microscopy was provided by a 100-W mercury arc lamp mounted on the side-arm of the Olympus microscope. This light was also filtered by dichroic infrared-reflecting filters and ultraviolet-absorbing filters. The light from this arc passed through a blue band-pass exciter filter (410–480 nm) and a high-pass dichroic mirror (cutoff wavelength 480 nm) that reflected the exciting light down through the objective onto the subpleural microcirculation beneath the window. Emitted light from the lung passed back through the objective, the dichroic mirror, and a yellow high-pass barrier filter (cutoff wavelength 510 nm). Video recordings of the subpleural microcirculation were made with a video recorder (model AU650 MII, Panasonic) and a Cohu (model 5510) intensified charge-coupled device camera that was attached to the microscope with a zoom adapter.

In each lobe, one to three microscopic fields were selected in which there was a venule that had two tributary branches forming a Y shape. While these fields were observed with fluorescence microscopy, test injections of dye (FITC conjugated to 70-kDa dextran, 10 mg/ml of 0.9% saline) were made using a loop just proximal to the lobar artery. Each limb of the loop contained a volume of ~25 ml and was controlled by a solenoid pinch valve on its downstream end. On one limb the valve was open when deenergized; on the other limb the valve was closed. The normally closed limb was loaded with a 1-ml dye bolus. When the solenoids were energized, blood flow was diverted through the dye-containing limb, thereby washing the dye into the lobar artery. In this way, the bolus of dye was rapidly introduced into the arterial circulation without the pressure increase or movement of the microscopic field that occurred when high-pressure injections were made directly into the lobar pulmonary artery. The passage of dye through the field was videotaped, and elapsed time in milliseconds was recorded on the videotapes by a time-date generator that was activated by the same switch that energized the solenoids of the injection loop.

The black level and gain of the camera and intensifier were adjusted according to these test injections of dye to maximize the contrast between the baseline brightness of the microscopic field before dye entered the circulation and the peak brightness during passage of dye through the microcirculation. After the test injections, three separate 1-ml dye injections were made for each venule during end expiration at an airway pressure of 5 mmHg (low airway pressure) and also at an airway pressure of 15 mmHg (high airway pressure), the

Fig. 1. Schematic of the experimental setup. PA, alveolar pressure; Ppa and Ppv, pulmonary arterial and venous pressure, respectively; ICCD, intensified charge-coupled device.
order of which was mixed. To eliminate the effect of atelectasis, lobes were inflated to 15 mmHg and then returned to 5 mmHg several times before the first recordings. Pump flow rate was set at 400 ml/min at baseline, and pulmonary venous pressure was set at 1 mmHg. To maintain a constant microvascular pressure when airway pressure was changed, pump flow rate was changed to maintain the pulmonary arterial-airway pressure gradient constant, and reservoir height was changed to maintain pulmonary venous pressure constant.

Dye-dilution curves were obtained by replaying the recordings and sampling image brightness at 30 Hz from rectangular areas over the venular lumens (referred to as vessel windows) and from areas over the adjacent alveoli (background windows) with a frame-grabber board interfaced with a microcomputer. The windows were movable and of adjustable size. To obtain a dye-dilution curve for each of the two tributary branches of the Y-shaped venules, the recordings of each injection were replayed twice, with sampling over a different branch each time.

The three-dimensional structure of the lung caused detectors placed over the venules to measure light emitted not only from dye within the venular lumens but also from dye in the surrounding alveolar capillaries. To obtain curves accurately reflecting the concentration of dye in the venular lumens at each instant in time, it was necessary to subtract light emitted by dye in the capillaries by use of the method of Presson et al. (11). In each animal, the background-corrected dye-dilution curves from each of the triplicate injections were aligned at the injection time and then averaged to produce a single average venular curve for each branch. The baseline segment of the average curve before dye entered the vessel was set to zero, and the tail of the curve (~5% of the area under the curve) was extrapolated to baseline as a monoexponential function. Finally, the area under the curve was set to unity. The mean appearance time (MT) from the time of injection was calculated as follows

\[
MT = \sum_{i=1}^{n} \frac{t_{i+1} + t_i}{2} \frac{I(t_{i+1}) - I(t_i)}{2}
\]  

where \( t_i \) was the time when the intensity of the dye-dilution curve (I) became greater than zero and \( t_n \) was the time when the curve returned to baseline. The percent difference in MT between the fast branch and the slow branch (\( \Delta MT \)) of a venular pair was calculated as follows

\[
\Delta MT(\%) = \frac{MT_{\text{slow}} - MT_{\text{fast}}}{(MT_{\text{slow}} + MT_{\text{fast}})/2} \times 100
\]

where \( MT_{\text{slow}} \) was the slow tributary branch and \( MT_{\text{fast}} \) was the fast tributary branch. We divided by the average of \( MT_{\text{slow}} \) and \( MT_{\text{fast}} \) as if flow were homogeneous. From Eq. 2,

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**Fig. 2.** Passage of fluorescein dye through a venular pair. **Top:** direction of flow is indicated by arrows; the base of the Y-shaped venule is not visible, because it flows directly down into the lung. **Middle:** passage of dye 8.98 s after injection, when the dye bolus was at the peak of its brightness during its passage through the faster branch; no dye had arrived in the slower branch. **Bottom:** 12.36 s after injection, the faster branch was nearly depleted of dye, and the slower branch was then filled with dye, at the peak of its brightness. Discrepancy between appearance times in these small venules draining adjoining neighborhoods can be significant. To the extent that there was uniform appearance of dye in the arteriolar branches feeding the acinus, the venular appearance time differences reflect acinar capillary transit time differences and, thus, uneven acinar blood flow with respect to acinar blood volume.
When $MT_{\text{low}} = MT_{\text{fast}}$ (homogeneous perfusion), $\Delta MT = 0\%$, i.e., the dye appeared simultaneously in each branch.

To test the hypothesis that derecruitment of septal capillaries would decrease venular perfusion heterogeneity, we selected only those venular branch pairs with $\Delta MT \geq 5\%$ at an airway pressure of 5 mmHg ($n = 15$). The tributary branches of the Y-shaped venules that met this criteria were similar in size ($48.2 \pm 12.4$ and $47.8 \pm 14.2$ (SD) $\mu$m). The parent vessel into which they drained averaged $67.8 \pm 20.6$ $\mu$m diameter.

**Measurement of capillary recruitment.** In 21 alveolar walls (9 lobes), the level of septal capillary recruitment was determined at airway pressures of 5 and 15 mmHg by recording the perfusion pattern of 1–3 alveoli in the field for 1 min. The videotapes from each 1-min observation period were replayed, and the perfused capillary segments were traced onto separate sheets of clear acetate placed over the video monitor. A capillary segment was considered to be perfused if one or more erythrocytes passed through the segment during the 1-min observation period. The length of the perfused capillaries was measured from the tracings with a digitizing pad, planimetry software, and a microcomputer. The area of the observed alveolar walls was measured with the same system. Because subpleural alveolar facets in the isolated lobe at an airway pressure of 5 mmHg can be approximated by flat disks with an average area of 8,000 $\mu$m², the alveolar wall area measured at 5 mmHg was divided by 8,000 $\mu$m² to obtain the number of average-sized alveolar walls in the observed alveolar facets. Because the alveolar diameters increased at an inflation pressure of 15 mmHg, the measured wall area was divided by the average wall area of 10,000 $\mu$m², the average area at this inflation pressure. This normalization permitted us to compare results between individual alveoli, between animals, and between treatments. Dividing the total length of perfused capillaries by the normalized alveolar area indicated how many times perfused capillaries crossed an average alveolar wall at its diameter. Defined mathematically, the capillary perfusion index (CPI) is

$$\text{CPI (}$\mu$m) = \frac{\sum \text{perfused capillary lengths (}$\mu$m)}{\text{alveolar wall area (}$\mu$m²)/average wall area (}$\mu$m²).}$$

The level of capillary recruitment can be readily estimated from the CPI.

**RESULTS**

An example of the passage of fluorescein dye through a venular pair is shown in Fig. 2. In Fig. 2, top, the base of the Y-shaped venule is not visible because it flows directly down into the lung. Passage of dye 8.98 s after injection is shown in Fig. 2, middle. At that time the dye bolus was at the peak of its brightness during its passage through the faster branch. No dye, however, had arrived in the slower branch. In Fig. 2, bottom, 12.36 s after injection, the fast branch was nearly depleted of dye, and the slower branch was now filled with dye, at the peak of its brightness. The discrepancy between appearance times through these small venules draining adjoining neighborhoods can be significant. The dye curves from these branches are shown in Fig. 3. $\Delta MT$ was 15.2% as calculated from Eq. 2.

When airway pressure was increased from 5 to 15 mmHg, two changes were observed in the pulmonary microcirculation of the nine animals studied. Perfusion heterogeneity between the venular tributaries decreased in 14 of 15 pairs and increased in 1 pair. On average, $\Delta MT$ decreased by 60% ($P < 0.01$) when airway pressure was increased from 5 to 15 mmHg.

**Statistics.** Measurements of cardiorespiratory variables, $\Delta MT$, and the CPI at low and high airway pressure conditions were compared with each other by use of paired two-tailed $t$-tests. Blood gases at the beginning and at the end of the study were also tested for differences with a paired two-tailed $t$-test. We accepted $P < 0.05$ as significant for all statistical tests.

**Table 1. Microcirculatory perfusion variables**

<table>
<thead>
<tr>
<th>Airway Pressure</th>
<th>$\text{CPI, } \mu$m</th>
<th>Perfusion heterogeneity, $\Delta MT$, %</th>
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<tr>
<td>5 mmHg</td>
<td>$296 \pm 33$</td>
<td>$11.8 \pm 1.4$</td>
</tr>
<tr>
<td>15 mmHg</td>
<td>$89 \pm 22$</td>
<td>$4.8 \pm 1.4$</td>
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</tbody>
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Values are means ± SE; $n = 21$ alveoli for capillary perfusion index (CPI) and 15 venular pairs for perfusion heterogeneity. $\Delta MT$, mean appearance time difference between fast and slow venular branches. $P$ values were obtained from 2-tailed, paired $t$-test.
way pressure was increased (Fig. 4, Table 1). Of the 21 alveoli studied in the same 9 animals, when airway pressure was increased, the CPI decreased in 18 alveoli, did not change in 2 alveoli, and increased in the remaining alveolus. On average, the level of capillary recruitment decreased by 70% ($P < 0.01$) with higher airway pressure (Fig. 5, Table 1).

Although pulmonary arterial pressure increased significantly when airway pressure was raised, the gradient between pulmonary arterial pressure and airway pressure was unchanged (Table 2). Pulmonary venous pressure was also held constant (Table 2). Blood gases did not change over the course of the study (Table 3).

**DISCUSSION**

We investigated the contribution of alveolar septal capillaries to intra-acinar perfusion heterogeneity by measuring appearance time differences in the tributary arms of small Y-shaped venules that drained a single acinus. When the complex septal capillary networks were derecruited by high positive airway pressure, venular perfusion became proportionally more homogeneous.

These results show a strong correlation, but the correlation does not establish cause and effect. Of the 15 venular pairs observed, 14 became more homogeneous when airway pressure was increased. In the odd case that became more heterogeneous with high airway pressure, one of the alveoli in the neighborhood of that venule had more septal capillary recruitment. Of course, each venule drains many alveolar walls, making the association limited. Nevertheless, it is intriguing that the only venular pair that behaved in the opposite manner from the others was associated with an alveolar recruitment pattern that was also opposite from other alveoli in this study. These associations are consistent and suggest that septal capillary pathlength and resistance differences are important contributors to intra-acinar perfusion heterogeneity.

We have considered several issues in reaching these conclusions. First, we assumed that the tributary branches of each Y-shaped venule drained the same acinus. Previously, we tested this assumption by comparing $\Delta MT$ of the largest venules with $\Delta MT$ the smallest venules on the surface of the lung (12), reasoning that the largest venules would be more likely to drain more than one acinus and would therefore be more likely to have dissimilar transit times. However, $\Delta MT$ of the largest branches was not different from that of the smallest branches. This result implied that all venular branches in this size range drain a single acinus. Even if the branches did drain more than one acinus, the decreased heterogeneity of perfusion still resulted from derecruitment of septal capillaries.

We also assumed that the results obtained from the isolated lobes are similar to results from intact animals. In pilot studies of intact dogs, $\Delta MT$ between venular branches was 0–5% in 40% of venular branch pairs and 5–10% in 35% of pairs ($n = 20$ pairs), results similar to the isolated lobe data previously reported (12). Although $\Delta MT$ values reported here were greater, because the venular branch pairs...
were selected for their heterogeneity to provide a clear test of the hypothesis, it still seems reasonable that these results would apply to intact animals.

Lamm et al. (8) previously showed that septal capillary blood flow is diverted through corner vessels when airway pressure is increased. Our results indicate that the pathways of perfusion through corner vessels are more spatially homogeneous than the pathways through septal capillaries. Increasing airway pressure, however, may have distended corner vessels. Permutt et al. (9) showed that extra-alveolar vessels (which likely include the corner vessels in our study) increase in volume when the lung is expanded by increased airway pressure. If increasing airway pressure did distend corner vessels, that effect may have contributed to the increased homogeneity of acinar perfusion; however, we have no evidence from this study bearing either way on that possibility.

Even when septal capillaries were significantly de-recruited by high airway pressure, there remained some heterogeneous venular appearance times. The remaining heterogeneity could have resulted from transit time differences in arteriolar inlet flow (3), which would have been unaltered by septal capillary derecruitment. Another possibility comes from the incomplete septal derecruitment at an airway pressure of 15 mmHg. Although a CPI of 89 μm at high airway pressure is a low value, i.e., a single perfused capillary pathway would not cross an average alveolar wall at its 100-μm diameter, some septal capillary perfusion remained. If we assume a linear relationship between septal capillary recruitment and perfusion heterogeneity, then we can speculate on the effect of complete capillary derecruitment. To estimate the effect of complete septal capillary derecruitment, we plotted CPI against perfusion heterogeneity (Fig. 6). When the relationship was extrapolated to zero CPI (no septal capillary perfusion), the y-axis was intercepted at 1–2%. If this extrapolation is valid, it suggests a number of interesting possibilities. First, it implies that septal capillaries account for nearly all acinar perfusion heterogeneity. Furthermore, it suggests that corner vessels are homogeneously perfused. Finally, the extrapolation suggests that whatever heterogeneity exists in arteriolar flow has little effect on acinar perfusion homogeneity, even under these conditions where observations are made on the surface of the lung, a location providing a maximal arterial length for dispersion to occur.

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REFERENCES