Red blood cell orientation in pulmonary capillaries and its effect on gas diffusion

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Departments of 1Biology and 2Mathematics, The Colorado College, Colorado Springs, Colorado 80903; 3Avon High School, Avon, Indiana 46123; and Departments of 4Anesthesiology, 5Physiology/Biophysics, and 6Pediatrics, Indiana University School of Medicine, Indianapolis, Indiana 46202-5120

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Nabors, L. Karina, William A. Baumgartner, Jr., Steven J. Janke, James R. Rose, Wiltz W. Wagner, Jr., and Ronald L. Capen. Red blood cell orientation in pulmonary capillaries and its effect on gas diffusion. J Appl Physiol 94: 1634–1640, 2003; 10.1152/japplphysiol.01021.2001.—When alveoli are inflated, the stretched alveolar walls draw their capillaries into oval cross sections. This causes the disk-shaped red blood cells to be oriented near alveolar gas, thereby minimizing diffusion distance. We tested these ideas by measuring red blood cell orientation in histological slides from rapidly frozen rat lungs. High lung inflation did cause the capillaries to have oval cross sections, which constrained the red blood cells within them to flow with their broad sides facing alveolar gas. Low lung inflation stretched alveolar walls less and allowed the capillaries to assume a circular cross section. The circular luminal profile permitted the red blood cells to have their edges facing alveolar gas, which increased the diffusion distance. Using a finite-element method to calculate the diffusing capacity of red blood cells in the broad-side and edge-on orientations, we found that edge-on red blood cells had a 40% lower diffusing capacity. This suggests that, when capillary cross sections become circular, whether through low-alveolar volume or through increased microvascular pressure, the red blood cells are likely to be less favorably oriented for gas exchange.

METHODS

Red blood cell orientation. Eight adult female Sprague-Dawley rats, 272 ± 18 (SD) g, were anesthetized by intraperitoneal injection of pentobarbital sodium (90 mg/kg) dissolved in 0.9% saline. After tracheal cannulation and sternotomy, the lungs were held for 10 s at an inflation pressure of either 6 or 20 cmH2O. These airway pressures were based on work done by Godbey et al. (4) on isolated dog lung lobes. Liquid propane, cooled to −189.9°C via liquid nitrogen, was steadily poured into the open thorax, rapidly freezing the lungs. Chiseled pieces of the frozen lungs were removed and immediately placed in liquid nitrogen. Only small lung pieces that had a pleural surface were quickly transferred to a freeze-substitution, solvent-fixative solution (1% HgCl2 in 100% ethanol) that was cooled to −70°C. The lung pieces were kept at −70°C for 7 days with four changes of fixative solution. After 7 days, the lung pieces were warmed to room temperature, rinsed with 100% ethanol, and infiltrated and embedded in Polysciences JB-4 medium. The lung pieces were oriented in the embedding medium so that each pleural surface would be cut at a right angle. Sections (1–2 μm thick, Sorvall MT-1 microtome) were stained with Biebrich’s scarlet-acid fuchsin.

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Multiple slides were made from the sampled lung of each rat. The sections were examined with a Leitz light microscope under oil immersion (objective ×100, aperture = 1.25; ocular ×16). Measurements were made with a calibrated ocular micrometer. A slide was picked at random, and measurements began at one edge of the lung piece and proceeded along the alveoli near the pleural surface to the opposite edge of the piece. We measured red blood cells (n = 50) in alveolar walls that were cut very close to perpendicular in cross section. We ensured perpendicular cuts by confining our measurements to walls that were uniformly thin relative to other walls in the histology slide. If the cuts were grazing, or at other than a right angle, the alveolar walls would appear thicker. Red blood cells in corner vessels were avoided by measuring only those cells that were >10 μm from an intersecting alveolar wall. No cells were measured that were further than three alveoli away from the pleural surface (∼0.3 mm) to ensure that freezing of the sampled capillaries was sufficiently rapid to achieve a vitrified state. Sometimes all 50 red blood cells measured from a single rat were from a single lung piece on a slide. More often a second slide, or less frequently a third slide, had to be used to accumulate 50 measured cells.

The orientation of red blood cells in alveolar walls was determined by measuring the length of the visual aspect of the cell that was parallel to the alveolar wall, the major axis, and the aspect perpendicular to the alveolar wall, the minor axis (Fig. 2). Then the major axis was divided by the minor axis to give an aspect ratio. A high-aspect ratio indicated that the diameter of the red blood cell was nearly parallel to the alveolar wall and thus had its broad sides directed toward alveolar gas. Conversely, a low-aspect ratio indicated that the red blood cell diameter was at a high angle with respect to the alveolar wall, and thus its broad sides were tipped away from the alveolar gas. By measuring the aspect ratios with respect to the alveolar wall, and not with respect to the capillary wall, red blood cell orientation was determined with respect to alveolar gas, which is what is important in this study.

The aspect ratios of the red blood cells in the two groups of rats (lungs inflated with 6 cmH₂O in one group, n = 4, and...
Finite-element analyses. Hsia et al. (8–10) have developed an application of finite-element analysis applied to models of red blood cells in pulmonary capillaries. Our finite-element modeling was based on their work. We compared two cases of red blood cell orientation in capillaries. The first orientation occurred in an oval-shaped capillary, causing a red blood cell to pass along the capillary with its broad side facing alveolar gas. Bottom: in the second case, a disk-shaped red blood cell had its edges toward alveolar gas, an orientation permitted by a circular capillary cross section. The model was simplified by making the surface of the red blood cells flat, instead of biconcave. Computing time was reduced by using only one-fourth of the red blood cell for the finite-element analysis. FEM area, synonymous with finite-element analyses.

With 20 cmH₂O in the other group, n = 4) were compared by using the nonparametric Wilcoxon–Mann–Whitney test.

Finite-element analysis. Hsia et al. (8–10) have developed an application of finite-element analysis applied to models of red blood cells in pulmonary capillaries. Our finite-element modeling was based on their work. We compared two cases of red blood cell orientation in capillaries. The first orientation occurred in an oval-shaped capillary, causing a red blood cell to pass along the capillary with its broad side facing alveolar gas (Fig. 3, top). In the second case, the disk-shaped red blood cell had its edge toward alveolar gas (Fig. 3, bottom), an orientation permitted by a circular capillary cross section. Although a circular cross-section capillary would not constrain the orientation of red blood cells flowing within it, we chose to model a red blood cell with its disk edge toward alveolar gas to determine the diffusing capacity for this least favorable orientation.

Because alveolar walls were observed in cross section in the histological slides, the microscopic view of the red blood cells was necessarily perpendicular to the direction of gas diffusion from alveoli into the red blood cells. This means that, when red blood cells appeared edge-on in the microscope slide, their broad sides actually faced alveolar gas (Fig. 3, top). Conversely, when the broad sides of red blood cells appeared in the microscopic view, their edges faced alveolar gas (Fig. 3, bottom).

We simplified the model by making the surface of the red blood cells flat, instead of biconcave. Computing time was minimized by using only one-fourth of the red blood cell for the finite-element analysis. Assuming symmetry about an x- and y-axis through the center of the red blood cell (Fig. 3), we found the diffusing capacity for a whole red blood cell by multiplying the calculated result by four.

As in the finite-element method of Hsia et al. (8), the geometric model used for the red blood cell with its edge toward alveolar gas was a 1-μm-thick section through the longitudinal axis of an alveolar capillary. The model red blood cell with its broad side toward alveolar gas had a smaller perimeter. Therefore, the thickness of the broad side model section was increased so that the model red blood cell had the same total membrane area as the model red blood cell with its edge facing alveolar gas. The transport of carbon monoxide from alveolar gas into a single red blood cell within the capillary was assumed to be due to diffusion down the carbon monoxide partial pressure (PCO) gradient that reached a steady state immediately. Each medium through which carbon monoxide diffused (alveolar air, tissue, plasma, and the red blood cell) was assigned a diffusion coefficient (5, 11) (Table 1). The red blood cell phase of carbon monoxide uptake (1/θCO) was modeled as a resistance to diffusion across a membrane 0.1 μm thick. The rate of carbon monoxide uptake depended on P O₂, according to the equation

$$\frac{1}{\theta_{CO}} = 0.929 + 0.0042P O_2$$  

as measured by Holland (7) in dog red blood cells at 39°C. The red blood cell uptake rate of carbon monoxide was converted to a diffusion coefficient (D) for the red blood cell membrane by the equation

$$D = \frac{\theta \cdot d}{\alpha A}$$  

where θ is rate of carbon monoxide uptake by a single red blood cell, d is membrane thickness, α is Bunsen solubility coefficient for carbon monoxide in lung tissue, and A is the area of membrane through which carbon monoxide diffuses. The boundary conditions of the model were that PCO = 1 Torr at a distance of 5 μm from the alveolar gas-tissue interface and PCO = 0 Torr at the inner boundary of the red blood cell membrane. Also there was no gas flux across the left and right boundaries of the model. The alveolar gas was assumed to be an infinite carbon monoxide source, and the red blood cell an infinite carbon monoxide sink.

Table 1. Dimensions and constants for red blood cell orientation computation model

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>RBC diameter</td>
<td>7.5 μm</td>
</tr>
<tr>
<td>RBC thickness</td>
<td>1.5 μm</td>
</tr>
<tr>
<td>Thickness of RBC membrane</td>
<td>0.1 μm</td>
</tr>
<tr>
<td>Circular cross-sectional capillary</td>
<td>8.0 μm</td>
</tr>
<tr>
<td>inside height</td>
<td></td>
</tr>
<tr>
<td>Oval cross-sectional capillary</td>
<td>2.0 μm</td>
</tr>
<tr>
<td>inside height</td>
<td></td>
</tr>
<tr>
<td>Length of air-tissue interface</td>
<td>13.4 μm</td>
</tr>
<tr>
<td>Closest distance between RBC membrane</td>
<td></td>
</tr>
<tr>
<td>and capillary wall</td>
<td>0.25 μm</td>
</tr>
<tr>
<td>Thickness of alveolar-capillary</td>
<td></td>
</tr>
<tr>
<td>tissue barrier</td>
<td>1.0 μm</td>
</tr>
<tr>
<td>DCO air*</td>
<td>2.41 × 10⁻⁶ m²/s²</td>
</tr>
<tr>
<td>DCO tissue* and DCO plasma*</td>
<td>2.45 × 10⁻⁶ m²/s²</td>
</tr>
<tr>
<td>Bunsen solubility coefficient of CO in lung tissue*</td>
<td>2.36 × 10⁻⁵ Torr</td>
</tr>
<tr>
<td>Bunsen solubility coefficient of CO in plasma*</td>
<td>2.49 × 10⁻⁵ Torr</td>
</tr>
<tr>
<td>θCO (at 80 Torr)*</td>
<td>2.47 × 10⁻⁵ s⁻¹·RBC⁻¹</td>
</tr>
</tbody>
</table>

*Values at 39°C. RBC, red blood cell; DCO air, diffusion coefficient of carbon monoxide in air; DCO tissue, diffusion coefficient of carbon monoxide in tissue; DCO plasma, diffusion coefficient of carbon monoxide in plasma; θCO, specific rate of carbon monoxide uptake by RBC and binding with hemoglobin.

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The diffusive transport of gas can be described by the equation

\[ (\alpha D_{CO} \nabla^2 P_{CO}) = 0 \]  

(3)

where \( D_{CO} \) is the diffusion coefficient for carbon monoxide, and \( \nabla^2 \) is the Laplace operator \( (\partial^2/\partial x^2 + \partial^2/\partial y^2) \) in the \( x-y \) plane. To solve Eq. 3 for our geometric model, we used the finite-element method (8) and the MatLab partial differential equations toolbox. The entire region of the model was divided into a minimum of 5,000 adjacent triangles (Fig. 4). Using Eq. 3, the software then found the \( P_{CO} \) at each of the nodal points formed by the mesh of triangles. After the spatial distribution of the \( P_{CO} \) gradient was determined, the carbon monoxide (Fig. 4) diffusive transport (\( CO_{\text{flux}} \)) for each element in the mesh was calculated as \( CO_{\text{flux}} = \alpha D_{CO}(\delta P_{CO}/\delta n) \), where \( \delta P_{CO}/\delta n \) represents \( P_{CO} \) gradients evaluated normal to the lines of equal \( P_{CO} \). The MatLab software ran on a personal computer with a 400-MHz Pentium II processor.

RESULTS

Figure 5 shows photomicrographs of red blood cells in alveolar walls in both high and low lung inflation groups. Aspect ratios were determined for 200 red blood cells in four rats (50 cells in each animal) that received 6-cmH2O pressure lung inflation and for 200 red blood cells in another four rats (also 50 cells in each animal) with 20-cmH2O pressure lung inflation. The average red blood cell aspect ratio was 1.44 ± 0.40 (SE) for the low-inflation pressure group and was 2.55 ± 0.06 (SE) for the high-inflation pressure group (\( P < 0.00001 \)). The distribution of aspect ratios for both groups of rats was skewed toward greater aspect ratios (Fig. 6). By finite-element analysis, the diffusing capacity for carbon monoxide (\( DL_{CO} \)) for a red blood cell with its broad side toward alveolar gas (Fig. 4, top) was 0.911 \( \mu m^3\cdot s^{-1}\cdot Torr^{-1} \). For a red blood cell with its disk edge toward alveolar gas (Fig. 4, bottom), the \( DL_{CO} \) was 0.543 \( \mu m^3\cdot s^{-1}\cdot Torr^{-1} \), a 40% decrease.

DISCUSSION

The way red blood cells are oriented with respect to alveolar gas depends on the level of alveolar inflation. We conclude this from rat lungs inflated with high airway pressure before they were rapidly frozen. The enlarged alveoli stretched capillaries into oval cross sections. The oval-shaped capillaries constrained the red blood cells to move through them with the broad sides of the cells oriented toward alveolar gas, thereby minimizing intravascular diffusion distance and reducing the total diffusion distance from gas into hemoglobin. In lungs rapidly frozen during low-pressure inflation, alveolar walls were not as stretched, and the capillaries assumed a nearly circular cross section. The circular shape permitted the red blood cells to flow through the capillaries in various orientations, including edge-on toward alveolar gas, an orientation that added to the intravascular diffusion distance for alveolar gas to reach hemoglobin. We determined the effect on gas uptake of these orientations by finite-element analysis to estimate the \( DL_{CO} \) of single red blood cells in broad-side vs. edge-on orientations. Red blood cells in the edge-on orientation had a 40% lower diffusing capacity.

There are several issues that need to be considered in interpreting these data. First is whether our rapid freezing technique arrested the moving red blood cells.
with sufficient rapidity to maintain their orientation. We optimized the rapid freezing in the following ways. 1) Rats were chosen for their small lung size. 2) The chest of each animal was widely opened to expose the lungs as completely as possible, and the chest was rapidly filled with liquid propane. 3) Liquid propane was used because of its wetting property. The propane was cooled 145.8 °C below its boiling point by placing its container in a bath of liquid nitrogen (−195.8 °C), which ensured that the propane would remain a liquid and continue to freeze the tissue as it warmed. Typically we cooled the liquid propane until propane ice crystals formed at −189.9 °C. 4) Only red blood cells that were within three alveoli of the pleural surface were measured. All red blood cells in these alveoli had smooth surfaces when viewed by light microscopy, indicating that the cells were frozen in a vitrified state. Some red blood cells in the walls of alveoli more distant from the pleural surface had pointed edges, suggesting that freezing was slow enough for ice crystals to form (15). The further inward from the pleural surface, the greater was the proportion of irregular-shaped red blood cells.

The estimated freezing time for blood in these near-surface alveoli was <100 ms (15). Presson et al. (12) showed that the average capillary transit time for red blood cells in pentobarbital-anesthetized dogs was 4.1 s when cardiac output was basal. During this time, the cells traveled across approximately three alveoli, each ~100 μm in diameter. Therefore, their velocity was ~73 μm/s (300 μm / 4.1 s). If we assume a similar velocity for red blood cells in an anesthetized rat, it would mean that a freezing time of ~100 ms would stop the cells completely in ~7 μm and would begin the slowing process in an even shorter distance. Because there are not large twisting forces affecting the red blood cells as they pass through the pulmonary capillaries, as shown by the lack of spinning and flipping of the cells viewed with in vivo microscopy, we think that the cells being frozen in place within their own body length is a short enough distance to maintain the in vivo orientation.

When the pulmonary microcirculation is viewed with in vivo microscopy, the red blood cells traversing the pulmonary capillaries on the upper surface of the lung are oriented with their broad sides toward alveolar gas. Although the orientation is visually obvious, the cells move too rapidly for quantitative measurements to be made. We have obtained a few high-speed photomicrographs, which demonstrate the orientation in the living lung (16). A typical example is shown in Fig. 7, which supports the idea that these orientations exist in intact animals.

To determine the importance of red blood cell orientation with respect to gas diffusion in pulmonary capillaries, we used a computer model to analyze how gas diffusion was influenced by different red blood cell orientations. Modeling of gas diffusion on a microlevel became possible with high-speed computers and the elegant application of finite-element analysis developed by Hsia et al. (8–10), which they applied to models of red blood cells in pulmonary capillaries. Our analysis was based almost entirely on their method. Once our computational routines were running, we checked our model by using the same variables they did (8) and obtained the same results. Hsia et al. (8) studied the effect of hematocrit on diffusing capacity using these methods and showed that, when red blood cells were close to each other, the cells competed for gas, which resulted in a decreased rate of uptake for each cell. In another study (9), they found that, when cells were distorted into paraboloids, a condition that exists in the systemic microcirculation (6, 14), they had a lower diffusing capacity for oxygen compared with disk-shaped cells. Later, they showed that cellular...
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cused our red cell distribution and shape. In our study, we fo-
ential orientations showed that the red blood cells. This adds to the list of pulmonary
intravascular factors shown by Hsia et al. (8–10) to
reduce diffusive gas uptake.

Fig. 7. Typical in vivo, high-speed photomicrograph of red blood cells (RBCs) traversing a well-expanded alveolus in a canine lung. This subpleural alveolar wall is flat with alveolar gas just below the optical plane. The disk-shaped objects are RBCs flowing through a single capillary. The orientation of the RBCs with respect to alveolar gas is uniform and shows that capillaries in a living lung under zone 2 conditions constrain the cells to be oriented with their broad sides toward alveolar gas.

diffusing capacity was reduced when red blood cells were clumped together compared with being evenly spaced (10). Their findings showed the importance of red cell distribution and shape. In our study, we fo-
cused our finite-element analysis on red blood cell orientation measured in the capillaries of rapidly fro-
zen lungs. In our diffusion analysis, for purposes of simplification, we ignored the effect of hematocrit by modeling only a single red blood cell in a capillary.

When we applied the analysis to our data, we found that the edge-on orientation produced a 40% decrease in diffusing capacity. Because the diffusing distance for alveolar gas appears to be significantly greater when red blood cells are in the edge-on orientation toward alveolar gas, we think the actual decrease is likely to be significantly greater. Our underestimate of the effect of orientation may be due, in some measure, to three simplifying assumptions. First, we calculated the DLCO, rather than the diffusing capacity for oxygen, which made the modeling simpler. In our calculations, we assumed, as did Hsia et al. (8), that the PCO every-
where within the red blood cell remained zero and that the diffusion path for carbon monoxide ended just inside the membrane. This assumption is justified by the high-binding affinity of hemoglobin for carbon monoxide and by the low PCO used in diffusing capacity measurements. Oxygen, however, would have to travel not only through the red blood cell membrane but also to all hemoglobin binding sites within the cell to satu-
rate the hemoglobin. In the broad-side orientation, when gas enters the cell through the broad side, the middle of the cell is much closer to the alveolar mem-
brane than when the gas enters the cell through the edge in the edge-on orientation. This would make the total diffusion path for gas even longer in the edge-on orientation than for the broad-side orientation, if we had applied the finite-element method to oxygen, in-
stead of carbon monoxide diffusion. The analysis for
oxygen, however, is a considerably more complicated problem (2), so we elected to focus on the simpler carbon monoxide model in this initial study. Second, the red blood cell orientation, and thus diffusion dis-
tance, would be more important in the case of oxygen than for carbon monoxide, because there is oxygen backpressure, but not carbon monoxide backpressure, within the red blood cell. Backpressure slows the net rate of diffusion and thus places greater importance on diffusion distance. Third, our finite-element anal-
ysis was based on a two-dimensional geometric model, which could not take into account the much larger surface area in close proximity to alveolar gas for the red blood cell in the broad-side orientation. For these reasons, we think the 40% decrease in diffusing capacity for the edge-on orientation is a lower bound estimate.

This is the first study to show by direct microvascu-
lar measurements in rapidly frozen lungs that the geometrical orientation of red blood cells with respect to alveolar gas depends on alveolar size. If alveoli are highly expanded, red blood cells will transit the resulting oval alveolar capillaries with their broad sides facing alveolar gas. We assume that the same capillary shape and red blood cell orientation occur whether small-animal lungs are expanded by high-airway pres-
sure, as in this study, or whether alveoli in the upper lung of larger animals are expanded by the weight of the lung below zone 2 (3). Figure 7 shows this red blood cell orientation by in vivo microscopy in the subpleural capillaries of alveoli in zone 2 of a dog. If alveoli are less expanded, either because of lower lung inflation or by being in zone 3 of a large animal (3), red blood cells may transit the more circular cross sections of alveolar capillaries with their edges facing alveolar gas. We have also observed this latter red blood cell orientation with in vivo microscopy in the subpleural capillaries of canine alveoli in zone 3, but we have not taken high-
resolution photographs of these fast-moving red blood cells. Our finite-element modeling of these two ex-
tremes in red blood cell orientation showed that the edge-on orientation reduces the diffusive gas uptake by the red blood cells. This adds to the list of pulmonary
During resting conditions or during moderate exercise, the orientation of red blood cells is of little consequence with regard to saturation with oxygen, because of the long capillary transit times (1, 12, 17). During heavy exercise, however, we think red blood cell orientation is likely to be of greater importance. One reason is the well-established observation that left atrial pressures rise to >30 mmHg during maximal exercise (13). That raises capillary transmural pressures, which will distend the capillaries into circular cross sections. Red blood cells are then free to traverse the capillaries in unfavorable orientations, including flowing in the middle of the stream surrounded by a relatively thick layer of plasma. This suggests that it may be a combination of too rapid a transit time and the unfavorable orientation of red blood cells that causes oxygen desaturation during maximal exercise and during some diseases in which capillary pressure is elevated. To our knowledge, this is a new and potentially important concept, i.e., that capillary cross-sectional shape determines the red blood cell orientation, which in turn can be a significant contributor to oxygen desaturation.

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REFERENCES