Capillary filtration coefficient, vascular resistance, and compliance in isolated mouse lungs

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The isolated perfused lung preparation has been widely used for studies of vascular function in sheep (2), dogs (11), ferrets (1), rabbits (4), rats (3), and, recently, mice (16). The capillary filtration coefficient ($K_{fc}$), a sensitive measurement of microvascular hydraulic conductivity, can be measured by gravimetric, indicator hemocoencentration, or tracer methods (9, 10) but has not previously been reported for mouse lungs. Although measurements of total pulmonary vascular resistance in isolated and in situ mouse lungs have recently been published (13, 16), the longitudinal distribution of segmental vascular resistances and pulmonary vascular compliance ($C_{vas}$) have not been determined for mouse lungs.

The purpose of the present study was to extend our isolated perfused lung preparation to mouse lungs to measure baseline vascular resistances and filtration coefficients and determine the responsiveness of the preparation to perturbations that increase vascular permeability and vascular tone.

METHODS

Isolated Lung Preparation

C57/BL6 male mice, weighing 29.3–38.9 g (34.2 ± 1.0 g), were anesthetized with an intraperitoneal injection of ketamine and xylazine. The trachea was cannulated, and the lungs were ventilated with a gas mixture of 20% O$_2$-5% CO$_2$-75% N$_2$ by using a piston-type respirator (model 683 rodent ventilator; Harvard, South Natick, MA). The tidal volume was adjusted to obtain a peak inflation pressure (PIP) of 10–15 cmH$_2$O at a respiratory rate of 65 breaths/min, with 3 cmH$_2$O positive end-expiratory pressure (PEEP). The chest was opened, heparin (100 IU) was injected into the right ventricle, and a suture was placed around the pulmonary artery and aorta. Cannulas were placed in the pulmonary artery and left atrium, and lungs and heart were removed en bloc and suspended from a balance beam attached to a force transducer (FT03D; Grass, Quincy, MA). The force transducer was positioned on the opposite side of the beam fulcrum from the lung. To improve weight sensitivity, the beam fulcrum from the lung. To improve weight sensitivity, the force transducer was one-third the distance from the fulcrum to the lung. Plastic drapes surrounded the preparation to eliminate weight artifacts caused by air currents. The initial 1–2 ml of perfusate, which contained residual blood cells and plasma, were discarded and not recirculated. Lungs were then perfused in a recirculating system with 5% bovine albumin in RPMI-1640 cell culture medium by using a roller pump (Minipuls 2; Gilson, Middleton, WI) at a flow rate of 0.5 ml/min. To minimize the system volume, the heat exchanger was removed and the lungs were perfused at 39°C. The venous outflow was collected in a reservoir, the height of which could be adjusted to increase venous pressure.

Recent developments in genetic engineering have resulted in a proliferation of transgenic mice, many of which include gene alterations that affect pulmonary vascular permeability or tone (13). Many studies of the effects of these gene manipulations concentrate on the observed histological features with few detailed studies of the functional physiological effects of gene expression on pulmonary microvascular permeability and vascular hemodynamic function in mouse lungs. This can be attributable to the small size of mice, which renders surgical procedures more difficult than in larger species. However, an additional advantage of mouse lungs used for isolated lung experiments is the small perfusion volume, which permits the use of small amounts of reagents that may have high cost or low availability.

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Arterial, venous, and airway pressures were continuously monitored by pressure transducers (Cobe, Lakewood, CO) that were zeroed at the midlung level, and pressures and lung weight were recorded on a Grass model 7D polygraph.

Pulmonary Vascular Resistances

Total ($R_T$) and segmental pulmonary vascular resistances were calculated from the perfusate flow and the differences between pulmonary artery ($P_{pa}$) and vein ($P_{pv}$), double-occlusion capillary ($P_{do}$ or $P_{pc}$), and arterial ($P_{ao}$) and venous ($P_{pv}$) occlusion pressures as previously described (11, 12, 17) as follows

$$R_T = (P_{pa} - P_{pv})/(\dot{Q}/100\, g)$$  

precapillary resistance ($R_a$)

$$R_a = (P_{pa} - P_{pc})/(\dot{Q}/100\, g)$$  

postcapillary resistance ($R_v$)

$$R_v = (P_{pc} - P_{pv})/(\dot{Q}/100\, g)$$  

large- and small-artery resistances ($R_{la}$ and $R_{sa}$, respectively)

$$R_{la} = (P_{pa} - P_{ao})/(\dot{Q}/100\, g)$$

$$R_{sa} = (P_{ao} - P_{pc})/(\dot{Q}/100\, g)$$  

small- and large-vein resistances ($R_{sv}$ and $R_{lv}$, respectively)

$$R_{sv} = (P_{pc} - P_{pv})/(\dot{Q}/100\, g)$$

$$R_{lv} = (P_{pv} - P_{pc})/(\dot{Q}/100\, g)$$  

and middle-compartment resistance ($R_{mc}$)

$$R_{mc} = R_{sa} + R_{sv}$$

where $\dot{Q}$ is perfusate flow.

$P_{do}$ has been shown to accurately predict the average capillary filtration pressure (11, 14), whereas the selective arterial and venous occlusions allow separation of segmental vascular resistances across large and small arterial and venous vessel segments. All resistances were normalized to predicted lung weight as $cmH_2O\cdot l^{-1}\cdot min^{-1}\cdot 100\, g^{-1}$.

$K_{fc}$

$K_{fc}$ (in $ml\cdot min^{-1}\cdot cmH_2O^{-1}\cdot 100\, g^{-1}$) is a sensitive measurement of endothelial hydraulic conductivity when capillary surface area is maintained constant (14). After an isogravimetric state is attained, $P_{pv}$ is increased by $-6\, cmH_2O$ for 20 min, and the change in capillary pressure ($dP_{pc}$) is determined by double occlusion before and after the $P_{pv}$ increase. The rate of lung weight gain ($dW/dt$) between 18 and 20 min is used to calculate $K_{fc}$ by using

$$K_{fc} = (dW/dt)/dP_{pc}$$

All $K_{fc}$ values were normalized to 100 g predicted lung weight on the basis of the ratio of lung weight to body weight in six control mice according to

$$PLW = 0.00452 \pm 0.0003\, BW$$

where $PLW$ and $BW$ are predicted lung weight and body weight, respectively.

$C_{vas}$

$C_{vas}$ was measured according to Rippe et al. (11) by using perfusate flow and the rate of increase in $P_{pv}$ ($dP_{pv}/dt$; in $cmH_2O/min$) after a venous occlusion

$$C_{vas} = \dot{Q}/(dP_{pv}/dt)$$

All $C_{vas}$ values were normalized to 100 g predicted lung weight and expressed as $ml\cdot cmH_2O^{-1}\cdot 100\, g^{-1}$.

Experimental Protocol

The general protocol consisted of an isogravimetric state for 20–30 min, followed by vascular occlusion pressure measurements and a $K_{fc}$ measurement. At 1 and 2 h after the initial $K_{fc}$ measurement, the occlusion pressure and $K_{fc}$ measurements were repeated for a total of three $K_{fc}$ measurements. Occlusion pressures were also measured during the increased venous pressure states during the $K_{fc}$ measurements and after the last $K_{fc}$ measurement.

Low-PIP control group. In five lungs, $PIP$ was maintained at $10\, cmH_2O$ and PEEP at $3\, cmH_2O$ throughout the $K_{fc}$ and occlusion pressure measurements. Occlusion pressures were repeated after the last (2 h) $K_{fc}$ measurement to serve as a time control for the phenylephrine-infusion group.

High-PIP injury group. In five lungs, PIP was increased to 30–31 $cmH_2O$ and PEEP to 6–7 $cmH_2O$ for 20 min after the second $K_{fc}$ measurement. After the third $K_{fc}$ measurement, $P_{pv}$ was returned to baseline and phenylephrine ($10\, M$) was infused into the venous reservoir. The vascular occlusion pressures were then repeated to determine $R_T$ and segmental vascular resistances.

Hemodynamic group. In five lungs, $PIP$ was maintained at $10\, cmH_2O$ and PEEP at $3\, cmH_2O$ for two $K_{fc}$ measurements 1 h apart. These baseline $K_{fc}$ and occlusion pressure measurements were similar to baseline values in the other two groups and are not presented. Between $K_{fc}$ measurements, stepwise increases in either flow or venous pressure were used to construct the vascular pressure-flow, pressure-resistance, flow-resistance, and pressure-compliance curves for the pulmonary circulation. The vascular pressure-compliance relationships were measured by using venous occlusions after step increases in venous pressure with constant flow. Experiments were then terminated, and the lungs were weighed.

Statistics

All values are expressed as means ± SE unless otherwise stated. The $K_{fc}$ values, pressures, and resistances were compared by using an ANOVA with repeated measures and a Newman-Keuls posttest with the use of CRUNCH4 statistical software on a Gateway 2000 digital computer. A significant difference was determined where $P < 0.05$.

RESULTS

$K_{fc}$

Figure 1 compares the $K_{fc}$ measurements in the high-PIP injury group (●) and the low-PIP control group (■). $K_{fc}$ was unchanged in either group at 0 and 1
h of low-PIP ventilation but increased significantly by 4.3-fold in the high-PIP injury group compared with paired baseline $K_{fc}$ and the low-PIP control group after 20 min of ventilation with a PIP of 32 cmH$_2$O. Terminal lung weights were $0.171 \pm 0.011$ g for uninjured lungs in the low-PIP control group and were significantly higher at $0.243 \pm 0.012$ g for the high-PIP injury group.

**Pulmonary Vascular Pressures**

Table 1 summarizes the vascular pressures and perfusate flow at high- and low-pressure states in the high-PIP injury group, after phenylephrine infusion, and the 2-h baseline pressures in the low-PIP control group. Vascular pressures in the low-PIP control group did not change significantly with time and were not significantly different from those in the high-PIP injury group. Isogravimetric capillary pressures averaged 5.5–5.9 cmH$_2$O in uninjured lungs at a perfusate flow of 0.51 ml/min. Baseline Ppa was significantly increased from baseline after PE infusion and from the low-PIP control group at the same time period.

Figure 2 indicates the vascular pressure-perfusate flow relationship for Ppa and Ppv as perfusate flow was increased from 0.3 to 1.0 ml/min.

**Vascular Resistance and Cvas**

Table 2 summarizes $R_T$, $R_a$, and $R_v$, $R_a/R_v$, and $C_{vas}$ at low and high venous pressures. There were trends toward a decrease in $R_T$ when venous pressure was increased during $K_{fc}$ measurement, but these did not reach significance. However, $C_{vas}$ decreased significantly after every venous pressure increase. After phenylephrine infusion $R_T$, $R_a$, and $R_a/R_v$ were significantly increased compared with values in the preceding low-venous-pressure state and the comparable time in the low-PIP control group.

$R_T$ decreased in response to increased perfusate flow (Fig. 3) and increased capillary pressure at constant

**Table 1. Vascular pressures and perfusate flows at all pressure states**

<table>
<thead>
<tr>
<th>Condition</th>
<th>Ppa</th>
<th>Pao</th>
<th>Pdo</th>
<th>Pvo</th>
<th>Ppv</th>
<th>Flow</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 h</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Baseline Ppv</td>
<td>8.56</td>
<td>6.68</td>
<td>5.92</td>
<td>4.96</td>
<td>3.64</td>
<td>0.52</td>
</tr>
<tr>
<td>High Ppv</td>
<td>13.56</td>
<td>12.16</td>
<td>11.68</td>
<td>11.24</td>
<td>9.60</td>
<td>0.52</td>
</tr>
<tr>
<td>1 h</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Baseline Ppv</td>
<td>7.76</td>
<td>6.28</td>
<td>5.54</td>
<td>4.72</td>
<td>3.08</td>
<td>0.51</td>
</tr>
<tr>
<td>High Ppv</td>
<td>13.22</td>
<td>11.88</td>
<td>11.62</td>
<td>10.98</td>
<td>9.48</td>
<td>0.51</td>
</tr>
<tr>
<td>Injury</td>
<td></td>
<td></td>
<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Baseline Ppv</td>
<td>8.85</td>
<td>6.93</td>
<td>5.88</td>
<td>5.08</td>
<td>3.58</td>
<td>0.52</td>
</tr>
<tr>
<td>High Ppv</td>
<td>14.85</td>
<td>12.53</td>
<td>11.95</td>
<td>11.05</td>
<td>9.88</td>
<td>0.52</td>
</tr>
<tr>
<td>Phenylephrine</td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Baseline Ppv</td>
<td>12.25</td>
<td>6.88</td>
<td>6.05</td>
<td>5.23</td>
<td>3.15</td>
<td>0.52</td>
</tr>
<tr>
<td>Time control</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Baseline Ppv</td>
<td>6.90</td>
<td>5.68</td>
<td>5.02</td>
<td>4.52</td>
<td>3.28</td>
<td>0.52</td>
</tr>
</tbody>
</table>

Values are means ± SE expressed as cmH$_2$O for all pressures and ml/min for flow. $n = 5$ lungs in all conditions. Ppa, Ppv, Pao, Pdo, and Pvo: pulmonary arterial, venous, arterial occlusion, double-occlusion, and venous occlusion pressure, respectively. *$P < 0.05$ vs. all similar pressure states within the same group and low-PIP control group. †$P < 0.05$ vs. the preceding low vascular pressure state.
perfusate flow (Fig. 4). Cvas also decreased in a curvilinear fashion as capillary pressure increased at a constant perfusate flow (Fig. 5).

Segmental Vascular Resistances

The segmental vascular resistance at high and low venous pressures in the high-PIP injury group, after phenylephrine infusion, and at 2 h in the low-PIP control group are summarized in Table 3. Rla and Rlv were significantly increased by phenylephrine infusion.

Figure 6 shows the changes in mean segmental vascular resistances between high- and low-pressure states and between phenylephrine infusion and control groups. Increased venous pressures decreased segmental pressures in small artery and venous segments, whereas phenylephrine infusion increased segmental Rla and Rlv.

### DISCUSSION

The isolated perfused lung preparation has become an accepted standard for physiological and pharmacological analysis of pulmonary vascular function (14). In the present study, we have adapted the same isolated lung preparation previously used in studies in dogs (11), rabbits (4), and rats (3) to the mouse lung. Although the small size of the mouse renders surgical isolation of the lung more difficult, isolated mouse lungs can be used to investigate the altered vascular function of targeted genes in relevant transgenic mouse models (13). In addition, the large array of immunological reagents available for mice and the small circulating perfusate volume in this preparation permit the use of reagents that are not available in other species, or the cost or availability of which may prohibit use in larger animal models. Although nominal perfusate...
volumes were 5 ml in these studies, a system volume of 2.5 ml was practical for perfusion at baseline vascular pressures. Therefore, these reservoir volumes approach the fluid volumes used in a cell culture plate. Modifications of our previous preparations that were used to prepare mouse lungs include the use of magnifying dissecting glasses, a cantilever-beam system to enhance the sensitivity of the force transducer, and drapes to block air currents. RPMI cell culture medium was used rather than Krebs or other physiological buffers to compare resistance values with those observed in the mouse lung preparation of von Bethmann et al. (16).

$K_{fc}$ is a sensitive measurement of transcapillary fluid conductance and has not previously been reported for mouse lungs (14). The normalized baseline $K_{fc}$ value for mouse lung of 0.33 ml min$^{-1}$ cmH$_2$O$^{-1}$ 100 g$^{-1}$ is similar to $K_{fc}$ values previously reported for isolated rat lungs (0.33 ± 0.03 ml min$^{-1}$ cmH$_2$O$^{-1}$ 100 g$^{-1}$) by Seibert et al. (12) and isolated rabbit lungs (0.33 ± 0.03 ml min$^{-1}$ cmH$_2$O$^{-1}$ 100 g$^{-1}$) by Hernandez et al. (4). However, exact quantitative comparisons of gravimetric $K_{fc}$ values are highly dependent on the portion of the weight gain curve used to represent transcapillary filtration (14). One of the advantages of using the rate of weight gain at 20 min after a venous pressure increase is that the slow component of vascular stress relaxation is minimized (9). However, the method used here for estimating $K_{fc}$ has been used in several previous isolated lung studies and produces stable and consistent values (7, 8).

We observed a 4.5-fold increase in $K_{fc}$ in mouse lungs after 20 min of ventilation with a PIP of 30.4 cmH$_2$O (PEEP 6.8 cmH$_2$O; mean airway pressure 14.8 cmH$_2$O) (Table 4). This compares with a 3.7-fold in-

Table 3. Segmental vascular resistances at all pressure states

<table>
<thead>
<tr>
<th>Condition</th>
<th>Rla</th>
<th>Rsa</th>
<th>Rsv</th>
<th>Rlv</th>
<th>Rmc/Rt</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 h</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Baseline Ppv</td>
<td>2.48±0.38</td>
<td>1.01±0.14</td>
<td>1.26±0.27</td>
<td>1.76±0.17</td>
<td>0.35±0.03</td>
</tr>
<tr>
<td>High Ppv</td>
<td>1.84±0.33</td>
<td>0.64±0.11</td>
<td>0.59±0.15</td>
<td>2.17±0.16</td>
<td>0.24±0.05</td>
</tr>
<tr>
<td>1 h</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Baseline Ppv</td>
<td>2.00±0.27</td>
<td>1.02±0.32</td>
<td>1.08±0.26</td>
<td>2.20±0.09</td>
<td>0.32±0.03</td>
</tr>
<tr>
<td>High Ppv</td>
<td>1.81±0.32</td>
<td>0.34±0.19</td>
<td>0.86±0.06</td>
<td>2.02±0.15</td>
<td>0.25±0.05</td>
</tr>
<tr>
<td>Injury</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Baseline Ppv</td>
<td>2.58±0.27</td>
<td>1.98±0.36</td>
<td>1.08±0.14</td>
<td>2.00±0.23</td>
<td>0.35±0.04</td>
</tr>
<tr>
<td>High Ppv</td>
<td>3.10±1.01</td>
<td>0.77±0.26</td>
<td>1.21±0.19</td>
<td>1.58±0.22</td>
<td>0.33±0.05</td>
</tr>
<tr>
<td>Phenylephrine</td>
<td>7.22±0.99*</td>
<td>1.11±0.55</td>
<td>1.11±0.17</td>
<td>2.79±0.11*</td>
<td>0.19±0.05*</td>
</tr>
<tr>
<td>Baseline Ppv</td>
<td>2.06±0.16</td>
<td>1.10±0.06</td>
<td>0.84±0.08</td>
<td>2.05±0.32</td>
<td>0.33±0.04</td>
</tr>
</tbody>
</table>

Values are means ± SE; n = 5 lungs in all conditions. Rla, Rsa, Rsv, Rlv, and Rmc: large artery, small artery, small vein, large vein, and middle-compartment resistance, respectively. *P < 0.05 vs. all similar pressure states within the same group and low-PIP control group.
crease in $K_r$ in isolated rat lungs after 30 min at a PIP of 35 cmH$2$O and a 9.7-fold increase after 60 min at a PIP of 30 cmH$2$O in isolated rabbit lungs (4, 7). Only minimal increases occurred in dog lungs at these PIP levels (10). Exact comparisons of injury are difficult because the degree of airway pressure-induced injury varies with peak pressure, duration and rate of ventilation, and mean airway pressure (6). However, mouse lungs are approximately as susceptible to ventilation-induced mechanical injury as are rabbit and rat lungs but have a greater susceptibility to injury than dog lungs. Mathieu-Costello et al. (5) also observed a higher threshold of dog lungs to mechanical stress injury due to vascular pressure compared with rabbit lungs, as evaluated by the number of epithelial breaks.

Pulmonary vascular resistance has recently been reported for partially isolated, perfused mouse lungs (16) and intact, open-chest mice (13). Normalized to cmH$2$O/$\text{min} \cdot 100 \text{g}^{-1}$, the R$T$ values of von Bethmann et al. (16) in perfused mouse lungs ranged from 7.4 to 10.2 cmH$2$O/$\text{min} \cdot 100 \text{g}^{-1}$ at a flow of 1.0 ml/min. Although the nominal perfusion rate was 0.5 ml/min in the present study, a comparable R$T$ of 11.3 cmH$2$O/$\text{min} \cdot 100 \text{g}^{-1}$ was obtained at a perfusion rate 1.0 ml/min. Correcting R$T$ for a 16% increase in viscosity due to perfusion at 29 rather than 37°C would result in an R$T$ of 9.5 cmH$2$O/$\text{min} \cdot 100 \text{g}^{-1}$, a value similar to that obtained by von Bethmann et al. In intact, open-chest mice, Steudel et al. (13) reported aortic flows of 6.2 to 9.3 ml/min and an average pulmonary artery pressure of 22.3 cmH$2$O. An R$T$ of 3.2 cmH$2$O/$\text{min} \cdot 100 \text{g}^{-1}$, or about one-third of that measured in isolated perfused lungs, can be calculated for these intact mice. A considerable difference in the slopes of the flow-pressure curves was obtained between their intact mouse lungs and the isolated mouse lungs of the present study (3.3 vs. 0.92 cmH$2$O/$\text{min} \cdot 100 \text{g}^{-1}$).

Pulmonary vascular resistance was inversely related to vascular pressure and flow in the present study (Figs. 3 and 4). Rippe et al. (11) reported a decreasing R$T$ as Ppc increased as well as significant decreases in both Rmc and Cvas at higher vascular pressures. However, the decrease in Rmc/R$T$ for dog lungs attained statistical significance only at higher venous pressures than those used in the present study (11). In the present study there was a trend for both R$T$ and Rmc/R$T$ to decrease at increased venous pressures, and Cvas decreased significantly with every increase in venous pressure. These decreases in vascular resistance and Cvas as vascular flow and pressure increase indicate the well-known effects of recruitment and distention of microvessels on vascular resistance and Cvas as perfusate flow and pressure increase (15).

Although segmental vascular resistances have not been previously reported for mouse lungs, we observed a longitudinal distribution of vascular resistance that was similar to that previously reported for the lungs of other species (Table 4). That is, vascular resistance was approximately equally distributed among large arteries, the middle compartment (small arteries and veins), and large veins. The absence of muscular arterioles such as are present in the systemic circulation results in a significant portion of the resistive pressure drop occurring across the microvessels in the lung (15). An increased percentage of R$T$ after phenylephrine infusion was attributable mainly to the significant increase in Rla and Rlv (Table 3). In a previous study in dog lungs, Rippe et al. (11) also observed increases in the large artery and vein components of R$T$ at constant flow with decreases in fractional Rmc in response to norepinephrine and serotonin in dog lungs. Histamine produced resistance increases primarily in the large veins in the above-mentioned study.

In conclusion, consistent baseline vascular resistance, Cvas, and microvascular permeability measurements could be obtained in the isolated mouse perfused lung preparation. These remained stable over 2 h and responded in a predictable manner to increases in vascular pressure and flow. The responses to injurious levels of high airway pressure and a vasoconstrictor agent were similar to those observed in other species. Thus this preparation can be readily used to assess pulmonary vascular effects of genetic alterations in mice or the vascular application of immunologic agents that require a small perfusate volume.


