The role of time and pressure on alveolar recruitment

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Submitted 9 June 2008; accepted in final form 4 December 2008

The role of time and pressure on alveolar recruitment. J Appl Physiol 106: 757–765; 2009. First published December 12, 2008; doi:10.1152/japplphysiol.90735.2008.—Inappropriate mechanical ventilation in patients with acute respiratory distress syndrome can lead to ventilator-induced lung injury (VILI) and increase the morbidity and mortality. Reopening collapsed lung units may significantly reduce VILI, but the mechanisms governing lung recruitment are unclear. We thus investigated the dynamics of lung recruitment at the alveolar level. Rats (n = 6) were anesthetized and mechanically ventilated. The lungs were then lavaged with saline to simulate acute respiratory distress syndrome (ARDS). A left thoracotomy was performed, and an in vivo microscope was placed on the lung surface. The lung was recruited to three recruitment pressures (RP) of 20, 30, or 40 cmH2O for 40 s while subpleural alveoli were continuously filmed. Following measurement of microscopic alveolar recruitment, the lungs were excised, and macroscopic gross lung recruitment was digitally filmed. Recruitment was quantified by computer image analysis, and data were interpreted using a mathematical model. The majority of alveolar recruitment (78.3 ± 4.8 and 84.6 ± 5.1%) occurred in the first 2 s (T2) following application of RP 30 and 40, respectively. Only 51.9 ± 5.4% of the microscopic field was recruited by T2 with RP 20. There was limited recruitment from T2 to T40 at all RPs. The majority of gross lung recruitment also occurred by T2 with gradual recruitment to T40. The data were accurately predicted by a mathematical model incorporating the effects of both pressure and time. Alveolar recruitment is determined by the magnitude of recruiting pressure and length of time pressure is applied, a concept supported by our mathematical model. Such a temporal dependence of alveolar recruitment needs to be considered when recruitment maneuvers for clinical application are designed.

alveolar mechanics; ventilator-induced lung injury; mathematical modeling; animal study; recruitment maneuvers; mechanical ventilation

The clinical impact of acute lung injury (ALI) and its more hypoxemic form of acute respiratory distress syndrome (ARDS) is significant, with an estimated 17,000–43,000 deaths per year (40). It is widely believed that improper mechanical ventilation of these patients can worsen ARDS mortality through a process of ventilator-induced lung injury (VILI) (14). As a result of this concern, investigators have proposed various “protective modes” of ventilation aimed at reducing morbidity and mortality in ALI secondary to VILI (4, 5). In fact, in a landmark study by the ARDS network, lower tidal volumes and reduced plateau pressures led to a reduction in overall mortality compared with standard-of-care ventilation (1). However, one potential and undesirable consequence of lower tidal volumes is progressive collapse or “derecruitment” of dependent lung (39), with the potential for repeated alveolar recruitment and derecruitment (R/D) over time (41).

There is sufficient evidence to suggest that repeated alveolar R/D can significantly contribute to VILI (23, 24, 37, 44), presumably through a shearing stress-induced trauma to the lung parenchyma when airways and alveoli are repeatedly forced open (37, 41, 44). Moreover, Slutsky and colleagues have shown that this mechanical injury to the lung can lead to a secondary activation of systemic inflammation, which could potentially lead to downstream multi-organ dysfunction (42). Steinberg et al. used in vivo microscopy in a porcine ARDS model to support the notion that alveolar instability (R/D) leads to VILI through increased mechanical stress (44). In addition, added positive end-expiratory pressure (PEEP) can stabilize alveoli, leading to a reduction in alveolar R/D (22, 29) and a reduction in serum inflammatory mediators (3, 15). Therefore, if alveolar R/D can be prevented with protective mechanical ventilation, there may be both pulmonary and systemic benefits resulting in reduced morbidity and mortality.

One widely espoused strategy to prevent VILI is to “open the lung and keep it open” (27). This strategy first requires a recruitment maneuver to open the lung, followed by sufficient PEEP to maintain the lung in its newly open state (4). Two components believed to be critical to recruiting the lung are the magnitude of the airway pressure increase and the time that the pressure is elevated (6), the latter being the primary impetus for sustained airway pressure elevations during recruitment (4, 9, 31). As pressure is applied, lung units open according to their critical opening pressure from lowest to highest in a sequence, as defined by the “avalanche theory” of lung inflation (2). Similarly, the closure of lung units during deflation, or derecruitment, is governed by alveolar critical closing pressure. Importantly, both critical opening and closing pressures are presumed to change appreciably in ALI and ARDS (6).

Animal studies have demonstrated that temporal recruitment occurs during sustained inflation (28, 33). Understanding the pressure and time dependence of alveolar recruitment is critical to the development of optimal recruiting strategies. Bates and Irvin have developed a mathematical model in which both pressure and time play key roles in the recruitment and derecruitment of lung units (6). Thus, according to this model, alveolar units not only have critical opening and closing pressures but also characteristic opening and closing times that change in the acutely injured lung (6). This is a novel and
important concept that has yet to be fully explored experimentally. We hypothesize that alveolar recruitment does not occur instantaneously when pressure is applied, but rather the recruitment is temporal, continuing gradually as the pressure is sustained.

In this study, we visually recorded the effects of an alveolar recruitment maneuver at both gross and microscopic levels over time in a lavage injured rat lung, and fit these data to a mathematical model of recruitment that incorporates both pressure and time. Recruitment of individual subpleural alveoli was visualized using in situ videomicroscopy. In the same injured lungs, gross recruitment was assessed ex vivo in terms of the amount of atelectasis visible on the pleural surface. We then interpreted these data using a mathematical model featuring distributions of critical opening pressures and opening velocities.

METHODS

Vertebrate animals. The protocol was approved by the Committee for the Humane Use of Animals at SUNY Upstate Medical University where all the animal experiments were performed. The experiments described in this study were performed in accordance with the National Institutes of Health guidelines for the use of experimental animals in research.

Surgical preparation. Male Sprague-Dawley rats (n = 6), weighing 371.1 ± 20.6 g received an intraperitoneal injection of ketamine (90 mg/kg) and xylazine (10 mg/kg) with additional dosing as needed to maintain anesthesia. A tracheostomy was established with a 2.5-mm pediatric endotracheal tube. A 5.0-Fr carotid arterial catheter was placed for blood gas analysis (model ABL5, Radiometer, Copenhagen, Denmark) and in-line measurement of systemic arterial pressure (TruWave, Baxter Healthcare, Irvine, CA). The external jugular vein was cannulated for fluid resuscitation and drug infusion. Perfusion was then achieved with intravenous pancuronium (0.8 mg/kg). After surgical preparation, the animals were placed on pressure control ventilation, PEEP 5 cmH2O, and control pressure (Pcontrol) of 15 cmH2O, inspiratory-to-expiratory ratio of 1:2, and inspired oxygen fraction of 1.0. (Servo® ventilator, Maquet, Bridgewater, NJ). A baseline blood gas was obtained, and the respiratory rate was adjusted to normalize baseline PCO2 between 35 and 45 cmH2O. Baseline blood pressure, blood gases, and pulmonary parameters (pH, PCO2, PO2, respiratory rate, and tidal volume) were obtained before injury.

Injury protocol. Lung injury was induced with saline lavage (16 ml/kg) via the endotracheal tube until the PO2 was ≤200 cmH2O. Postinjury hemodynamic and pulmonary parameters were recorded. The animals were killed (barbiturate overdose, 150 mg/kg), and the left lung was exposed with a sternotomy and left thoracotomy. Great care was used not to injure the lungs during surgical exposure. The postinjury lung surface had inhomogeneous areas of gross atelectasis (Fig. 1; see movie 2 in supplemental material, which is available online at the Journal of Applied Physiology’s website). The ventilator was switched to airway pressure release ventilation (APRV) or inverse ratio mode (Servo® Bivent). An initial recruitment maneuver was performed at 40 cmH2O for 40 s to normalize lung volume history before initiating the protocol.

In situ microscopy. A microscopic coverslip mounted on a ring was lowered onto the pleural surface, and the lung was held in place by gentle suction (≤5 cmH2O) at end inspiration for placement of an in situ videomicroscope (Olympus, epi-objective microscope with epi-illumination). Each placement of the videomicroscope was done randomly on the lung surface. Microscopic images of alveoli in the same microscopic field during the entire recruitment maneuver were viewed at a final magnification of ×130 with a color video camera (model CCD SSC-S20, Sony) and stored in digital format on a personal computer (Dell OPTIPLEX GX620). Each microscopic field measured 1.22 × 106 μm².

Microscopic recruitment. Following lung injury, the animals (n = 6) were placed on inverse ratio mode (Bivent) Pressurelow 20 cmH2O, Pressurerise 0 s, Timeon 0.2 s, and Timetop 1 s. The lung was allowed to derecruit for 5 min to a pressure of 5 cmH2O to standardize the lung volume history. Sequential recruitment maneuvers were made with three different recruitment pressures (RP) of 20, 30, and 40 cmH2O for 40 s in random order, with a derecruitment for 5 min at 5 cmH2O between each recruitment maneuver. Video recordings of subpleural alveoli in the left lung were taken in real time with the in situ microscope throughout the 40-s recruitment periods (see movie 1 of supplemental material, available online at the Journal of Applied Physiology’s website). Postexperimental video analysis was performed, and two randomly selected video frames were extracted for alveolar recruitment measurement by computer image analysis (Empire Imaging Systems, Image Pro, Syracuse, NY) at 0, 2, 5, 10, 20, 30, and 40 s during the 40-s recruitment maneuver. At each of these time points, the recruited alveoli were circled and the area (μm²) within each circle was calculated using the computer image software. All the areas of recruited alveoli were added to yield the sum area of alveolar recruitment per microscopic field. The total number of alveoli analyzed per animal using this method ranged from 2,207 to 3,549 alveoli. The data were expressed as the percentage of the entire microscopic field occupied by recruited alveoli.

Percent recruitment (alveoli) = \frac{\text{Sum area of all alveoli (μm}²\text{)}}{\text{Microscopic field area (μm}²\text{)}} \times 100

Microscopic field area. A small majority of the microscopic field area (μm²) is made up of capillaries and interstitium not incorporated into the traced alveolar area. Therefore, the lung could approach, but never achieve, 100% recruitment. The microscopic field was maintained throughout each recruitment maneuver, and then a new random field was chosen for the subsequent RP maneuver. Therefore, three random locations were used for each recruitment pressure in each animal.

Macroscopic recruitment. Lungs (n = 5) were excised following the microscopic measurements for gross lung recruitment filming. One lung was damaged during removal and could not be used, so the number of lungs tested in the macroscopic protocol was one less than in the microscopic protocol. The lungs were suspended via the tracheostomy tube, which was attached to the ventilator (see movie 2 of supplemental material, available online at the Journal of Applied Physiology’s website). The excised lungs were ventilated using the Servo® ventilator in Bivent mode with the same settings as used during the microscopic measurements. The lungs were allowed to derecruit to 5 cmH2O between each recruitment maneuver to standardize lung volume history. This derecruitment pressure was only held for 1 min in the ex vivo lungs in an attempt to prevent drying out and desiccation that occurs when exposed to air. Desiccation of the lung was also prevented by periodically wetting with normal saline. In an identical manner to the microscopic images, photos were captured every 2 s during the 40-s recruitment maneuver.

The lungs were randomly subjected to the identical RP of 20, 30, and 40 cmH2O and held for 40 s while gross video recording was taken (Sony digital video camera recorder, model DCR-TRV33) of the lung surface. Postexperimental video analysis was performed by computer image analysis (Empire Imaging Systems, Image Pro, Syracuse, NY) on individual photographs taken from the video recording at 0, 2, 5, 10, 20, 30, and 40 s during the 40-s recruitment maneuver. The areas of visible lung that were inflated (i.e., pink) were circled (Fig. 1, gross lung photos; see black lines circling the inflated tissue), and the area within each of these circled areas was measured (mm²) using the computer image software. All areas were added to yield the sum area of inflated lung. The data were expressed as the percentage...
of the entire visible lung surface that was occupied by inflated (pink) areas of lung (Fig. 1):

Percent recruitment (gross lung)

\[ \text{Percent recruitment} = \frac{\text{Sum area of visible inflated (pink) lung (mm}^2) \times 100}{\text{Area of entire visible lung surface (mm}^2) \times 100} \]

Statistical analysis. All results are presented as means ± SE. Statistical analyses were performed using a multiple-measures ANOVA, followed by pairwise means comparisons using Tukey’s post hoc tests (JMP software, version 5 Cary, NC). We accepted a \( P \) value of <0.05 as statistically significant.

Modeling. Following the original suppositions of Bates and Irvin (6), the dynamic nature of opening and closing was modeled by associating each lung unit (i.e., single independent segment of open lung) with a virtual trajectory \((x)\) that takes values from 0 to 1. This trajectory does not correspond to any physical quantity or process, yet it is motivated by the considerations of liquid bridge formation discussed previously (6). Movement along the trajectory occurs at a velocity proportional to the difference between the pressure \((P)\) applied to the unit and a critical opening pressure \((P_0)\). The constant of proportionality is \(s_o\). That is,

\[ \frac{dx}{dt} = s_o(P - P_0) \quad (1) \]

The unit is either open or closed, depending on which extreme of the trajectory, either \(x = 1\) or \(x = 0\), was last visited. The dynamics of the model are governed by probability distribution functions for \(P_0\) and \(s_0\), that is

\[ P_0 \in A(P_0) \quad (2) \]

\[ s_0 \in B(s_0) \quad (3) \]

The experiments reported here involved suddenly elevating a lung from 5 cmH\(_2\)O to a recruiting pressure of \(P_r\). This leads to the eventual recruitment of a fraction \(F(P_r)\) of the lung given by

\[ F(P_r) = F(5) + \int_{5}^{P_r} A(P)dP \quad (4) \]

where \(F(5)\) is the fraction of lung already recruited by maintaining pressure at 5 cmH\(_2\)O for an extended period of time. The fraction of recruited lung at time \(t\), however, depends on how rapidly the...
individual units move from $x = 0$ to $x = 1$. The velocity of movement of a unit along its virtual trajectory is given by Eq. 1, so the time, $t_0$, taken to open a unit with opening pressure $P_o$ is

$$t_0 = \frac{1}{s_d(P_r - P_o)}$$

(5)

Therefore, for a unit to be open at time $t$, it must satisfy the two conditions: $P_0 < P_r$ and $s_d > 1/[t(P_r - P_o)]$. If we assume that $A$ and $B$ are independent (that is, the value of $s$ that a unit has does not influence its value of $P_0$), then

$$F(P_r, t) = F(5) + \int_{[P_r - P_o]}^{P_r} B(s)ds \frac{dP}{A(P)dP}$$

(6)

Having the model simulate the time course of recruitment thus comes down to the question of choosing the distributions $A(P)$ and $B(s)$. The cumulative distribution of opening pressures, which is the integral of $A(P)$ with respect to $P$, describes how percent recruitment at infinite time depends on $P_r$. Insofar as recruitment is complete by 40 s after each step change in pressure, a plot of percent recruitment at 40 s vs. $P_r$ gives the integral of $A(P)$ for the data of the present study.

The appropriate distribution for $B(s)$ is less obvious. Bates and Irvin (6) used a hyperbolic distribution for $B(s)$ in their original numerical study, but this is problematic for the analytical development of the model because the integrated area under a hyperbola between 0 and infinity is infinite, which means it cannot feasibly be used as a probability distribution function in Eq. 6. We therefore decided to make $B(s)$ exponential, since this functional form is also monotonically decreasing but can be scaled to have unity area. That is:

$$B(s) = b e^{-\lambda s}; \quad 0 \leq s \leq \infty$$

We adapted Eq. 6 for the present study by incorporating the above equation for $B(s)$ and using a functional form for $A(P)$ that was determined from our experimental data.

RESULTS

Hemodynamic and ventilatory parameters. Table 1 displays baseline and postinjury blood gas measurements, mean arterial blood pressure, respiratory rate, and tidal volume. The pH and Po2 were significantly lower after injury, indicative of ARDS.

We adapted Eq. 6 for the present study by incorporating the above equation for $B(s)$ and using a functional form for $A(P)$ that was determined from our experimental data.

<table>
<thead>
<tr>
<th>Time</th>
<th>pH</th>
<th>Pco2, Torr</th>
<th>P02, Torr</th>
<th>MAP, Torr</th>
<th>RR, breaths/min</th>
<th>Vl, ml</th>
</tr>
</thead>
<tbody>
<tr>
<td>Baseline</td>
<td>7.38±0.03</td>
<td>38±4.17</td>
<td>533.4±34.4</td>
<td>78.4±19.2</td>
<td>22.6±3.89</td>
<td>5.6±1.96</td>
</tr>
<tr>
<td>Postinjury</td>
<td>7.27±0.10*</td>
<td>42.1±13.4</td>
<td>131.7±52.7*</td>
<td>73.7±16.9</td>
<td>37.4±13.5*</td>
<td>5.6±1.98</td>
</tr>
</tbody>
</table>

Values are means ± SE. MAP, mean arterial pressure; RR, respiratory rate; Vl, tidal volume. *Significant difference vs. baseline ($P < 0.05$).
Table 2. Percent alveolar recruitment in the first 2 s

<table>
<thead>
<tr>
<th>RP</th>
<th>T0</th>
<th>T2</th>
<th>%Δ</th>
</tr>
</thead>
<tbody>
<tr>
<td>20 cmH2O</td>
<td>20.5 ± 6.0</td>
<td>51.9 ± 5.4*</td>
<td>30.5 ± 7.2†</td>
</tr>
<tr>
<td>30 cmH2O</td>
<td>49.8 ± 18.7</td>
<td>78.3 ± 7.4</td>
<td>47.0 ± 10.1</td>
</tr>
<tr>
<td>40 cmH2O</td>
<td>26.6 ± 10.6</td>
<td>84.6 ± 5.1</td>
<td>66.0 ± 10.6</td>
</tr>
</tbody>
</table>

Values are means ± SE. RP, recruitment pressure; T0, before application of RP; T2, 2 s after application of RP; %Δ, the percent increase in alveolar recruitment from T0 to T2. *Significant difference compared with other pressures (P < 0.05). †Significant difference compared with 40 cmH2O (P < 0.05).

Both the microscopic and gross recruitment data, together with their combined regression line given by

\[
\text{Percent recruitment} = 14.8 + 2.0P
\]  
\[
(8)
\]

The positive intercept in Eq. 8 implies that ~15% of the alveoli are already recruited even when pressure is zero, which means that to completely collapse the lung one would have to apply a pressure of about ~7 cmH2O. Also shown in Fig. 3 is a cumulative Gaussian distribution spanning the same range of pressure as the data points (mean of the Gaussian is 16 cmH2O, standard deviation is 12 cmH2O). Whereas the straight line passes through the middle of the data, the cumulative Gaussian swings systematically wide of the data. This means that, rather than being Gaussian, as assumed in the original model of Bates and Irvin (6), the distribution of opening pressures pertaining to the data of the present study is roughly constant. That is, \( A(P) \) is constant between the limits -7.4 and 42.6 cmH2O thus:

\[
A(P) = \frac{1}{50}; \quad -7.4 \leq P \leq 42.6
\]  
\[
0; \quad P < -7.4, \quad P > 42.6
\]  
\[
(9)
\]

With this choice for \( A \) and the equation for \( B \) (see Eq. 7), Eq. 6 now becomes

\[
F(P_r, t) = 0.248 + \int_{-\infty}^{\infty} \int_{[P_r,P_{\text{crit}}^{-1}]} be^{-b} dP \frac{1}{50} dP
\]

\[
= 0.248 + \frac{1}{50} \int_{P_r}^{\infty} e^{-b} dP
\]  
\[
(10)
\]

so long as \( P_r \) is <42.6 cmH2O, which is the case for the data of the present study.

We fit Eq. 10 to both the microscopic and gross data sets by adjusting the value of \( b \) for each set so as to minimize the sum of squared residuals between the predicted values of \( F \) and the experimental measurements for all values of \( t \) and \( P_r \). For the microscopic data, we obtained a value of \( b = 0.01 \text{s}^{-1}\text{cmH}_2\text{O}^{-1} \) with a root mean-squared residual between the model predictions and the data of 8 cmH2O. For the gross data, \( b = 0.10 \text{s}^{-1}\text{cmH}_2\text{O}^{-1} \) with a root mean-squared residual of 10 cmH2O. These differences in \( b \) for the two data sets give us at least some idea of the range of values this parameter might take, given that the microscopic and gross data sets reflect only small samplings of the entire lung. Indeed, when \( b = 0.10 \text{s}^{-1}\text{cmH}_2\text{O}^{-1} \), the model predictions of \( F(P_r, t) \) match both the microscopic and gross percent recruitment experimental data to within the differences between the two data sets themselves. This indicates that the model has captured the essential aspects of the recruitment dynamics in the saline lavage-injured rat lung.

Table 3. Percent alveolar recruitment from 2 to 40 s

<table>
<thead>
<tr>
<th>RP</th>
<th>T2</th>
<th>T40</th>
<th>%Δ</th>
</tr>
</thead>
<tbody>
<tr>
<td>20 cmH2O</td>
<td>51.9 ± 5.4*</td>
<td>63.1 ± 9.0*</td>
<td>20.9 ± 4.6</td>
</tr>
<tr>
<td>30 cmH2O</td>
<td>78.3 ± 7.4</td>
<td>89.0 ± 5.5</td>
<td>12.0 ± 2.8</td>
</tr>
<tr>
<td>40 cmH2O</td>
<td>84.6 ± 5.1</td>
<td>99.8 ± 0.2</td>
<td>15.0 ± 5.1</td>
</tr>
</tbody>
</table>

Values are means ± SE. T40, 40 s after application of RP; %Δ, the percent increase in alveolar recruitment from T2 to T40. *Significant difference compared with other pressures (P < 0.05).

Table 4. Percent gross lung recruitment in the first 2 s

<table>
<thead>
<tr>
<th>RP</th>
<th>T0</th>
<th>T2</th>
<th>%Δ</th>
</tr>
</thead>
<tbody>
<tr>
<td>20 cmH2O</td>
<td>26.0 ± 9.9</td>
<td>35.8 ± 10.2†</td>
<td>9.8 ± 3.2*</td>
</tr>
<tr>
<td>30 cmH2O</td>
<td>21.7 ± 9.0</td>
<td>56.3 ± 10.2</td>
<td>34.6 ± 6.7</td>
</tr>
<tr>
<td>40 cmH2O</td>
<td>20.2 ± 9.1</td>
<td>77.0 ± 6.8</td>
<td>57.7 ± 8.6</td>
</tr>
</tbody>
</table>

Values are means ± SE. *Significant difference compared with other pressures (P < 0.05). †Significant difference compared with 40 cmH2O (P < 0.05).
Table 5. Percent gross lung recruitment from 2 to 40 s

<table>
<thead>
<tr>
<th>RP</th>
<th>T2</th>
<th>T40</th>
<th>%Δ</th>
</tr>
</thead>
<tbody>
<tr>
<td>20 cmH2O</td>
<td>35.8±10.2*</td>
<td>38.4±11.4*</td>
<td>2.7±1.3</td>
</tr>
<tr>
<td>30 cmH2O</td>
<td>56.3±10.2</td>
<td>63.5±12.5</td>
<td>7.2±2.7</td>
</tr>
<tr>
<td>40 cmH2O</td>
<td>77.6±6.8</td>
<td>94.9±3.7</td>
<td>17.4±6.3</td>
</tr>
</tbody>
</table>

Values are means ± SE. *Significant difference compared with 40 cmH2O (P < 0.05).

DISCUSSION

The data collected in the present study both by in situ microscopy and from gross pleural measurements of reversing atelectasis show that the amount of recruited lung depends both on the magnitude of the recruitment pressure and on the length of time over which this pressure is applied. This is demonstrated by the increased recruitment that occurred as the inflation pressure of the lungs was progressively increased from 5 cmH2O to 20, 30, and 40 cmH2O, and also by the temporal increases in recruitment that occurred out to 40 s after each step change in pressure, as shown in Fig. 2, A and B. With suitable choices for the distributions A(P) and B(s), this behavior is also predicted by a mathematical model of recruitment that incorporates pressure and time (6) as demonstrated in Fig. 2C.

There are probably several different mechanisms that can give rise to a time dependence of recruitment and derecruitment, but the best studied is the formation of liquid bridges across the lumen of small airways. It has been shown in vitro (35) that a compliant conduit lined with fluid will eventually become occluded by a liquid bridge when the pressure applied to it falls below a certain value. Closure does not take place immediately, however, because it takes time for the fluid to flow into the bridge (36). Similarly, when the pressure exceeds a certain pressure, the airway will eventually re-open, but this is proposed to take time as the fluid occluding the lumen is pushed axially and redistributed back along the walls of the conduit (19).

Nevertheless, the precise mechanisms by which recruitment takes place in the lung remain controversial. Indeed, it is still not entirely clear how changes in lung volume occur at the alveolar level during inflation. The two principal competing hypotheses are 1) expansion of individual alveoli and alveolar ducts and 2) increases in the number of open alveoli and alveolar ducts (i.e., R/D). There is evidence supporting both hypotheses (10, 18). Some investigators believe that alveolar R/D is the main mechanism of normal lung volume changes during tidal ventilation (8, 11, 42). In a slightly refined description of this model, Namati et al. demonstrated the recruitment of “daughter” alveoli once a threshold airway pressure is achieved in the feeding primary “mother” alveolus (32). Alternatively, others argue that the majority of volume change during tidal inflation is accommodated by changes in the size of the alveolar mouth (26), but not all experts accept this notion (25).

The R/D of an alveolus is generally believed to be governed by its critical opening and closing pressures (13, 32, 38). In particular, it is commonly postulated that, when the critical opening pressure of an alveolus, alveolar duct, or small airway is reached, it instantly “pops” open (2) and then continues to expand until maximally inflated. As a result, some mathematical models have focused exclusively on transmural pressure as being the sole controlling variable (23, 45). In the model by Suki et al., for example, airways pop open instantaneously once they reach their critical opening pressures, leading to avalanches of distal airway openings (46). Data from the present study, however, suggest that more precise modeling of alveolar R/D must incorporate both pressure and time. Accordingly, alveolar R/D in the present model is not instantaneous but rather occurs over a time scale that may range from seconds to minutes (6).

The alveolar opening measurements in our animal model of saline lavage show that the majority of recruitment, both microscopic and macroscopic, occurred within the first 2 s following application of all three RPs. Nevertheless, recruitment continued up to 40 s at all RPs, demonstrating the existence of a range of airspace opening rates. These data support the hypothesis of Bates and Irvin that recruitment is not instantaneous but rather has a time scale that may extend over many seconds (6). It is not intuitively obvious what the functional form for the distribution of opening velocities, $B(s)$, should be from inspection of these data. However, in their original modeling study, Bates and Irvin (6) found that a hyperbolic distribution for $s_p$ gave model predictions reminiscent of data in the literature. In the present study, we employed a decaying exponential function for $B(s)$ because it has the same monotonically decreasing characteristic as the hyperbolic function but has the advantage that it can be scaled to have an area of unity (Eq. 7) and therefore conveniently serves as an analytic expression for a probability distribution function.

The notion that the lung has a distribution of regional opening pressures is not entirely novel. However, whereas other studies have suggested that this distribution is Gaussian (13, 38), the cumulative distribution of opening pressures in the present study appears to be linear. Of course there is considerable scatter in the data shown in Fig. 3, so we cannot discount the possibility that the cumulative distribution is not exactly linear. However, it is clear from Fig. 3 that the data are certainly not described by a cumulative Gaussian. Also, when...
a Gaussian distribution of opening pressures was used in the
model, we were able to simulate recruitment-time curves that
resembled the data as closely as those in Fig. 2C. This implies
that the distribution of opening pressures, \( A(P) \), was close to
being uniform over a wide range of pressure for the rat model
of lavage injury we investigated. However, it is entirely plau-
sible that other opening pressure distributions such as the
Gaussian may be more appropriate for other different kinds of
lung injury or in other species.

The similarity between the micro- and macro-recruitment
during delivery of the RP-40 is striking (Fig. 2, A and B),
suggesting that near maximal recruitment is achieved at this
pressure. The atelectatic patches seen grossly on the pleural
surface tended to disappear suddenly following lung inflation
(see movie 2 of supplemental material, available online at the
Journal of Applied Physiology’s website), likely corresponding
to the opening of the rather large airways that supply these
segments. These findings support the avalanche theory of
alveolar recruitment (45). When a gross lung unit “pops” open,
we can microscopically detect a large percentage of alveolar
recruitment (Fig. 1). Since almost 80% of both gross and
microscopic achievable recruitment occurs within 2 s during
the RP-40 maneuver (77.6 and 84.6%, respectively), it is likely
that our microscopic field was in a location that grossly
recruited. This suggests that, as the gross lung goes from
atelectatic (dark red) to inflated (pink), ~80% of achievable
alveolar recruitment occurs briskly yet still requires almost 40 s
to achieve near maximal recruitment. Correspondingly, other
investigators have found that up to 86% of gross lung recruit-
ment occurs as early as within 0.5 s at 50 cmH2O of continuous
pressure (28). On the other hand, only 35.8% gross recruit-
ment and 51.9% microscopic recruitment occurred within 2 s when
applying 20 cmH2O. During the RP-20, we observed a gradual
increase in alveolar recruitment over time that never ap-
proached 80% achievable recruitment.

The greater discordance between total gross and microscopic
recruitment observed at lower recruiting pressures is likely due
to suboptimal resolution of the gross ex vivo lung assessment
technique when recruitment is submaximal. This suggests
that there is a component of microscopic alveolar recruit-
ment occurring in the atelectatic dark red segments of lung
before we can detect gross lung recruitment manifesting in
an abrupt change from red to pink lung (see movie 2 of
supplemental material, available online at the Journal of
Applied Physiology’s website). Furthermore, in the areas
that do grossly recruit, there appears to be continued micro-
scopic recruitment over at least 40 s. Interestingly, regard-
less of the recruiting pressures applied, roughly 80–90% of
the total achievable recruitment by 40 s was typically
achieved within the first 2 s, at both the gross and the micro-
scopic level. This suggests that, despite differences in absolute
values for gross and microscopic recruitment, both processes
are governed by the same physiological dynamics.

When the majority of recruitment occurs within the first 2 s
depth of inflation, one also needs to consider whether such a
rapid recruitment process generates increased shearing forces
that could promote additional lung injury. However, Gadiali
et al. demonstrated in their in vitro model that shear forces
generated by rapid bubble propagation down an artificial air-
way lead to negligible epithelial injury compared with the
vertical strain imposed on the same cells when bubbles are
propagating more slowly, the latter phenomenon mimicking
slower airway recruitment (47). Furthermore, others have dem-
onstrated that, when such aggressive deep inflation is delivered
infrequently, it generates negligible biotrauma compared with
when it is delivered with every breath (3), possibly due to
plasticity of cell membranes and a critical period of cellular
recovery time (16).

The clinical relevance of these findings ultimately depends
on the application of our improved understanding of alveolar
recruitment dynamics to the design of maneuvers that maxi-
mize alveolar opening while minimizing mechanical stress (3).
Currently, there is no clinical consensus as to the best recruit-
ment strategy. The most commonly used strategy is the sus-
tained application of 40 cmH2O over 40 s (4), a technique
similar to the most effective strategy employed in the present
study. Clinical trials using this recruitment protocol have
yielded mixed results (4, 9, 17, 21, 31). Grasso et al. used a
recruitment maneuver of 40 cmH2O for 40 s with low tidal
volumes and found that only patients with early ARDS re-
sponded favorably (21). Subsequent clinical studies have cor-
robated the finding that significant variation in response to
recruitment exists within the general ARDS population (9, 17).
The most recent large randomized clinical trial using a com-
bination of low tidal volumes, combined with high PEEP and
sustained recruitment maneuvers, failed to demonstrate a sig-
ificant impact on all-cause mortality (31). However, the group
receiving this intervention did demonstrate a reduction in
refractory hypoxemia and use of rescue therapies (31). Data
from the present study suggest that, at least in our model, a
40-cmH2O maneuver over 40 s does indeed open most of the
lung, whereas 20 cmH2O for 40 s does not.

Sustained pressure is thus clearly important in achieving
maximal recruitment, but the clinical efficacy of this practice is
unclear when the majority of recruitment occurs within the first
2 s of pressure application. It is not clear from our data that
such a significant amount of recruitment would be achieved
during the first few seconds of recruitment in all forms of
injury, but previous studies do suggest this to be the case in
other models as well (33). By contrast, the effect of sustained
recruitment pressures on the dynamics of alveolar and small
airway closure (derecruitment) is largely unknown. Although
nearly 80% of maximally achievable recruitment occurs within
the first 2 s of applied pressure in our model, the effect of 38
additional seconds of sustained pressure on subsequent dere-
cruitment during the next passive exhalation is unclear. It has
been shown in vitro (35) that a compliant conduit lined with
fluid is eventually occluded by a liquid bridge across the lumen
when applied pressure falls below a certain value. This process
does not take place immediately since it takes time for the fluid
to flow into the bridge. In theory, a sustained increase in airway
pressure during recruitment could change the distribution of
airway surface lining fluid or the distribution of surfactant at
the air liquid interface such that subsequent liquid cross-bridge
formation during deflation is retarded (12). Future studies need
to focus on the recruitment (and antiderecruitment) potential
of applying less elevated pressures over longer periods of time,
as in high-frequency oscillatory and airway pressure-release
modes of ventilation or delivering higher but more cyclic
recruiting pressure in conjunction with higher PEEP (7).

Caveats of the methods. Only subpleural alveoli can be
measured with our in vivo microscopic technique since our
depth of field is limited to 70 μm, limiting analysis of dynamic alveolar mechanics to two dimensions. Subpleural alveolar mechanics might differ mechanically from those within the lung parenchyma since there is less structural support of subpleural alveoli. That is, subpleural alveoli are not surrounded on all sides by adjacent alveoli (i.e., one wall of a subpleural alveolus is attached to the visceral pleura rather than another alveolus), and thus these alveoli lose a degree of structural interdependence. Reduced interdependent support may cause subpleural alveoli to recruit at different pressures and with different time constants than those within the lung (30). Mead et al., however, were able to demonstrate using a lung model that the subpleural and internal alveoli are subjected to equal pressures (30). In addition to this, Gil et al. showed that the alveolar surface area-volume relation was the same between subpleural and parenchymal alveoli (20). Both of these studies support our belief that alveolar mechanics are similar between subpleural and deep alveoli and that subpleural alveoli can be used to accurately represent the mechanics of all alveoli.

Another issue is that the suction used to stabilize the alveoli under the cover slip might alter alveolar mechanics. However, we have shown that this suction only slightly increases both alveolar size and stability; alveolar size changed from expiration to inspiration and was 1.1% with the suction and increased 8.3% without suction (34). We acknowledge that our assessment of gross lung recruitment is somewhat crude, but it is nevertheless practical for helping to corroborate our findings at the microscopic level. Assessing the change in visible lung surface color from dark red (collapsed) to pink (open) is by no means a sophisticated way of measuring lung recruitment and yields only inferential evidence for recruitment throughout the lung. Nevertheless, our technique of filming the gross visible lung surface yielded corroborative visual insight into the temporal mechanism of lung recruitment, as various segments of lung were observed popping open throughout the 40-s recruitment maneuver (see movie 2 of supplemental material, available online at the Journal of Applied Physiology’s website).

Finally, alveoli may behave in a mechanically different manner when the chest is open compared with when the lung is enclosed within an intact chest wall. This could potentially affect the assessment of both micro- and macro-lung recruitment. Furthermore, the tethering effects of blood flow through neighboring alveolar capillaries on alveolar structure and function are not specifically addressed with our technique. In any case, we must accept these technical limitations since we currently have no way of performing videomicroscopy of the pleural surface with the chest wall in place.

In conclusion, this study provides direct evidence of the temporal behavior of alveolar recruitment in an animal model of ARDS. The speed, timing, and effectiveness of recruitment all depend on the magnitude of the recruitment pressure and the length of time that this pressure is applied. Mathematical modeling of our data suggests that the temporal dependence of recruitment in our model of injury is best described in terms of a linear distribution of critical opening pressures and an exponential distribution of virtual trajectory velocities for airspace opening. We believe that our findings further our understanding of the dynamic physiology of alveolar recruitment, which could influence the design and delivery of optimal recruitment maneuvers in the setting of ARDS. This will help to improve lung function while minimizing mechanical stress and potential for VILI.

ACKNOWLEDGMENTS

The authors thank Kathleen Snyder for dedicated assistance with surgical procedures and experimental setup.

GRANTS

This study was funded in part by National Heart, Lung, and Blood Institute Grant R01 HL-075593.

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