Transient mechanical benefits of a deep inflation in the injured mouse lung

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Received 28 May 2002; accepted in final form 24 June 2002

Allen, Gilman, Lennart K. A. Lundblad, Polly Parsons, and Jason H. T. Bates. Transient mechanical benefits of a deep inflation in the injured mouse lung. J Appl Physiol 93: 1709–1715, 2002.—The lasting effects of a recruitment maneuver (RM) in the injured lung are not well characterized. We speculated that the reduction in respiratory elastance (H) after a deep inflation (DI) is transient in nature and should be sustained longer at higher positive end-expiratory pressure (PEEP). Thirteen ventilated mice were given 2 DIs at various levels of PEEP before and after saline lavage. Forced oscillations were used to measure H periodically over 7 min after the DIs. Time constants (τ) were estimated for the post-DI recovery in H. Values for τ before lavage (80–115 s) were reduced after lavage (13–30 s) at all levels of PEEP (P ≤ 0.0001). PEEP did not significantly influence τ before or after lavage. The plateau level and total recovery in H after a DI were significantly influenced by PEEP and lavage (P < 0.0001). Our results suggest that for a DI to be beneficial in the injured mouse lung, it may have to be applied several times a minute.

Physicians have known for a long time that atelectasis plays a pivotal role in the development of hypoxemia during mechanical ventilation, both in general anesthesia for routine surgery and in various instances of acute lung injury. Recent practice has now moved toward the use of lower tidal volumes (VT) in patients with acute lung injury and acute respiratory distress syndrome (ARDS) after two important studies demonstrated lower mortality outcomes by using this strategy (1, 1a). However, because of the progressive alveolar derecruitment that is often encountered with the use of lower VT (27), there has been a renewed interest in the use of recruitment maneuvers (RMs) as a means of reopening these regions of atelectasis. Many studies have demonstrated improvement in oxygenation, respiratory system compliance, and even lung volume after sustained or deep inflations (DIs) in many different scenarios (3–5, 17, 18, 26, 28), but some of these same studies were unable to show any lasting improvement when RMs were delivered during conventional positive pressure ventilation (4, 18, 25).

These apparently conflicting results may be explained by the different experimental situations involved, as the effect of a RM is likely to depend on the size and duration of the inflation, the mode of ventilation in which the inflation is delivered, and the level of positive end-expiratory pressure (PEEP) employed. Furthermore, if the effects of a RM are transient, then its apparent benefits will depend on when they are measured. Different researchers have measured the effects of a RM at different time points after its administration (3, 4, 17, 26, 28), yet there has been little attempt to characterize the rate at which the benefits of a RM are lost in the period immediately after its delivery.

The goal of our study, therefore, was to characterize the effects of a DI on lung mechanics during conventional mechanical ventilation in a mouse model of lung injury. We followed a measure of respiratory elastance (H) as an index of ongoing derecruitment after DI, both at baseline and after saline lavage at various levels of PEEP. We hypothesized that elastance would be decreased transiently by DI and that the rate and magnitude of subsequent recovery to its resting level would be increased in lung injury. A secondary hypothesis was that PEEP would retard the rate of recovery in elastance after DI. We also speculated that this influence of PEEP on the recovery of elastance would be more significant after lavage because an injured lung will be more prone to atelectasis.

METHODS

Animal preparation. We studied thirteen 8- to 9-wk-old BALB/c female mice (Jackson Laboratories, Bar Harbor, ME) weighing 19.8 ± 0.8 g. Each mouse was anesthetized with pentobarbital sodium via intraperitoneal injection at a dose of 90 mg/kg and then underwent tracheostomy with a secured 18-gauge metal cannula, and then connected to a flexiVent (SCIREC, Montreal, Canada) computer-controlled...
small animal ventilator. The mice were ventilated in a quasi-
sinusoidal fashion at a rate of 200 breaths/min. Cylinder
piston displacement was set at 0.25 ml, which resulted in a
Vt of 0.20 ml (~10 ml/kg) when gas compression was ac-
counted for. PEEP was controlled by submerging the expira-
tory limb from the ventilator into a water trap. The animals
were allowed 5 min to adjust to the ventilator at a PEEP of 3
cmH2O and were then paralyzed with an intraperitoneal
injection of pancuronium bromide (0.5 ml/kg). To ensure
adequate anesthesia, heart rate was monitored via continu-
ous pulse plethysmography that was measured transcutane-
ously across the femoral crease. Halfway through the proto-
col, an additional dose of pentobarbital sodium (30 mg/kg)
was administered for maintenance of deep anesthesia.

Experimental protocol. After the initial stabilization pe-
riod, the level of PEEP was set at 1 cmH2O, and two DIs were
delivered at constant flow with a pressure limit of 25 cmH2O.
Each DI lasted 2 s. The mouse was then returned to quasi-
sinusoidal ventilation at 200 breaths/min with a Vt of 0.20
ml. Respiratory system input impedance (Zrs) was measured
via a forced-oscillation technique (described in Data Analysis
below) 4 s after the two DIs, then subsequently every 15 s for
5 min, and then every 30 s for an additional 2 min. The entire
protocol was timed by a computer and repeated at a PEEP of
3 and 6 cmH2O. The order of PEEP was not randomized to
minimize the possible damaging effects of the higher PEEP
levels on measurements at lower PEEP. After completion of
the protocol at each of the three different levels of PEEP, the
mouse was disconnected from the ventilator, and 0.5 ml of
PBS was instilled via the tracheal cannula and slowly suc-
tioned back for a return of ~0.3 ml in every animal. The
mouse was then allowed 5 min to restabilize on the ventila-
tor, and the same protocol was repeated at a PEEP level of 5,
1, and 6 cmH2O. Again, PEEP levels were not randomized,
and this time a PEEP of 3 cmH2O was used first because the
animals tended to do poorly at 1 cmH2O.

Data analysis. Zrs was determined by measuring piston
volume displacement and pressure in the ventilator cylinder
while delivering 2-s oscillatory volume perturbations to the
airway opening. These perturbations were composed of 13
superimposed sine waves of varying amplitude and fre-
quency, ranging from 1 to 20.5 Hz. The frequencies were set
at mutually primed values to reduce harmonic distortion that
can occur in nonlinear systems (10). Before beginning the
protocol, we obtained dynamic calibration signals necessary
to correct for the physical characteristics of the flexiVent
in subsequent measurements of Zrs (13, 32). Zrs itself was de-
determined via Fourier transformation of the signals of ventilator
piston volume and cylinder pressure as described previously (9,
13). Zrs was interpreted by being fit with the model

\[ Z_{rs} = \frac{G - iH}{(2\pi f)^\alpha} \]  

where

\[ \alpha = \frac{2}{\pi} \arctan \left( \frac{H}{G} \right) \]  

The parameters Raw and Iaw largely characterize the resistive and inertive properties, respectively, of the airways,
whereas G characterizes the dissipative properties of the lung tissues (10). The symbol f represents frequency, and i is
the square root of -1. In the present study, we focus our attention on H, which is essentially the conventional elas-
tance of the respiratory system. (Indeed, it is precisely equal
to H at an oscillation frequency of 1/2π Hz.) We thus obtained
a set of H values vs. time for 7 min after the DIs in each
mouse at each level of PEEP. Each data set began immedi-
ately after the two DIs at the lowest value for H and then
increased toward a plateau as time progressed. Under base-
line conditions, the data for H over time could be satisfac-
tory fitted by a first-order exponential function, which gave
the time constant (τ). After saline lavage, the curves for H
over time could not be well fit to an exponential function, and
an effective τ was determined as the time needed for H to
achieve 67% of its total excursion over the 7-min measure-
ment period. Values for τ were also calculated from the
combined data at each level of PEEP, both before and after
lavage. All statistical analyses were performed by using SAS
statistical analysis software (ver. 8.1, SAS Institute, Cary,
NC). Data sets were subjected to natural logarithmic trans-
formation before comparing differences between pre-
and postlavage conditions and different levels of PEEP. Repea-
ted-measures ANOVA was used to examine the within-subject
effects of PEEP and lavage on the values for τ, baseline and
plateau values for H, and a total recovery in H after the DI.
Differences were considered significant at a P value of <0.05.
This was followed by Tukey’s procedure to compare means at
each level of PEEP, before and after lavage, to determine
between-condition differences.

RESULTS

After lavage, three mice died and three others began to
spontaneously breathe or twitch, most often when
placed on PEEP of 1 cmH2O. This made their data
uninterpretable and presumably occurred because the
amount of lung collapse was greatest at a PEEP of 1
cmH2O. This left interpretable data for n = 13 mice at
every level of PEEP before lavage and n = 12, 7, and 10
at PEEPs of 3, 1, and 6 cmH2O, respectively, after
lavage. Grouped values for H vs. time after two DIs for
all interpretable data sets, both before and after la-
vage, are shown in Figs. 1 and 2. Values of τ for each
averaged data set are listed in Figs. 1 and 2 and show
that elastance approached its plateau value substan-
tially more quickly after saline lavage compared with
control conditions.

To avoid bias during statistical analysis, data from a
mouse were discarded unless the mouse had a com-

Fig. 1. Mean elastance (H ± SE) over time at various levels of
positive end-expiratory pressure (PEEP) before saline lavage. Values
for the time constant (τ) for each of the compiled data at each level of PEEP
are shown above the respective curves. DI, deep inflation.
complete set of interpretable data at each level of PEEP, both before and after lavage. Furthermore, analysis of the remaining seven mice showed one animal with a value for $\tau$ at a PEEP of 3 cmH$_2$O that was well beyond 2 standard deviations from the mean. We suspect this may have been due to spontaneous movement that we had not been previously aware of, so data from this animal were omitted, as well, leaving a final $n$ value of 6 mice. This significantly reduced variation in $\tau$ at a PEEP of 3 cmH$_2$O after lavage but did not change the overall results or conclusions from analysis using the initial $n = 7$ mice.

Saline lavage exhibited a significant effect on the values for $\tau$ overall ($P = 0.0001$), but no similar effect was demonstrated by the level of applied PEEP ($P = 0.16$). When data from individual animals were compared, no significant differences in $\tau$ were observed between any two levels of PEEP before or after lavage (Fig. 3). Baseline level of $H$ was significantly increased by saline lavage ($P = 0.0003$) and significantly reduced by higher PEEP ($P < 0.0001$). When mean baseline levels of $H$ from each level of PEEP were compared, the only nonsignificant difference was between a PEEP of 1 and 3 cmH$_2$O before lavage ($P = 0.31$; Fig. 4). The plateau level of $H$ was also significantly increased by lavage and reduced by higher PEEP ($P < 0.0001$). When separate levels of PEEP were compared, the only nonsignificant difference was again found between a PEEP of 1 and 3 cmH$_2$O before lavage ($P = 0.26$; Fig. 5). Total recovery in $H$ (difference between plateau and baseline) was significantly increased by lavage and reduced by higher PEEP ($P < 0.0001$). However, when separate levels of PEEP were compared, the total recovery in $H$ was only significantly reduced by a PEEP of 6 cmH$_2$O, both at baseline (PEEP 6 vs. 1 cmH$_2$O, $P < 0.05$) and after lavage (PEEP 6 vs. 1 and 3 cmH$_2$O, $P < 0.0001$) (Fig. 6).

**DISCUSSION**

Recent clinical trials showing a mortality benefit from low VT ventilation (1a, 2) have refocused investi-
Nonsustained DIs in our study did reduce $H$, albeit significantly less than $H$ produced by sustained DIs (26), as seen in lung injury models (21, 29–31). Nevertheless, nonsustained DIs have been shown before to provide benefits in acute injury models (1a, 29–31). However, this effect is probably impractical and may even be injurious.

Of course, the above considerations only apply to the brief DIs that we delivered to our mice. There is evidence that the majority of recruitment can occur as early as 1–2 s into an inhalation during volume-controlled ventilation of an injured lung (24). However, it is also possible that a more sustained DI may have produced a greater effect on $H$. Traditionally in human subjects, DIs are pressure limited at a range between 30 and 40 cmH$_2$O and, if sustained, are held for 15–40 s (1a, 29–31). Nevertheless, nonsustained DIs have been shown before to provide benefit (26), and the nonsustained DIs in our study did reduce $H$, albeit transiently, even after a lavage.

PEEP also has the potential to modulate the effect of a DI by preventing at least some parts of the lung from ever falling below their critical closing pressures (21, 28). The role of the RM is then to reach the higher degree of open lung would be achieved in the injured lung by delivering a DI every 10–30 s, depending on the level of PEEP. Such a high frequency of DI delivery is probably impractical and may even be injurious.

The transience of the improvement in $H$ derived from a DI in this study suggests that for any significant effect to be achieved through RMs, DI would need to be delivered frequently to maintain a significant fraction of the initial degree of open lung that existed immediately after its delivery. We can explore this issue quantitatively as follows. $H$ is a measure of stiffness of the respiratory system; so at a given level of PEEP, we assume that it is inversely proportional to the amount of open lung. Because $H$ is a function of time, we can describe the time course of the amount of open lung ($\bar{O}$) relative to that immediately after the DI at time $t = 0$ as

$$\bar{O}(t) = \frac{H(0)}{H(t)}$$

(3)

where $t$ is time. If we give a DI every $T$ seconds, then $\bar{O}(t)$ is a periodic function with each period beginning at a value of 1 and reaching $H(0)/H(T)$ at time $T$. The initial value of 1 is a relative value describing the maximum amount of open lung achieved after a DI at a given level of PEEP. The mean value of $\bar{O}(t)$, when DIs are given every $T$ seconds, is equal to its mean value over a single period of $T$ seconds, which is

$$\bar{O}(T) = \frac{1}{T} \int_0^T \frac{H(0)}{H(t)} \, dt$$

(4)

Figure 7 plots $\bar{O}(T)$ vs. $T$ for both the control and saline-lavaged mice at each level of PEEP calculated by using the numerical integral of the averaged data for $H$ over time (Figs. 1 and 2) in Eq. 4. Figure 7 shows, for example, that to maintain a normal lung at an average of 90% of its maximal open state, a DI would have to be given about every 300 s. Furthermore, these data indicate that even if no DIs are given, the normal lung would remain in a predominantly open state at all levels of PEEP. This suggests that the DI may be of minimal clinical benefit in the normal lung. Furthermore, the DI may not need to be frequently delivered in a normal lung, a notion that is supported by the general anesthesia literature (31). In contrast, the same degree of open lung would be achieved in the injured lung by delivering a DI every 10–30 s, depending on the level of PEEP. Such a high frequency of DI delivery is probably impractical and may even be injurious.
critical opening pressures for segments not opened during normal tidal inflations (6, 23). This means that the amount of recruitable lung decreases with increasing PEEP (5, 17, 27). This was clearly the case in the present study after lung injury because we found that increasing PEEP markedly reduced baseline \( H \) (Fig. 4), its plateau level (Fig. 5), and particularly its total recovery (Fig. 6). This effect of PEEP achieved greater statistical significance after saline lavage (Figs. 4–6), implying that PEEP has more measurable effects when the lung is injured and prone to collapse. We initially hypothesized that increasing PEEP would slow down the closure process in general, thereby lengthening the benefits of a DI. Interestingly, this was not the case, either at baseline or after lavage (Fig. 3). This finding contrasts with that of a much earlier study in lambs and rabbits (35), although a more recent investigation using computer tomography scanning in injured pig lungs (24) found no significant differences between values for \( \tau \) at different levels of PEEP. However, we are not able to conclude that closure in the lung is a pressure-independent process just because \( \tau \) does not depend on PEEP. Because total recovery in \( H \) that follows a DI is smaller at higher levels of PEEP (Fig. 6), it could be that this phenomenon, when combined with lengthened closure times, results in a value for \( \tau \) that is relatively unchanged by increasing PEEP.

If closure in the lung is as strongly time dependent as our results indicate, it is also likely that the benefit derived from a DI will depend on the volume history of the lung before the point when the DI is given. This suggests that the effects of a DI may depend to some extent on the mode of ventilation being utilized. Indeed, studies have demonstrated reductions in lung compliance and increased lung volumes after RMs delivered during high-frequency ventilation (3, 4, 18) but not during conventional positive pressure ventilation (4, 18). In addition, sustained DIs have been shown to result in greater recruited lung volumes when using low \( V_T \) or low levels of PEEP but not with high \( V_T \) or high PEEP (17). Nevertheless, despite using conventional positive pressure ventilation with a moderate \( V_T \) in our study, we were still able to demonstrate an improvement in \( H \) after a DI both at baseline and after lavage. Our positive observations were perhaps due in part to the fact that we made frequent measurements of mechanics with a very sensitive technique. Had we measured \( H \) less frequently, beginning some minutes after DI, we might have missed observing any effect. For example, Pelosi et al. (26) were able to demonstrate immediate improvement in end-expiratory lung volume and elastance in ARDS patients during a frequent-sigh period of mechanical ventilation. However, their earliest follow-up measurements were made 30 min after sigh interruption, at which time these effects were lost. In another study involving saline-lavaged dogs, improvement in the arterial partial pressure of oxygen seen after a sustained DI was maintained at 15 min, but only in those animals ventilated with the lowest \( V_T \) and PEEP (17).

This raises the point of how to define optimal PEEP. Conventionally, PEEP is chosen as the pressure at or just above the lower inflection point (LIP) of the inspiratory limb of the quasi-static pressure-volume curve (1a, 26, 27). LIP is generally thought to correspond to the major recruitment events occurring during inflation. However, several studies have shown evidence of recruitment beyond LIP (21, 23, 27). The concept of ongoing recruitment throughout lung inflation has also been supported by mathematical models (12), human studies utilizing computer tomography of the chest (8), and animal models (33). Thus controversy already exists over how recruitment is best served by PEEP. The results of the present study raise another issue that complicates the question of optimal PEEP even further. That is, if recruitment depends on time, then the position of LIP during a quasi-static stepwise inflation of the lung may have little relevance for the recruitment that takes place during a normal, mechanically ventilated breath. This means that optimizing PEEP may need to be based on more than a static view of the lung and require a consideration of the dynamics of opening and closing of air spaces.

Our study thus makes the case for recruitment and derecruitment in the lung being dynamic phenomena. This clearly has implications for optimizing mechanical ventilation. However, there clearly are anatomic differences that limit extrapolation of these results directly to humans. For example, there is obviously a significant size difference between humans and mice. In humans, gravity has a significant effect on regional distribution of critical opening pressures and recruitment from PEEP (6). In the mouse, regional differences throughout the lung due to gravity are presumably negligible. It has also been suggested that smaller animals may have a more rapid rate of loss in compliance due to the smaller surface area of their alveoli and subsequent rapid change in the proportional air-space configuration (35). Indeed, previous human studies suggest that the benefits of sustained inflations can persist as long as 40 min in normal subjects under general anesthesia (31) but not without some degree of decay over time. Another possible limitation is that we were unable to randomize the order in which the different levels of PEEP were delivered in our study. This was because our mice frequently did not tolerate being placed on a PEEP of 1 cmH\(_2\)O immediately after lavage. The likelihood that order played a significant role was, in part, diminished by starting each protocol with two DIs and essentially beginning with a maximally inflated lung at each level of PEEP. Furthermore, in some mice, PEEP was returned from 6 to 3 cmH\(_2\)O at the end of the experiment (data not shown), and the resulting \( H \) vs. time data were similar to those obtained at the initial PEEP of 3 cmH\(_2\)O. Another possible limitation to our study is not having measured oxygenation. However, previous studies have demonstrated a significant correlation between RM-induced improvements in compliance and oxygenation (26, 34).

It should also be noted that, in our study, we experimented with only a single type of lung injury, whereas...
it has been shown that RMs have different effects in different types of lung injury (7, 17, 18, 26). Extrapulmonary forms of ARDS involve greater degrees of edema and alveolar collapse and are thus more amenable to recruitment than direct lung injury, which is characterized more by air-space consolidation (7, 26). We suspect that our model more resembles extrapulmonary ARDS since saline lavage is likely to predominantly produce surfactant depletion and atelectasis (19, 22). Other models of lung injury that more closely mimic pneumonia and consolidation may behave differently. Increases in end-expiratory lung volumes seen with higher PEEP in the latter scenario could potentially be less due to alveolar recruitment and more due to overdistension of the less consolidated and more compliant regions. This could potentially result in higher, instead of lower, H with increasing PEEP (7, 34). The lower values for H observed with increasing PEEP in our study argue more for improved alveolar recruitment than alveolar overdistension. If this protocol were to be repeated in a different model of lung injury, such as one more consistent with pneumonia, the findings could be significantly different. Furthermore, measuring lung volumes in both types of lung injury at various levels of PEEP could help to better clarify the question of recruitment vs. overdistension.

Another key point about our discussion thus far is that we have assumed that the transients we observed in H after the DIs were entirely due to progressive derecruitment of the lung. However, the mechanisms responsible for the long-term transients in H seen during mechanical ventilation remain controversial. For example, one possible explanation for the observed recovery in H over time under baseline conditions could be stress adaptation within the tissues (15). The difference in shape between curves derived under baseline conditions and those after lavage suggest possible differences in the mechanisms at play, with derecruitment likely playing a greater role in the injured lung. Because mechanical stretch is known to stimulate secretion of surfactant by type II alveolar cells (37) and may lead to more effective dispersion of existing surfactant, an alternative mechanism is the gradual loss of surfactant from the air-liquid interface. Williams et al. (35) ascribed slow changes in H entirely to this latter mechanism, whereas Horie and Hildebrandt (14) proposed that a combination of surfactant dynamics together with airway closure at low lung volumes could be responsible. With the recent interest in optimal ventilation of critically ill patients, the attention of the scientific community seems to have focused on recruitment/derecruitment as an important process in the lung (12, 16, 18, 21, 23, 27, 28), particularly to describe the greatly increased hysteresis of the quasi-static pressure-volume curve in lung injury (18, 28). Even so, Wilson et al. (36) have just proposed a model that ascribes the knee in the pressure-volume curve from edematous lungs to the nonlinear coupled behavior of the parenchyma and alveolar fluid, which does not invoke recruitment/derecruitment at all. The truth may well involve contributions from all these mechanisms. Nevertheless, after lung injury, the rate and magnitude of increase in H are so dramatic after a DI that it is hard to imagine that derecruitment is not the principal process involved. Certainly, lung volume is significantly reduced by lavage at any given inflation pressure, as our laboratory has recently shown (20) by using body-box plethysmography in saline-lavaged mice.

In summary, we have shown that DI in mice produces a transient decrease in H that returns rapidly toward its pre-DI value, with a rate of return that is greatly accelerated in lavage-induced lung injury. When we equate increases in H with decreases in the amount of open lung, we predict that DIs would have to be delivered several times per minute to keep an injured lung significantly more open than would be the case if no RMs were given at all. We conclude that higher levels of PEEP can significantly reduce the baseline, recovery, and final plateau of H after a DI, especially in an injured lung. However, added PEEP does not significantly reduce the rate of recovery in H after a DI. This means that PEEP does not reduce the frequency with which DIs need to be delivered in this model of lung injury.

Statistical analysis was performed by Dr. Janice Yanushka Bunn, Research Assistant Professor, Medical Biostatistics, University of Vermont.

This work was supported by AstraZeneca and National Institute of Health Grants RO1 HL-67273 and NCRR-COBRE P20 RR-15557-01.

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