Design of a new variable-ventilation method optimized for lung recruitment in mice

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Thammanomai A, Hueser LE, Majumdar A, Bartolá-Suki E, Suki B. Design of a new variable-ventilation method optimized for lung recruitment in mice. J Appl Physiol 104: 1329–1340, 2008. First published March 13, 2008; doi:10.1152/japplphysiol.01002.2007.—Variable ventilation (VV), characterized by breath-to-breath variation of tidal volume (VT) and breathing rate (f), has been shown to improve lung mechanics and blood oxygenation during acute lung injury in many species compared with conventional ventilation (CV), characterized by constant VT and f. During CV as well as VV, the lungs of mice tend to collapse over time; therefore, the goal of this study was to develop a new VV mode (VVN) with an optimized distribution of VT to maximize recruitment. Groups of normal and HCl-injured mice were subjected to 1 h of CV, original VV (VV0), CV with periodic large breaths (CV1Lb), and VVN, and the effects of ventilation modes on respiratory mechanics, airway pressure, blood oxygenation, and IL-1β were assessed. During CV and VV0, normal and injured mice showed regional lung collapse with increased airway pressures and poor oxygenation. CV1Lb and VVN resulted in a stable dynamic equilibrium with significantly improved respiratory mechanics and oxygenation. Nevertheless, VVN provided a consistently better physiological response. In injured mice, VV0 and VVN, but not CV1Lb, were able to reduce the IL-1β-related inflammatory response compared with CV. In conclusion, our results suggest that application of higher VT values than the single VT currently used in clinical situations helps stabilize lung function. In addition, variable stretch patterns delivered to the lung by VV can reduce the progression of lung injury due to ventilation in injured mice.

Acute respiratory distress syndrome; lung injury; elastance; gas exchange

ACUTE RESPIRATORY DISTRESS syndrome (ARDS), a severe form of acute lung injury (ALI), is a critical respiratory disease that results in high mortality rate (18). Despite almost 40 years of effort to improve the outcome of ARDS, the main supportive therapy remains mechanical ventilation, a method used to replace or assist spontaneous breathing. Paradoxically, although mechanical ventilation is essential for life support, nonphysiological mechanical forces are invariably present in the lung during prolonged mechanical ventilation and can lead to ventilator-induced lung injury (VILI) (47, 50). The physiological consequences of VILI include a decrease in lung compliance, impaired gas exchange, alveolar atelectasis, pulmonary edema, and surfactant dysfunction (48, 56). The commonly used standard protocol for patients with ARDS/ALI is “lung protective ventilation,” which was shown by the ARDS network (1) and Amato et al. (6) to significantly reduce mortality rate in patients with the application of a low (6–8 ml/kg) tidal volume (VT) accompanied by moderate positive end-expiratory pressure (PEEP) compared with high-VT (10–15 ml/kg) ventilation. However, the mortality rate in ARDS/ALI patients undergoing low-VT mechanical ventilation is still high, (30–40%) (17, 19). Furthermore, this approach remains controversial, since three other randomized trials have shown no beneficial effect of low-VT ventilation over the higher-VT ventilation (14, 15, 52). Additionally, application of low VT can also lead to respiratory acidosis, which could be a consequence of insufficient VT (26, 27). It is therefore important to seek alternative ventilation modes that can avoid the adverse consequences and improve patient care.

In 1996, Lefevre et al. (35) proposed an alternative approach to mechanical ventilation by introducing “biologic variability” of natural breathing to mechanical ventilation, whereby the breathing rate (f) and VT were varied on a cycle-by-cycle basis. They showed that incorporation of such variability into mechanical ventilation improved oxygenation and lung compliance in an oleic acid-induced porcine model of ALI. Subsequently, Suki et al. (53) proposed a mechanism to explain how addition of variability, which they called noise, to peak inspiratory pressure can improve arterial PO2 (Pao2). Several beneficial physiological effects of this “noisy ventilation,” which is also termed “variable ventilation” (VV), have since been shown to occur in healthy and ARDS/ALI models in pigs (11, 40, 41), dogs (42), guinea pigs (7, 8), sheep (10), and, more recently, normal human subjects (12). In addition to the beneficial effects on respiratory function, VV has also been demonstrated to favorably influence surfactant secretion in healthy guinea pigs (8).

The mouse has been a popular and often preferred species in medical research because of the relative ease with which various models of lung diseases can be generated. Indeed, mice have increasingly been used in respiratory research, including the lung injury model (2, 5). The chest wall of the mouse is relatively compliant (31, 51), resembling that of preterm or newborn infants (45). Consequently, the alveoli of the mouse lung, even the unjured lung, are prone to collapse during mechanical ventilation. It is thus conceivable that the mouse lung can easily develop VILI via atelectrauma.

Therefore, the purpose of the present study was to investigate the physiological effects of VV during short-term ventilation of healthy and injured mice. Our preliminary results suggested that the current form of VV used in larger animals (7, 8, 10) is unable to recruit the lung and keep it open. Hence, we developed a new VV (VVN) method and optimized its parameters to recruit the lung and keep it open in normal mice. The costs of publication of this article were defrayed in part by the payment of page charges. The article must therefore be hereby marked “advertisement” in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

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and mice with HCl-induced injury. We then tested the effects of VVN on mechanics, gas exchange, and IL-1β, an inflammatory cytokine known to accumulate in lung injury (21). The performance of VVN was systematically compared with that of the following ventilation modes: conventional ventilation (CV) with fixed VT and f, original VV (VV0) as developed by Arola et al. (8), and CV with added periodic large breaths (CVLsB) with amplitudes that matched the largest VT in VVN. The latter ventilation mode was included to test the hypothesis that the beneficial effects of VV are due to the variability in VT as characterized by its distribution, and not just by the presence of a few large breaths.

METHODS

Development of the VVN Method

In a pilot study, we found that to prevent the progressive collapse of alveoli during ventilation of normal mice with CV, we had to deliver unexpectedly large VT to sufficiently recruit the lung. Briefly, several mice were ventilated with CV at 8 ml/kg superimposed on 3 cmH2O PEEP. Respiratory mechanics were measured, and respiratory elastance increased 6% in the first 5 min. A deep inspiration (DI) always returned the lung to baseline, characterized by a low elastance, suggesting that the increase was due to gradual lung collapse (2, 3, 5, 31). For determination of the smallest large VT that could recruit the lung, the animals were ventilated with CV for 1 min and given a large (e.g., 16 ml/kg) test VT, and elastance was measured to assess recruitment. The size of the test VT was then progressively increased to several times the baseline VT. We found that the smallest large VT that could return elastance to within 10% of its baseline was three to four times the baseline VT of 8 ml/kg.

The distribution of VT in VV0 developed for guinea pigs (Fig. 1) was based on a uniform distribution of peak airway pressures, which was shown to result in an inverse power-law distribution of VT values over a relatively narrow range (8). Consequently, the VT distribution in VV0 may not be able to provide adequate recruitment and/or keep the recruited regions of the mouse lung open. Thus, in order for VV to recruit the lung, larger VT values would have to be incorporated in the VV by extension of the tail of the VT distribution. However, if the shape of the distribution, a single power law as in VV0, and the mean VT (VTmean) were maintained, stretching of the tail to high VT values would also require an extended low-VT region that would not be able to sustain adequate ventilation of the animal. Therefore, the distribution of VT needs to be redesigned by stretching the tail while limiting its minimum value.

On the basis of these considerations, we developed a new VT distribution with the following characteristics. For low values of VT, the distribution is uniform between the minimum VT (Vmin) and the peak VT (Vp) followed by a power-law decay up to a maximum value (Vmax). The corresponding probability density distribution function is written as

\[
p(V_T) = \begin{cases} 
  p_0 & \text{for } V_{\text{min}} \leq V_T \leq V_p \\
  p_0 \left(\frac{V_p}{V_T}\right)^{-\delta} & \text{for } V_p \leq V_T \leq V_{\text{max}}
\end{cases}
\]

where \(\delta\) is a parameter and \(p_0\) is the normalization constant. Using the shape defined by Eq. 1, one can conveniently control the minimum and the maximum, as well as the range, of "low"-VT values by adjustment of Vmin, Vp, and Vmax. The sequence of VT values from this distribution ventilates the animals mainly in the lower range of VT values while occasionally larger VT values are also delivered. The mean and standard deviation (SD) of the distribution are given by Eqs. 2 and 3.

\[
VT_{\text{mean}} = \frac{1}{2} \left( \frac{V_{\text{min}} + V_{\text{max}}}{2} - \delta \right) + \frac{\delta}{2 - \delta} \left( \frac{V_{\text{min}} + \delta}{2} - \frac{\delta^2}{2 - \delta} \right)
\]

\[
\text{SD}(VT) = \frac{1}{3} \left( \frac{V_{\text{min}} + 3 \delta}{3 - \delta} \right) - V_{\text{mean}}^2
\]

Four independent parameters of the density distribution in Eq. 1, Vmin, Vmax, Vp, and the exponent \(\delta\), determine the shape of the distribution, as well as VTmean and SD of the distribution. To compare the effects of the various ventilation protocols, we set VTmean to be the same as VT in the CV group chosen on the basis of the weight of the animal. Vmin was fixed at 0.7 VTmean to avoid too-low VT values during ventilation as well as to match Vmin in the VV0 distribution. On the basis of preliminary computer simulations, we found that in order for a substantial increase in Vmax and constant VTmean and Vmin, Vp and \(\delta\) were constrained within a small range. Thus we had to choose Vp = 0.9 VTmean, a value slightly smaller than VTmean, while \(\delta\) was set to 5.1, which resulted in a much faster decrease of p(VT) in VVN than in VV0 (Fig. 1). This latter feature of the VV0 distribution allowed us to achieve much higher values of Vmax than in VV0 while maintaining VTmean and Vmin. To determine the optimum Vmax, a group of mice was ventilated with VVN with several sequences of VT values with similar shape and identical mean but different Vmax.

Animal Preparation

Male C57BL/6 mice (22–26 g body wt; Charles River Laboratories, Wilmington, MA) were used throughout the studies. The protocol was approved by the Animal Care and Use Committee of Boston University. The mice were anesthetized with pentobarbital sodium (70 mg/kg ip), tracheostomized with an 18-gauge metal cannula, and placed on a heated pad to maintain a constant body temperature (37°C) throughout the experiment. Extra doses of pentobarbital sodium (20 mg/kg) were administered every 20 min to keep the animal in a deeply anesthetized state. The tracheal cannula was later connected to the outlet of a small
animal ventilator-oscillator system (flexiVent, SCIREQ, Montreal, PQ, Canada).

Acid Aspiration Lung Injury

A mouse model of lung injury was obtained by intratracheal induction of 0.1 M HCl (pH 1.25) in 1 μl/g increments, each separated with a bolus of air, for a total of 3 μl/g. Lung collapse was prevented by connecting the mice to the ventilator, immediate administration of two DIs, defined here as a ramp increase in volume to 1 ml in 4 s, and administration of two additional DIs at 5 and 10 min after treatment. The animals were then ventilated using a constant VT of 8 ml/kg for a stabilization period of 20 min at 3 cmH2O PEEP.

Optimization of the Parameters in VVN

As described by Eq. 1, the distribution of VT values in VVN consists of a flat region between Vmin and Vf following a power-law decay up to Vmax. A group of normal mice (n = 4) and a group of HCl-injured mice (n = 2) were ventilated with CV (VT = 8 ml/kg and f = 240 breaths/min) at 3 cmH2O PEEP for 15 min followed by a series of 15-min sessions of VVN with identical Vmin, Vf, δ, and VTmean but different Vmax. For the normal mice, the tested Vmax levels were 4, 3.75, 3.5, or 3.25 times VTmean of 8 ml/kg. For the injured mice, however, we found that it was sufficient to test lower Vmax levels, including 2.5, 2.25, 2, and 1.5 times VTmean. These sessions with different Vmax levels were randomized in order. After each set of trials, a DI was given to fully recruit the lung. Respiratory mechanics were measured at the beginning, before, and during (at 5, 10, and 15 min) each VVN session.

Comparison of the Effects of Ventilation Modes

Volume history was standardized by administration of two DIs to normal (n = 32) and injured (n = 32) mice. Mice were randomly selected for 60 min of CV, VVO, CVLB, or VVN (for each mode n = 8 each for normal and injured mice). PEEP was always set at 3 cmH2O. In a previous study, we found that the VT delivered by the flexiVent significantly depended on the load impedance (55). Hence, under all ventilation modes, Vf was monitored by the flexiVent and, when necessary, manually adjusted to ensure that the proper VT was delivered to the animal independent of its mechanical impedance.

CV mode. Mice were ventilated with constant VT at 8 ml/kg and f of 240 breaths/min.

VVO mode. VTmean was 8 ml/kg (SD 1.6), and f was adjusted on a cycle-by-cycle basis to obtain a minute ventilation equal to that during CV. This VVO was based on the original design by Arold et al. (8). An example of the corresponding VT sequence is shown in Fig. 2A.

CVLB mode. Mice were ventilated mostly with a VT of 8 ml/kg and a constant f of 240 breaths/min. Twice in every minute, the animals also received a large breath. These large breaths resulted in SD values of 1.6 and 0.8 ml/kg for normal and injured mice, respectively. The size and timing of the large breaths were matched with the two largest VT values of the VVN mode in 1 min. An example of the VT sequence is shown in Fig. 2B.

VVN mode. With use of Eq. 2, VTmean was fixed to be 8 ml/kg and f was adjusted on a cycle-by-cycle basis to obtain a minute ventilation of 240 breaths/min. The parameters were as follows: Vmin = 0.7 VTmean, Vf = 0.9 VTmean, and δ = 5.1. Vmax was set to 3.5 and 2.25 times VTmean for normal and injured mice, respectively, with the corresponding distributions shown in Fig. 1. An example of the VT sequence is shown in Fig. 2C. The SD values of VT were calculated from Eq. 3 (3 and 2 ml/kg for healthy and injured mice, respectively).

At the conclusion of the experiment, arterial blood from the carotid artery was collected and analyzed for arterial P02 (PaO2), PCO2 (PaCO2), blood pH, and percent O2 saturation with an I-STAT blood gas analyzer (Abbott Laboratories, Abbott Park, IL). A lavage sample was also collected from each mouse at the conclusion of the ventilation session by instillation of 1 ml of warm saline through the tracheal cannula and slow retrieval of ~0.9 ml. The lavage sample was then centrifuged, and the cell-free supernatant was frozen at −20°C until further analysis. After the animals were killed, their lungs were perfused, removed, and homogenized in 2 ml of PBS (pH 7.2) and centrifuged. The supernatant was transferred to microcentrifuge tubes and stored at −20°C.

A group of C57BL/6 mice (n = 4, 20–22 g body wt) were anesthetized with pentobarbital sodium (70 mg/kg ip), tracheostomized, and immediately lavaged as described above. This group served as an unventilated control group. Another group of C57BL/6 mice (n = 4, 20–22 g body wt) was treated with HCl as described above and ventilated using a constant VT of 8 ml/kg for an additional 20 min at 3 cmH2O PEEP. The
animals were lavaged immediately after completion of the injury induction steps. This group served as a baseline injury group without the additional 60-min ventilation session. The lavage samples and lung homogenate samples from both groups were centrifuged, and the supernatants were stored as described above.

**Impedance Measurements**

Respiratory mechanics were determined using the optimum ventilation waveform (OVW), which is a broadband waveform designed to estimate lung impedance via forced oscillations while simultaneously delivering tidal-like volume excursions (37). The energy in the OVW was distributed among five discrete frequencies from 2 to 31 Hz, which were selected to eliminate harmonic distortion and minimize cross talk according to a nonsum-nondifference principle (54). The phase angle for each frequency component was adjusted to minimize the peak-to-peak pressure of the oscillatory waveform. For each mouse, the peak-to-peak amplitude of the oscillatory waveform was adjusted to match the VT delivered during mechanical ventilation to minimize the disruption of ventilation during impedance measurements. Every 5 min, two complete 1-s cycles of OVWs were delivered. The piston displacement and pressure in the ventilator cylinder were recorded by the flexiVent, and the data were corrected for the effects of gas compression in the valves and the resistance of the cannula. Calculations were done offline using software developed in the laboratory. Fast Fourier transform was used to obtain the cross- and auto-power spectra of pressure and flow from overlapping data segments. The respiratory input impedance (Zrs) was calculated at each input frequency as the ratio of the cross-power spectrum of pressure and flow to the auto-power spectrum of flow.

**Modeling**

The Zrs spectra were fit by a model consisting of an airway compartment, characterized by the airway impedance (Zaw), and a tissue compartment, described by a tissue impedance (Zti), connected in series. Thus Zrs is calculated as Zaw + Zti. Zaw is further partitioned to airway resistance (Raw) and inertance (law)

\[
Zaw = Raw + jo_{aw}
\]

where \(o_{aw}\) is the circular frequency. Zti was modeled as a constant-phase impedance, which was introduced by Hantos et al. (24) and has been widely used to estimate Zti in animal models (22–24) and humans (33, 34). The constant-phase model characterizes Zti by a tissue damping coefficient \((G)\) and a tissue elastance coefficient \((H)\) as follows

\[
Zti(o_{aw}) = \frac{G - jH}{(o_{aw})^2}
\]

where \(o_{aw} = o/o_{0}\) is the normalized circular frequency with \(o_{0} = 1\) rad/s (13). The exponent \(alpha\) characterizes the frequency dependence of tissue resistance \([Rti = G(o_{aw})^\alpha]\) and tissue elastance \([Eti = H(o_{aw})^{\beta - 1}\)]. All parameters were estimated using a global optimization algorithm, which minimized the root-mean-squared (RMS) error between data and model (16).

**Airway Pressure Measurement**

A separate pressure transducer (World Precision Instruments, Sarasota, FL) attached to the tracheal cannula was used to monitor and record the airway pressure throughout the experiments. The mean peak airway pressure was calculated for every 5-min period.

**Western Blot Analysis**

To test whether the ventilation mode had an effect on lung injury, Western blot analysis was carried out for IL-1β as follows. Each lavage sample was mixed with the corresponding homogenized lung sample at a ratio of 1:1. The mixed samples for each lung were further diluted at a ratio of 1:5. Equal volumes from the mixed samples were processed by electrophoresis and Western blot analysis. A polyclonal anti-rabbit anti-IL-1β antibody (Chemicon, Temecula, CA) at a dilution of 1:1,000 in Tris-buffered saline + Tween 20 at room temperature was used to identify the presence of IL-1β in the samples. A chemiluminescent agent (Bio-Rad Laboratories, Hercules, CA) was applied to produce luminescence in proportion to the amount of protein on a photographic film. The relative intensity was quantified by densitometry after removal of the background intensity using software developed in the laboratory.

**Statistical Analysis**

The statistical differences among parameters under different conditions were tested using ANOVA (1-way ANOVA, 1-way repeated-measure ANOVA, 2-way ANOVA, and Tukey’s pairwise comparison) and t-test (SigmaStat, SPSS, Chicago, IL). When the data were not normally distributed, one-way ANOVA on ranks was used to analyze statistical differences. \(P < 0.05\) was used to establish statistical significance.

**RESULTS**

**Optimization of \(V_{\text{max}}\) in VVN**

During CV, \(H\) increased linearly; during VVN, \(H\) eventually reached a plateau level for certain values of \(V_{\text{max}}\). The plateau in \(H\) and the time required to reach the plateau strongly depended on the value of \(V_{\text{max}}\). For the normal mouse, within 15 min of ventilation, \(H\) reached a plateau when \(V_{\text{max}}\) was ≥3.5 times VTmean. For the injured mouse, \(H\) reached its plateau level when \(V_{\text{max}}\) was ≥2.25 times VTmean. On the basis of similar results in four normal and two injured mice, the final \(V_{\text{max}}\) was set to 3.5 and 2.25 times VTmean for normal and injured mice, respectively, for the rest of the study.

**Effects of Ventilation Mode on Respiratory Mechanics**

The respiratory mechanical parameters in normal mice (Fig. 3) show that DIs can successfully recruit the collapsed regions and standardize volume history, because at time 0, \(H\) had similar low values in all animals. The RMS error was similarly low, \(<0.04\text{ cmH}_2\text{O} \cdot \text{s} \cdot \text{ml}^{-1}\), among all ventilation modes. Law was small and did not change consistently; hence, it is not reported. During the 60-min ventilation period, all other parameters (Raw, G, and H) for animals in the CV, VVO, and CVLB groups significantly increased with time \((P < 0.001)\). For animals in the VVN group, only Raw and \(H\) depended significantly on time \((P < 0.001)\). All parameters in the CV and VVO groups increased linearly throughout the experiment, whereas in the CVLB and VVN groups, they reached a plateau within 30 min of ventilation. Thus only the latter two ventilation modes were able to stabilize the mechanical condition of the lung within 60 min of ventilation.

At baseline and at 30 min, there was no significant difference in any parameters among the groups. Nevertheless, the percent increase in \(H\) from baseline (%\(H\)) was statistically significantly lower in the CVLB and VVN groups than in the CV \((P < 0.005\) and \(P < 0.05\), respectively) and VVO \((P < 0.001\) and \(P < 0.005\), respectively) groups. At the conclusion of the experiment, even though Raw increased similarly by 30% in the CV and VVO groups and by 10% in the CVLB and VVN groups, this increase was not statistically significant. \(G\) and \(H\) statistically significantly depended on the mode of ventilation \((P < 0.01\) and \(P < 0.001\), respectively) at 60 min.
On average, $G$ increased from baseline by 34%, 39%, and 6% in the CV, $VVO$, and $CVLB$ groups, respectively. During $VVN$, $G$ increased only by 2% from baseline, which was not significant. In the CV and $VVO$ groups, $H$ increased linearly from baseline, with corresponding $\%H$ values of 80% and 83%; in the $CVLB$ and $VVN$ groups, $H$ increased until it reached a plateau at 15 and 10 min, respectively, with corresponding $\%H$ values of 25% and 20% (Fig. 3). Pairwise comparisons showed that the increases in $\%H$ were statistically significant ($P < 0.001$); however, there were no differences between CV and $VVO$ or between $CVLB$ and $VVN$. Pairwise comparisons also showed that $H$ values were significantly smaller in the $CVLB$ and $VVN$ groups than in the CV and $VVO$ groups after 45 min ($P < 0.05$). $\%H$ was systematically smaller during $VVN$ than during $CVLB$. Indeed, when the large intergroup variability was eliminated by a comparison only between the $VVN$ and $CVLB$ groups and the data in the plateau region between 30 and 60 min were pooled, $\%H$ was significantly smaller during $VVN$ than during $CVLB$ ($P < 0.001$).

The mechanical parameters during ventilation of HCl-injured mice are shown in Fig. 4. The RMS errors for the CV and $VVO$ groups were higher than those of the normal mice ($P < 0.05$). Nevertheless, the model was still able to fit the $Zrs$ spectra relatively well. All parameters ($Raw$, $G$, and $H$), including the RMS error for all ventilation groups, significantly increased with ventilation time ($P < 0.001$). At baseline, there was no difference in any parameters among the groups. On average, the baseline $Raw$ was similar between the normal and injured groups, whereas the baseline $G$ and $H$ were 20% and 25% higher, respectively, than the baseline levels in the normal mice ($P < 0.001$). All respiratory mechanical parameters in the CV group increased linearly with time; during $VVO$, $CVLB$, and $VVN$, they reached a plateau level, indicating that variability in $VT$ during $VVO$ and $VVN$, as well as periodic large breaths in $CVLB$, were able to stabilize lung mechanics in injured mice.

At 30 and 60 min, $G$ and $H$ significantly depended on the ventilation mode ($P < 0.001$). At 30 min, $G$ and $H$ were higher in the CV and $VVO$ groups than in the $CVLB$ and $VVN$ groups ($P < 0.05$ for CV and $P < 0.005$ for $VVO$). At 60 min, $G$ and $H$ statistically significantly lower in the $CVLB$ and $VVN$ groups than in the CV and $VVO$ groups ($P < 0.005$). Also, the corresponding percent increases in $G$ and $H$ from baseline were significantly higher in the CV group than in the $CVLB$ and $VVN$ groups ($P < 0.01$), whereas $Raw$ was significantly higher in the CV group than in the $VVO$, $CVLB$, and $VVN$ groups, respectively. Additionally, pairwise comparisons showed that $H$ reached a plateau after 15 min during $VVO$, 25 min during $VVO$, and 30 min during $CVLB$. The statistical analyses also showed that $H$ was significantly smaller in the $CVLB$ and $VVN$ groups than in the CV group after 40 and 35 min, respectively. Furthermore, similar to the data in Fig. 3, $\%H$ was systematically lower in the $VVN$ group than in the $CVLB$ group. When analyzed separately including data only from the $VVN$ and $CVLB$ groups after 30 min of ventilation, $H$
Effects of Ventilation Mode on Airway Pressures

The mean peak airway pressure and its percent increase averaged over 5-min intervals are shown in Fig. 5 for the normal and injured mice. For each mode of ventilation and in normal and injured mice, mean peak airway pressure was significantly affected by ventilation time ($P < 0.001$).

In the normal group, there was no significant difference in mean peak airway pressure among ventilation groups at any time point (Fig. 5A). Mean peak airway pressure increased linearly in the CV and VVO groups, whereas it reached a plateau in the CVLB and VVN groups. Nevertheless, when examined separately during only the last 5-min period (corresponding to 55–60 min), percent mean peak airway pressure significantly depended on the ventilation mode ($P < 0.001$). Pairwise comparisons showed that percent mean peak airway pressure was significantly higher in the CV and VVO groups than in the VVN group ($P < 0.02$ and $P < 0.005$, respectively) and significantly lower in the CVLB group than in the VVO group ($P < 0.05$). When the data corresponding to the plateau were combined, mean peak airway pressure (Fig. 5A) and percent mean peak airway pressure (Fig. 5B) were statistically significantly lower during VVN than during CVLB ($P < 0.01$). Mean peak airway pressure was only 8% higher in the injured groups than in the normal groups (10.4 vs. 9.3 cmH$_2$O, $P < 0.001$). In period 1 (corresponding to 0–5 min), there was no significant difference among the groups. Between 25 and 30 min, period 6, mean peak airway pressure was significantly higher in the VVO group than in the CVLB and VVN groups ($P < 0.05$). However, percent mean peak airway pressure in the VVN group increased at a significantly slower rate than in the CV and VVO groups ($P < 0.01$ and $P = 0.05$, respectively). During the last period, 55–60 min, mean peak airway pressure and percent mean peak airway pressure significantly depended on the mode of ventilation ($P < 0.001$). Pairwise comparisons showed higher mean peak airway pressure in the CV and VVO groups than in the CVLB and VVN groups ($P < 0.005$). Also, percent mean peak airway pressure was significantly higher during CV than during any other ventilation mode ($P < 0.05$ for VVO and $P < 0.001$ for CVLB and VVN). Finally, from period 8 in the normal and injured mice, mean peak airway pressure was consistently lower in the VVN group than in any of the other groups and percent mean peak airway pressure nearly doubled during CVLB compared with VVN, a statistically highly significant difference ($P < 0.001$).

Effects of Ventilation Mode on Blood Gases

$\text{PaCO}_2$ and $\text{PaO}_2$ are shown in Fig. 6 for the normal and injured mice. For the normal mice, $\text{PaO}_2$ and $\text{PaCO}_2$ were significantly better in the CVLB and VVN groups than in the CV and VVO groups ($P < 0.01$). Moreover, the alveolar-arterial gradient was significantly lower in the VVN and CVLB groups than in the VVO group (Table 1; $P < 0.02$). Direct comparison of the alveolar-arterial gradient between the VVN
and CVLB groups showed that the alveolar-arterial gradient in the latter group was statistically significantly higher (P < 0.05). For the injured mice, PaO2 was significantly higher in the CVLB and VVN groups than in the CV group (P < 0.05). However, PaCO2 and the alveolar-arterial gradient were not different among the various ventilation groups. Additionally, the ratio of PaO2 to fraction of inspired O2 (P/F) was significantly higher in the CVLB and VVN groups than in the CV group in normal and injured animals (P < 0.05) but significantly higher in the CVLB and VVN groups than in the VVO group only in normal mice (P < 0.02).

DISCUSSION

We have developed a new VV method, VVN, optimized for the mouse. VVN delivers randomly arranged larger-than-average breaths while mainly ventilating with lower, but variable, VT. We investigated the effects of VVN on respiratory mechanics, airway pressures, gas exchange, and a lung injury marker in normal mice and mice with HCl-induced lung injury over 1 hour of ventilation. The HCl aspiration-induced injury mimics ALI following gastric content aspiration, which has been identified as an important risk factor in the development of ARDS (46). The HCl aspiration treatment has been shown to result in ARDS-like conditions in many animal models, including mice (25, 29, 43, 44, 57). We also compared the performance of VVN with that of constant-VT ventilation, or CV; a previously published VV approach, VVO (7, 8); and a specific ventilation mode that combines low VT with periodic large breaths, CVLB. The primary findings of the present study are as follows: 1) VVN needs to be separately optimized for normal and injured mice; 2) for normal and injured mice, VVN and CVLB improved and stabilized respiratory mechanics, airway pressure, and blood gases compared with CV or VVO; 3) in injured mice, VVO or VVN, but not CVLB, also resulted in significantly less IL-1β-related lung injury than CV; and 4) once the lung reached a stable state, VVN outperformed CVLB.

Fig. 5. Mean peak airway pressure and percent increase in mean peak airway pressure in normal (A and B) and injured (C and D) mice. Each point represents mean of peak airway pressure in a 5-min ventilation period.

Effects of Ventilation Mode on IL-1β

Western blots for IL-1β in the normal and injured mice are shown in Fig. 7. For statistical analysis, the amounts of IL-1β in the normal group were normalized with the mean value of the unventilated normal group (Fig. 8). In baseline injury samples, IL-1β expression was on average five times higher than in the unventilated normal group. One-way ANOVA showed no effect of ventilation mode on IL-1β levels in the lungs of normal mice. After injury, the dependence of IL-1β levels on ventilation mode was statistically significant (P < 0.01). Pairwise comparisons showed significantly higher IL-1β levels in the CV group than in the baseline injury, VVO, and VVN groups (P < 0.02, P < 0.05, and P < 0.01, respectively). However, interestingly, the IL-1β level in the CVLB group was not different from that in the CV group.
The study by Arold et al. (8) in normal guinea pigs showed little difference in H over 3 h of ventilation, despite the improvement in oxygenation and increase in surfactant release, suggesting a dissociation between these indexes of lung function. Our preliminary results in normal mice and in a mouse lavage model of lung injury showed that H progressively increased during CV and VVO, when variability in VT was 25% around the mean, and no difference between the two ventilation modes (data not shown). In normal mice, there was no difference in mechanical parameters during CV and VVO, even at 60% variation around VTmean. This led us to a new design, VVN, in which the tail of the distribution of VT was stretched to accommodate larger VT values (Fig. 1).

We optimized the parameters in VVN for maximum recruitment and stability of lung elastance using VVN with similar shape and VTmean but with different Vmax values. To reduce interanimal variability, we used the same animal to test the effect of Vmax on respiratory mechanics. Within 15 min of ventilation, H plateaued at Vmax >3.5 times VTmean for normal mice and Vmax >2.25 times VTmean for injured mice. These are surprisingly large VT values compared with the modest 40% variability in VT during VVO in guinea pigs (8). However, the alveoli of the mouse lung are smaller (36); hence, surface forces at the air-liquid interface are expected to be larger than in other animals. When the larger surface tension forces are combined with an extremely floppy chest wall in the supine mouse (32), the result is a strong tendency of the lung to collapse at low and even moderate PEEP levels. Another possibility is that the surface film itself stiffens over time. Horie and Hildebrandt (28) observed that dynamic lung compliance (inverse of H) of isolated air-filled lungs fell by 10–30% during 20 min of ventilation. Since the drop in compliance was significantly attenuated in saline-filled lungs, they concluded that the mechanism responsible for these observations was related to viscoelastic adaptation of the surface film. However, they also acknowledged that when a small VT was superimposed on a low end-expiratory lung volume, which also produced the largest drop of 30% in compliance, the results could be influenced by small airway closure. Allen et al. (2, 3, 5) found trends in H similar to those in our study during CV, and they interpreted their results in terms of derecruitment, which is also supported by recent in vivo microscopy of the periphery of the lung during ventilation (4, 49). Using computerized tomographic scanning, Gattinoni et al. (20) demonstrated that the pressure-volume (P-V) curve in human patients with ARDS is significantly affected by recruitment-derecrut-
The injured regions in the ARDS/ALI lung are heterogeneously distributed, including almost-normal regions, mildly injured regions, and severely injured regions (20). Therefore, it is possible that the part of the injured lung that is collapsed cannot be recruited. This explains why the baseline $H$ values were higher after the injury (Figs. 3 and 4). An intermediate $V_T$ in $V_{VN}$ could be sufficient to recruit the normal and perhaps part of the mildly injured regions of the lung. The largest $V_T$ values could perhaps also recruit most of the mildly injured regions. The presence of collapsed regions may result in slight overinflation of the neighboring open regions in the injured lung. Thus the $V_{\text{max}}$ necessary to achieve this may be smaller in the injured than in the normal lung. It is important to point out that the $V_{\text{max}}$ used in the normal mice was 3.5 times $V_T^{\text{mean}}$, which was generally between 0.2 and 0.22 ml. Therefore, $V_{\text{max}}$ was ~0.7–0.8 ml, which is still lower than our DI, defined as 1-ml ramp increase in lung volume. In the injured mice, $V_{\text{max}}$ was 0.5–0.6 ml, which was well below the DI level.

**Effects of Ventilation Mode in Normal Mice**

Because of the significant stretch of the tail of the $V_T$ distribution in $V_{VN}$, especially for the normal mice, it is crucial to ensure that the effects of $V_{VN}$ are due to the variability in $V_T$ and not simply a few large $V_T$ values. Therefore, we also implemented $CV_{LB}$, in which $CV$ was accompanied with large breaths that had the same large $V_T$ values delivered exactly at the same time points as in $V_{VN}$ (Fig. 2).

Respiratory mechanics. Raw and $G$ barely increased over 60 min of ventilation during $CV_{LB}$ and $V_{VN}$, whereas during $CV$ and $VVO$, these parameters increased linearly (Fig. 3). $H$ increased in all groups until 25 min. Although $H$ continued to increase in the $CV$ and $VVO$ groups, suggesting no stable equilibrium, $H$ during $CV_{LB}$ and $V_{VN}$ developed a plateau. In the plateau region (at 30–60 min), $\%H$ was significantly smaller during $V_{VN}$ than during $CV_{LB}$ ($P < 0.001$). Thus, although the large breaths in $CV_{LB}$ and $V_{VN}$ are needed to reach a stable dynamic equilibrium (the plateau in $H$) that is similar to a fixed point in a nonlinear dynamic system, the presence of intermediate $V_T$ values in $V_{VN}$ leads to a different dynamic equilibrium with a larger number of open alveoli. The lung behaves nonlinearly (38), especially after injury (30). Thus differences in the stable dynamic equilibrium are expected, since in a nonlinear system the output depends not only on the mean, which is matched between $CV_{LB}$ and $V_{VN}$, but the higher-order moments of the input signal, in this case, the shape of the $V_T$ distribution. Since the application of a DI at the conclusion of the experiment was able to return all mechanical parameters to their baseline values, we conclude that changes in mechanics were due largely to derecruitment of alveoli.

The mean peak airway pressure showed a trend similar to $H$, but with smaller increases in percent mean peak airway pressure (5% and 16%) than in $\%H$ (20–80%). These results imply that dynamic elastance is a far more sensitive indicator of physiological changes than airway pressure. The reason is that airway pressure is influenced by visco components of airflow (Raw) and tissues ($G$), whereas $H$ is selectively sensitive to tissue viscoelasticity and the number of open alveolar units. The smallest percent mean peak airway pressure was seen during $V_{VN}$ and was significantly smaller in the plateau region than during $CV_{LB}$. This further strengthens the conclusion that variability leads to a different dynamic equilibrium, which brings about additional improvements in lung function over regularly delivered large breaths in $CV_{LB}$.

Gas exchange and IL-1$\beta$. Blood gas measurements at the conclusion of the experiment showed a significant improvement in $P_{aCO_2}$, $P_{aO_2}$, and $P/F$ in the $V_{VN}$ and $CV_{LB}$ groups compared with the CV and $VVO$ groups. This improvement was due to the presence of more and better-aerated regions in the $V_{VN}$ and $CV_{LB}$ groups. Furthermore, the alveolar-arterial gradient during $V_{VN}$ was about half of that during $CV_{LB}$ (9.8 vs. 18.5), and this difference was statistically significant (Table 1).

Western blot analyses showed no significant difference in IL-1$\beta$ in any of the ventilation groups compared with the unventilated group. This is consistent with the study of Allen et al. (5), who showed no significant difference in the mean tissue mRNA levels for IL-1$\beta$ between conventional low $V_T$ with and without periodic recruitment over 2 h of ventilation. Thus, despite the increase in $H$ and the decline in blood oxygenation, after 60 min of ventilation, there was no detectable sign of IL-1$\beta$-related inflammation in normal mice due to any of the ventilation modes.

**Effects of Ventilation Mode in HCl-Injured Mice**

Respiratory mechanics. Our mouse model of acid aspiration adapted from Nagase et al. (43) was used to mimic the conditions that prevail during ARDS/ALI. Nagase et al. found an almost threefold increase in elastance compared with the control group. We also found similar increases in $H$ immediately after the application of HCl. However, with subsequent DIs, $H$ dropped to a value only ~25% higher than in normal mice. Thus the threefold increase in elastance (43) may have been due to fluid blockage as a result of incomplete recruitment. In our HCl-induced injury, we allowed 30 min for the HCl to take effect. Our results showed that, despite the close proximity between normal and injury baseline levels, $H$ increased significantly faster in the injured mice (Fig. 4 vs. Fig. 3), suggesting that some fundamental mechanical properties of the lung, most likely related to surfactant and subsequent development of edema, had already been altered as a result of injury.

In contrast to normal mice, the mechanical parameters in the $VVO$ group during injury were different from those of the CV group, in that they reached a stable dynamic equilibrium marked by the plateau in $\%H$ (Fig. 4D). Since after HCl injury the level of variability in $V_{VN}$ needed to stabilize the lung was only 2.25 times $V_T^{\text{mean}}$, the variability in $VVO$ appeared to be sufficient to achieve such a stable dynamic equilibrium in 60 min, albeit one with a much higher $\%H$ (Fig. 4D). Since HCl causes direct injury to the parenchyma on contact, it is conceivable that the largest breaths in $VVO$ were sufficient to prevent collapse of parts of the lung compared with the single $V_T$ in CV but were not as efficient as the $V_{VN}$ method. Raw and $\%H$ were the smallest during $V_{VN}$, and, in the plateau region, $H$ and $\%H$ were significantly lower during $V_{VN}$ than during $CV_{LB}$. Thus, similar to normal mice, $V_{VN}$ was able to...
reach a stable dynamic equilibrium with a higher recruited lung volume than \( CV_{LB} \). Furthermore, percent mean peak airway pressure nearly doubled during \( CV_{LB} \) compared with \( VV_N \), which was statistically highly significant \((P < 0.001)\). This again may be a potentially important improvement over \( CV_{LB} \), especially if ventilation was continued beyond 60 min.

Gas exchange and IL-1\(\beta\). \( PaO_2 \) and \( P/F \) were significantly higher in the \( VV_N \) and \( CV_{LB} \) groups than in the \( CV \) group, but not in the \( VVO \) group. Also, there were no signs of any differences during \( CV_{LB} \) or \( VV_N \). However, in contrast to the consistent differences in mechanical properties, the variation in \( PaO_2 \) (Fig. 6) and alveolar-arterial gradient (Table 1) was quite high. Although the reason for this is unclear, it is possible that drawing a small amount of blood (100 \( \mu \)l) was inherently accompanied by large interanimal variability.

In patients with ARDS, elevated IL-1\(\beta\) was associated with poor survival rate (39). In the present study, Western blot analyses showed that IL-1\(\beta\) levels were significantly higher than baseline in the \( CV \) group (Fig. 8). This may be due to the overstretching of the aerated regions during CV. Interestingly, IL-1\(\beta\) levels were not elevated compared with baseline in the \( VVO \) group. Thus the variability in \( VVO \) and \( VV_N \) was able to further minimize IL-1\(\beta\)-related injury compared with CV. In fact, although not significant, the level and variability of IL-1\(\beta\) were smallest in the \( VV_N \) group. Finally, it is striking that IL-1\(\beta\) in the \( CV_{LB} \) group was not different from that in the \( CV \) group. It is possible that the variability in \( VV_O \) and \( VV_N \) helps prevent development of unphysiological shear stresses, because the same alveolar regions do not undergo repeated opening and closure as in CV. However, the large breaths in \( CV_{LB} \) alone are unable to eliminate such shear stresses when the lung is ventilated with CV between these large breaths, and consequently inflammation-related injury can develop.

Possible Mechanism

More than half of the breaths were smaller during \( VV_N \) than during \( CV_{LB} \). However, these small breaths in \( VV_N \) are mixed with the intermediate breaths. The amount of lung that can be recruited by these intermediate breaths depends on the size of the breath as well as the degree of collapse at the end of the previous expiration. Suki et al. (53) proposed a simple nonlinear mathematical model of the P-V curve to show how variability in peak pressures during VV can increase lung volume. Briefly, during CV, airway pressure increases from end-expiratory pressure to end-inspiratory pressure, which is equal to peak airway pressure. The corresponding regional lung volume increases to a value \( V_0 \). However, during VV, peak airway pressure varies on a cycle-by-cycle basis around its mean with a given SD. The corresponding regional recruited volume also varies. Because of the nonlinearity of the regional

<table>
<thead>
<tr>
<th></th>
<th>Stiff Region</th>
<th>Intermediate Region</th>
<th>Open Region</th>
<th>Sum of All Regions</th>
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<tr>
<td>( VV_N )</td>
<td>0.16</td>
<td>0.26</td>
<td>0.37</td>
<td>0.79</td>
</tr>
<tr>
<td>( CV_{LB} )</td>
<td>0.15</td>
<td>0.24</td>
<td>0.34</td>
<td>0.72</td>
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Table 2. Estimated average recruited volume along each P-V curve from Fig. 9B for \( VV_N \) and Fig. 9C for \( CV_{LB} \) and corresponding average recruited volume ratio of \( CV_{LB} \) to \( VV_N \)

Table 3. Percent improvements in various parameters during \( VV_N \) and \( CV_{LB} \) compared with CV

<table>
<thead>
<tr>
<th></th>
<th>Normal</th>
<th>Injured</th>
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<tr>
<td>( H_\infty )</td>
<td>(-33)</td>
<td>(-38)</td>
</tr>
<tr>
<td>( H_\infty )</td>
<td>(-75)</td>
<td>(-71)</td>
</tr>
<tr>
<td>Mean peak airway pressure</td>
<td>(-66)</td>
<td>(-77)</td>
</tr>
<tr>
<td>Arterial PCO(_2)</td>
<td>(-18)</td>
<td>(-22)</td>
</tr>
<tr>
<td>Arterial PO(_2)</td>
<td>(+39)</td>
<td>(+35)</td>
</tr>
<tr>
<td>%SO(_2)</td>
<td>(+5)</td>
<td>(+8)</td>
</tr>
<tr>
<td>Alveolar-arterial gradient</td>
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<td>(-32)</td>
</tr>
<tr>
<td>IL-(\beta)</td>
<td>(-41)</td>
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\( H_\infty \), elastance coefficient at 60 min; \%SO\(_2\), arterial O\(_2\) saturation. Values are specified for only those parameters that demonstrated a statistically significant improvement.
P-V curve, the recruited volume following a larger-than-average peak airway pressure will be significantly amplified; hence, the average recruited volume over a sufficiently large number of breaths will increase from $V_0$ to $V_1$, while the average peak airway pressure is maintained. Suki et al. (53) showed that, depending on the regional P-V curve, $V_1$ can be $>200\%$ larger than $V_0$. Recently, Bellardine Black et al. (9) used CT images to measure the regional P-V curves in a sheep saline lavage-induced ARDS model. They found a nearly linear P-V curve at the top of the lung, which was more aerated, but a strongly nonlinear P-V curve at the bottom of the lung, which was much less aerated. Thus the regional P-V curves can be very different in the injured lung, with highly nonlinear shapes reminiscent of the curve used in the modeling study of Suki et al.

Although we could not acquire regional P-V curves, we measured global P-V curves in several mice at three different time points (Fig. 9A). The “open” P-V curve was obtained during baseline, when the animal’s lung was intact and fully recruited. The “stiff” P-V curve was obtained after 30 min of ventilation following induction of HCl injury. At this stage, a significant portion of the lung was collapsed. The “intermediate” P-V curve was obtained immediately after the stiff P-V curve, which also served to recruit the animal’s lung. To determine the average recruited lung volumes, we selected a series of measured peak airway pressure values from the $V_{VN}$ and $CV_{LB}$ groups and used these time series as inputs to the three measured P-V curves. The corresponding volumes obtained from each P-V curve as a function of peak airway pressure are shown in Fig. 9, B and C, and the average recruited volume from each curve is summarized in Table 2. These simulations show that although the average peak airway pressure was lower in the $V_{VN}$ group (10.9 cmH$_2$O) than in the $CV_{LB}$ group (11.6 cmH$_2$O), the average recruited volume was consistently higher, by $\sim8\%$, during $V_{VN}$ in reasonable agreement with the lower $\%H$ (Figs. 3D and 4D). This is a consequence of the nonlinearity of the P-V curves. Therefore, the lung was more protected from overstretching, as well as repetitive closing and opening, because of many small VT values during $V_{VN}$ than during $CV_{LB}$. Furthermore, the presence of the intermediate breaths in $V_{VN}$ also resulted in higher average recruited lung volume. These simulations provide some limited mechanistic insight as to how the variability in $V_{VN}$ makes it a better ventilation mode than $CV_{LB}$ and, hence, support the original mechanism of stochastic resonance (53).

We also note that important time-dependent effects, such as the rate of recollapse (2, 10) or tissue viscoelasticity, are not included in this analysis. Incorporation of the rate of collapse in the model could explain more accurately the difference in recruitment between $V_{VN}$ and $CV_{LB}$. Although the difference between the various performance features of $V_{VN}$ and $CV_{LB}$ may seem small, many of them, such as the plateau values of the elastance and airway pressures or alveolar-arterial gradient in normal mice, are statistically significant. Furthermore, in Table 3, we summarize the relative improvements of $V_{VN}$ and $CV_{LB}$ over CV with median values of 38% and 30%, respectively, a difference that was statistically significant ($P = 0.012$, by paired t-test on ranks). Consequently, in the present study, $V_{VN}$ outperformed $CV_{LB}$ in many parameters during a 60-min ventilation period. Moreover, it is likely that if the lungs were ventilated over a longer time period, even the apparently small differences could easily add up and be beneficial for patients.

If we define the ventilated patient as a system in which the input is the time sequence of VT values, the state variables are alveolar collapse and surfactant and IL-1$\beta$ levels, and the output is recovery rate, then such a system is highly nonlinear. Hence, any small changes in the initial and boundary conditions can lead to drastically different outcomes. It is thus clear that $V_{VN}$ and $CV_{LB}$ are not identical and that the nature of the distribution of VT can have a significant impact on survival rate during long-term mechanical ventilation.

Summary

We developed a new mode of $VV$, $VV_N$, and compared its performance in mice with that of the constant low-VT ventilation, $CV$, the original design of $VV$, $VVO$, and a low-VT ventilation mode accompanied with large breaths, $CV_{LB}$. We found that $V_{VN}$ and $CV_{LB}$ significantly outperformed $CV$, the current clinical method, as well as $VVO$. $V_{VN}$ and $CV_{LB}$ lead to a stable dynamic equilibrium in alveolar recruitment, but during $V_{VN}$, this equilibrium corresponds to an improved mechanical lung condition. Taken together, our results imply that, during ventilation of normal and injured mice at a moderate PEEP, lung function significantly benefits from periodic large breaths, whereas variability provides an additional improvement in mechanics and airway pressures and protects the lung from heterogeneities and inflammation-related injuries. These results may have clinical implications for the ventilation of infants with premature lung conditions.

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