Effects of various modes of mechanical ventilation in normal rats

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ABSTRACT

Background: Recent studies in healthy mice and rats have reported that positive-pressure ventilation delivered with physiological tidal volumes at normal end-expiratory volume, worsens lung mechanics and induces cytokine release, thus suggesting that detrimental effects are due to positive-pressure ventilation *per se*. The aim of this study in healthy animals is to assess whether these adverse outcomes depend on the mode of mechanical ventilation.

Methods: Rats were subjected to 4 h of spontaneous, positive-pressure, and whole-body or thorax-only negative-pressure ventilation (N=8 per group). In all instances the ventilatory pattern was that of spontaneous breathing. Lung mechanics, cytokines concentration in serum and broncho-alveolar lavage fluid, lung wet-to-dry ratio, and histology were assessed. Values from eight animals euthanized shortly after anesthesia served as control.

Results: No evidence of mechanical ventilation dependent lung injury was found in terms of lung mechanics, histology, or wet-to-dry ratio. Relative to control, cytokine levels and recruitment of polymorphonuclear leucocytes increased slightly, and to the same extent with spontaneous, positive-pressure, and whole-body negative-pressure ventilation. Thorax-only negative-pressure ventilation caused marked chest wall and lung distortion, reversible increase of lung elastance, and higher polymorphonuclear leucocyte count and cytokine levels.

Conclusion: Both positive and negative pressure ventilation performed with tidal volumes and timing of spontaneous, quiet breathing neither elicit an inflammatory response nor cause morpho-functional alterations in normal animals, thus supporting the notion of the presence of a critical volume threshold above which acute lung injury ensues. Distortion of lung parenchyma can induce an inflammatory response, even in the absence of volotrauma.

Introduction

Positive-pressure mechanical ventilation (PPMV) is widely used as a life-saving intervention, as well as a valuable tool during anesthesia. Nonetheless, this intervention is regarded as potentially harmful even in healthy lungs, when ventilator-induced lung injury (VILI) can arise from overstretching of lung parenchyma, leading to surfactant inactivation, failure of the alveolar-capillary barrier, and edema,[1-3] or abnormal stresses due to cyclic opening of closed airways, inducing epithelial necrosis and sloughing and airway-parenchymal uncoupling.[4-6] Furthermore, VILI is usually,[6-8] though not always,[3,9] accompanied by the release of pro-inflammatory cytokines, possibly because of cellular mechano-transduction processes.[10,11]

If overstretching and cyclic airways closing and opening were the major triggers of the pathological cascade of VILI, then a safe PPMV would be obtained by using appropriate tidal volumes (V_T) and end-expiratory volumes (EELV). This task could be challenging or even impossible in the clinical settings, where lung parenchyma is often heterogeneous.[12,13] On the other hand, no harm should result from the PPMV of normal lungs, if V_T and EELV are similar to those of spontaneous, quiet breathing. Indeed, the conclusion has been drawn that ventilation becomes injurious to normal lungs only above a relatively high, species dependent V_T threshold, usually >15 $ml \cdot kg^{-1}$.[3]

In contrast with this conclusion, recent studies indicate that a safe threshold in terms of V_T and EELV does not exist for PPMV. In healthy rats, an increased expression of proinflammatory cytokines has been observed in macrophages of bronchoalveolar lavage fluid (BALF) after 2 h of PPMV with a V_T of 10 $ml \cdot kg^{-1}$,[14] and disruption of the extracellular matrix, with perivascular space engorgement, cuff formation, and substantial alterations of lung mechanics was found after 4 h of PPMV with V_T of 8 $ml \cdot kg^{-1}$ and zero end-expiratory

pressure (ZEEP).[15] In normal mice, PPMV with a V_T of 7.5-8 $ml \cdot kg^{-1}$ and positive end-expiratory pressure of 2-4 cmH_2O for 4-6 h caused an increased expression in lung tissue homogenates, and higher concentration in serum and BALF of several inflammatory cytokines,[16-18] besides recruitment of pulmonary granulocytes,[16,18] moderate lung edema,[16,17] and increased permeability of the alveolar-capillary barrier.[18]. These studies suggest that even the ventilatory pattern adopted by the spontaneously breathing animal becomes injurious if produced in the anesthetized, paralyzed animal by means of conventional positive-pressure ventilators. Indeed, a recent study has shown that in surfactant-depleted, paralyzed rabbits negative-pressure ventilation improves oxygenation and lessens parenchymal and airway injury relative to conventional PPMV.[19]

With the aim of assessing whether mechanical ventilation with the V_T's of spontaneous, quiet breathing causes *per se* detrimental effects on lung functions in normal animals, the outcomes of prolonged spontaneous ventilation (SV) in anesthetized rats have been compared to those obtained with PPMV and negative pressure mechanical ventilation of corresponding duration, negative pressure having been applied either to the whole body ($NP_{WB}MV$), as in the iron lung, or to the thorax only ($NP_{TO}MV$), as with cuirass or poncho-like devices.

Materials and Methods

Animal Preparation. Forty male Sprague-Dawley rats (weight range 365-440 g) were premedicated with diazepam (10 $mg \cdot kg^{-1}$) and anesthetized with an intraperitoneal injection of pentobarbital sodium (40 $mg \cdot kg^{-1}$) and chloral hydrate (170 $mg \cdot kg^{-1}$). The animals were kept supine and breathed room air at ZEEP throughout the experiment. The rectal temperature was maintained at 37°C with a heating pad. The trachea was cannulated, and a balloon-tipped catheter placed in the lower third of the esophagus, the appropriate positioning of the balloon being tested with the occlusion method.[20] A pentobarbital (~4 $mg \cdot ml^{-1}$) saline solution was continuously infused (~8 $ml \cdot kg^{-1} \cdot h^{-1}$) into a tail vein.

The animals were handled according to the National Institute of Health guiding principles. The study was approved by Ministero della Salute, Rome, Italy.

Procedure and data analysis. Airflow, tracheal (Ptr) and esophageal pressure signal were measured, digitized, and stored as previously described.[5,6] Transpulmonary pressure (P_L) was obtained as Ptr-esophageal pressure, and volume changes (ΔV) by integration of the digitized airflow signal.

The whole experimental procedure is shown in figure 1. After a 10 min period of spontaneous ventilation (point A), the animals were either killed immediately with an overdose of anesthetics (C group) or randomly assigned to one of the four ventilation modes (SV, PPMV, $NP_{WB}MV$, and $NP_{TO}MV$ group; 8 rats each). Animals were paralyzed (fig. 1, squares) with pancuronium bromide (1 $mg \cdot kg^{-1}$), and ventilated with the pattern observed during spontaneous breathing. Adequateness of anesthesia was repeatedly checked from the

absence of sudden changes of heart rate and paw pinch reflex in the spontaneously breathing animals.

Positive pressure ventilation was performed using a specially designed, computer-controlled ventilator.[5] Negative pressure ventilation with the required V_T and timing was obtained by means of an adjustable negative pressure source and computer-controlled valve group connected to perspex chambers containing either the whole body or its upper part only, in which case a rubber diaphragm was made to fit the animal's body just below the xiphoid process. In spontaneously breathing animals, application of the diaphragm alone had little constraining effects, as lung elastance increased by ~2%. Ports in the chamber walls provided the connections to the tracheal cannula, esophageal balloon, venous catheter, and rectal probe.

To ensure the same volume history and alveolar recruitment, two inflations at Ptr of ~20 cmH_2O were performed before the collection of mechanical measures (fig. 1, hatched areas). Lung dynamic elastance (E_L) and resistance (R_L) were computed using the subtraction method for SV, $NP_{WB}MV$ and $NP_{TO}MV$ groups,[21] and the rapid end-inflation occlusion method for PPMV group,[5] while chest wall elastance (Ew) was computed according to the subtraction method in all but the SV group. The difference between the end-expiratory and residual volume was assessed by connecting the expiratory port to a drum in which pressure was set at –10 cmH_2O and used as an index of EELV. After the 4 h ventilation period, all animals were subjected to PPMV for about 7 min (crossed area in fig. 1), for the assessment of pulmonary and chest-wall quasi static elastance ($Est_{,L}$ and Est,w, respectively), interrupter resistance of the lung ($Rint_{,L}$; essentially airway resistance), respiratory viscoelastic resistance (Rvisc,rs) and time constant (τvisc,rs) according to the rapid end-inflation occlusion method,

as previously described.[5] Two inflation pressure-volume (P-V) curves were also obtained by slowly inflating (~1 $ml \cdot s^{-1}$) the respiratory system to a Ptr of 40 cmH_2O.

After collection of 1.5–2 ml of blood from the heart (point B in fig. 1), the animals were killed with an overdose of anesthetic, the main left bronchus was cannulated, and the left lung removed, weighed immediately, lavaged with 4.3 $ml \cdot kg^{-1}$ of normal saline in two aliquots, left overnight in an oven at 120°C, and weighed again to compute the wet-to-dry ratio (W/D). The effluents were pooled, centrifuged (Harrier 18/80, Sanyo Gallenkamp PLC, Loughborough, UK) at 2,000 rpm for 10 min, and the supernatant was frozen and stored at -20°C.

Cytokine (tumor necrosis factor-α, interleukine (IL)-1β, -6, and -10, macrophage inflammatory protein-2) analysis and assessment of albumin concentration was carried out in duplicate in blinded fashion on BALF and serum, as previously described.[6] Sandwich enzyme immunoassay of E-Selectin was performed using goat anti-rat E-selectin antibodies (R&D Systems Inc, MN, USA). Absorbance was read at 405 nm. A pool of sera from 20 anesthetized healthy rats was used as the standard control, and the readings from individual specimens were expressed as percent of control.

The right lung was fixed by intratracheal instillation of a 8% formaldehyde, 0.1% glutaraldehyde solution with the pressure maintained at 20 cmH_2O for 24 h, and processed for histological examination according to standard methods, as previously described.[5,6,22] Histologic evaluation was performed in a blind fashion by a single observer, using an image analysis system (IMAQ; National Instruments, Austin, TX). The following measures were obtained a) mean linear intercept (Lm), a measure of air-space enlargement, and its coefficient of variation as a measure of dispersion; *b)* percent ratio of abnormal to total (normal and

abnormal) bronchiolar-alveolar attachments, and distance between normal attachments as indices of airway-parenchymal mechanical uncoupling; *c)* percent ratio of lesioned (epithelial necrosis and sloughing) to total membranous bronchioles, the bronchiolar injury score, as an index of small airway injury; *d)* percent ratio of cuffed to total arterioles (50-250 μm in diameter) as an additional index of edema; and *e)* polymorphonuclear leukocyte count in the alveolar septa as an index of parenchymal inflammation.

Statistics. Analyses were performed using SPSS 18.0 (SPSS Inc., Chicago, IL). Results are presented as mean±SD for mechanical parameters, W/D and BALF to serum albumin concentration ratio, and as median and range for cytokine and E-selectin concentration, and histologic parameters. Based on the percent difference between spontaneous and mechanical ventilation of the variables measured in previous studies[15,16,17] (W/D; polymorphonuclear count; dynamic P_L), and the mean and standard deviation of the corresponding variables assessed in this laboratory[7,9,22], the statistical power computed for $\alpha=0.05$ and $N=8$ for each group was 0.99. Comparisons of P-V curves, breathing pattern and mechanical parameters during prolonged ventilation with various modes was performed using mixed between within group factorial ANOVA for repeated measurements. One-way ANOVA was used to detect between-group differences for final measures of respiratory mechanics, W/D and BALF-to-serum albumin concentration ratios. In all instances, two tailed testing was performed. Analysis of non parametric variables was made with the Kruskal-Wallis and Mann-Whitney test. When post hoc tests were performed, the Bonferroni correction was applied. The level for statistical significance was taken at $P\leq0.05$.

Results

Breathing pattern (V_T, T_I and T_E) and lung mechanics (E_L and R_L) did not differ statistically among the various groups, during the initial period of spontaneous breathing (table 1). In forty animals, V_T, both absolute and per kg body weight, T_I, T_E, E_L and R_L averaged 2.52±0.25 ml, 6.2±0.6 ml·kg^{-1}, 0.23±0.01 s, 0.44±0.07 s, 1.89±0.41 cmH_2O·ml^{-1}, and 0.111±0.026 cmH_2O·s·ml^{-1}, respectively.

Prolonged and final ventilation period. Lung dynamic elastance was similar in the SV, PPMV and $NP_{WB}MV$ groups, but larger in the $NP_{TO}MV$ group by ~60% at all points in time (P=0.001); it increased progressively in all groups by the same relative amount (P<0.001), which averaged 17±15% (fig. 2). In contrast, R_L was constant and similar in all groups (fig. 2; P=0.342, 0.538 and 0.591 for time, time·group, and group, respectively). Ew was also time independent; it did not differ significantly between the PPMV and $NP_{WB}MV$ group (0.81±0.27 and 0.72±0.25 cmH_2O·ml^{-1}; P=0.515), but was markedly larger in the $NP_{TO}MV$ group (1.29±0.39 cmH_2O·ml^{-1}; P=0.005). EELV was similar in all groups (P=0.873), and time independent (P=0.878) averaging 1.96±0.28 and 1.99±0.28 ml at the beginning and end of the ventilation period, respectively.

When respiratory system mechanics was reassessed during the final PPMV period (fig. 1, crossed area), no statistically significant differences in $Est_{,L}$, $Est_{,w}$, $Rint_{,L}$, $Rvisc_{,rs}$, and $\tau visc_{,rs}$ were found among the 4 groups (table 2). Similarly, the inflation P-V curves of the respiratory system, chest wall and lungs of the various groups were almost superimposed (fig. 3).

W/D ratio, albumin BALF to serum ratio and cytokines. No difference in the W/D ratio and that between BALF and serum albumin concentration occurred among groups (P=0.846 and 0.169, respectively), indicating that interstitial or alveolar edema was absent. The mean values of these variables were 4.24±0.25 and 0.70±0.48%, respectively,

Cytokine concentrations in serum and BALF and serum levels of E-selectin are shown in figure 3. Serum concentration of IL-1β, IL-6 and IL-10 in the SV, PPMV, NPWBMV and NPTOMV groups was significantly higher than that of C group, whereas that of IL-1β and IL-10 was higher in the NPTOMV than SV, PPMV, and NPWBMV groups, in which it was similar. In BALF, no statistically significant changes from control levels were observed, except for IL-1β and IL-6 concentration in NPTOMV group, which were increased significantly. Serum E-selectin concentration was similar in C, SV, PPV and NPWBMV, but significantly higher in the NPTOMV group.

Histology. The results of histologic measurements are reported in table 3. None of the measured variables differed significantly among the various groups, except polimorphonuclear leukocyte count. Indeed, the number of polymorphonuclear leukocytes in the alveolar septa per unit length of the alveolar wall was similar in groups SV, PPMV, and NPWBMV (P=0.318), lower in group C (P=0.004 or less), and higher in group NPTOMV (P<0.001) than in SV, PPMV, and NPWBMV groups.

In all groups, no signs of focal alveolar collapse, edema, or hemorrhage were present.

Discussion

This study in healthy rats has investigated the effects of different types of prolonged ventilation that occurred with the same V_T, timing, and EELV of the anesthetized, spontaneously breathing animal, showing that: *1)* compared to spontaneous breathing, PPMV causes neither lung damage nor inflammatory reaction; *2)* NPWBMV is equivalent to PPMV; and *3)* NPTOMV induces lung distortion and a mild inflammatory response.

No change in lung mechanical properties occurred because of prolonged SV, PPMV or NPWBMV (fig. 2 and 3; table 2), though differences could have resulted from lung distortion due to different changes between spontaneous and positive-pressure ventilation of the vertical gradient of P_L,[23] rib cage dimensions in anesthetized rabbits and dogs,[24] and regional diaphragmatic displacement in anesthetized humans.[25] On the contrary, NPTOMV should have caused a kind of cylindrical deformation of the lungs,[26] as the rib cage expanded and the abdominal content shifted cranially with each inflation; indeed, E_L increased immediately with NPTOMV, remained elevated throughout the 4 h of mechanical ventilation (fig. 2), and returned normal on removal of distortion (table 2). Similar distortion should also occur in dogs and humans, as can be inferred from the smaller pressure changes required to produce equal changes in EELV with positive end expiratory pressure than grid and wrap devices or cuirass.[27-29] Furthermore, lung distortion can induce cyclic closing and opening of small airways, in spite of preserved EELV, with possible epithelial cell damage and cytokine release.[4-6,30]

In contrast with Mead and Collier[31] findings in dogs and present results in rats (fig. 2), Moriondo *et al.*[15] have reported that for the same V_T, P_L swings are 5 fold larger in mechanically ventilated than spontaneously breathing rats, implying a nearly 7 times greater

E_L with PPMV. These results are difficult to explain. Such very large swings of P_L were observed only in surfactant depleted, open-chest rats and rabbits during PPMV at ZEEP, and were associated with markedly greater W/D and BALF to serum albumin concentration ratio, heavy signs of airway and parenchymal damage, and prominent inflammatory response.[6,32]

The effects produced by $NP_{WB}MV$ never differed from those with PPMV (table 3 and fig.4), as predictable on the basis of the similar increases of P_L and Pw, that also ensure similar cardiovascular responses.[33] Indeed, the differences between negative and positive pressure ventilation reported by Grasso *et al.*[19] were not reproduced by a recent study.[34]

PPMV and $NP_{WB}MV$ neither impaired the integrity of the alveolar capillary barrier, because both the W/D and BALF to serum albumin concentration ratio were similar to those of the C and SV group, nor caused histologic evidence of airway, parenchymal, and vascular damage (table 3). Serum levels of IL-1β, IL-6 and IL-10 were, however, higher in the PPMV and $NP_{WB}MV$ than C group, but similar to those of the SV group (fig.4), consistent with previous findings of similar tumor necrosis factor-α, IL-6, IL-10, and keratinocyte-derived chemokine concentration in serum or lung homogenates of spontaneously breathing and mechanically ventilated rats.[35] The same applies to recruitment of polymorphonuclear leukocytes in the alveolar septa (table 3), suggesting that inflammatory response during SV, PPMV, and $NP_{WB}MV$ is related to anesthesia, surgery, and/or animal's instrumentation.[36] Hence, the comparison of cytokines levels between control and mechanically ventilated animals can lead to an erroneous evaluation of the pro-inflammatory effects of MV. This may have happened in the studies on mice, where the SV group was lacking,[16] or different doses of anesthetics had been administered.[17] Furthermore, it seems unlikely that the discrepancy between these and present studies can be attributed to the somewhat longer period of

mechanical ventilation used in mice,[16,17] because increased expression of inflammatory cytokines in BALF macrophages of rats and elevation of pulmonary and systemic cytokine levels in mice are already observed within 120 and 60 min from the onset of PPMV with non-injurious tidal volumes.[14,16] At any rate, the kind of response to PPMV observed in mice vanished completely after 24 h.[16]

In contrast with PPMV and NPWBMV, NPTOMV elicited a significant increase of polymorphonuclear leukocyte count in the alveolar septa (table 3), as well as of IL-1β, IL-10, and E-selectin concentration in serum and IL-1β and IL-6 concentration in BALF relative to corresponding levels in the SV group (fig.4). The presence of IL-1β and IL-6 in BALF was probably the consequence of local production in the airspaces, because their BALF and serum concentrations did not correlate (P>0.70), consistent with the preserved integrity of the alveolar-capillary barrier. Cytokines release with NPTOMV cannot be attributed to anesthesia, surgery, animal's instrumentation, and breathing pattern, because they were the same in all groups. A possible explanation can be provided by the presence with NPTOMV of lung distortion, leading to pro-inflammatory cytokine release via cellular mechanotransduction.[10,11] This inflammatory response, however, had no apparent, short term consequences, because none of the functional and morphological variables differed among all groups at the end of the experiments, (table 2 and 3). On the other hand, it remains to be seen whether with a more protracted NPTOMV, the inflammatory reaction can eventually cause mechanical and/or histologic alterations.

Interestingly, serum E-selectin, an endothelial cell adhesion molecule mediating leukocyte rolling and phagocyte chemotaxis,[37] was increased with NPTOMV only, and its levels did in fact correlate with the degree of lung distortion (P=0.035) indexed by the ratio

between E_L at the onset of NPtoMV and the preceding SV period. This suggests that distortion could have activated the endothelial cells by changing their shape either directly or through alterations of the blood flow regime in their proximity.[38,39]

In conclusion, both prolonged positive and negative pressure ventilation performed in normal animals with the V_T and timing of spontaneous, quiet breathing neither elicit a pro-inflammatory response nor cause functional and morphological alterations. In view of the adverse effects of conventional mechanical ventilation with large tidal volumes, this supports the notion of the presence of a critical volume threshold above which acute lung injury ensues. However, an inflammatory reaction with cytokines production and/or release can be induced by distortion of the pulmonary parenchyma, even in the absence of volotrauma in normal lungs, as it occurs with poncho-like devices. This could have important implications in the presence of pathological, heterogeneous alterations of lung mechanics, when distortion can be induced by conventional positive pressure ventilation in spite of the use of low tidal volumes.

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Figure Legends

Fig. 1. Time line representation of the main procedures used in rats subjected to various ventilation modes. The animals of the control group (C group) were killed immediately after the initial period of spontaneous ventilation. Squares indicate induction of paralysis. BALF, broncho-alveolar lavage fluid; E_L and R_L, lung dynamic elastance and resistance.

Fig. 2. Lung mechanical properties during the 4 h period of spontaneous ventilation (SV), positive pressure (PPMV), and whole-body ($NP_{WB}MV$) or thorax-only negative pressure ventilation ($NP_{TO}MV$). A: dynamic elastance (E_L), and B: resistance (R_L). Closed symbols indicate values obtained during the preceding 10 min of spontaneous breathing. Symbols were shifted slightly along the time axis to avoid excessive overlapping. Bars: SD; *P<0.05 and **P<0.01, significantly different from the corresponding value at t=0; °P<0.01, significantly different from the corresponding values of the other groups.

Fig. 3. Quasi-static inflation pressure-volume curves of lung (A) and respiratory system (B) obtained after 4 h of spontaneous ventilation (continuous line in all panels), positive pressure (PPMV), and whole-body ($NP_{WB}MV$) or thorax-only negative pressure ventilation ($NP_{TO}MV$). P_L and Prs, transpulmonary pressure and pressure across the respiratory system, respectively. Both for P_L- and Prs-volume curves, no significant difference was found among groups (P=0.628 and 0.542, respectively). Bars: SD.

Fig. 4. Box-plot (median, 25-75th percentiles) of cytokine concentration in serum and broncho-alveolar lavage fluid (BALF) and E-selectin in serum of rats killed immediately (C), or subjected to a 4 h period of spontaneous ventilation (SV), positive pressure (PPMV), and whole-body negative ($NP_{WB}MV$) or thorax-only negative pressure ventilation ($NP_{TO}MV$). Macrophage inflammatory protein (MIP)-2, interleukine (IL)-1β, -6, and -10, and tumor

necrosis factor (TNF)-α. P values in panels pertain to Kruskal-Wallis test; *P<0.05, **P<0.01, significantly different from C group; °P<0.05 and °°P<0.01, significantly different from SV, PPMV and NPWBMV groups.

Table 1. Breathing pattern and lung mechanics during the initial ten min period of spontaneous breathing

Group	n	V_T ml	V_T ml·kg^{-1}	T_I s	T_E s	E_L cmH$_2$O·ml^{-1}	R_L cmH$_2$O·s·ml^{-1}
C	8	2.4±0.2	6.0±0.3	0.23±0.02	0.48±0.06	1.92±0.32	0.128±0.027
SV	8	2.6±0.4	6.5±0.9	0.22±0.02	0.40±0.05	1.81±0.39	0.116±0.026
PPMV	8	2.6±0.2	6.4±0.3	0.22±0.01	0.42±0.04	1.75±0.40	0.108±0.022
NP$_{WB}$MV	8	2.5±0.2	5.9±0.7	0.24±0.02	0.43±0.12	1.94±0.62	0.094±0.027
NP$_{TO}$MV	8	2.5±0.2	6.0±0.7	0.23±0.03	0.45±0.11	2.03±0.35	0.109±0.025
P		0.314	0.314	0.207	0.321	0.718	0.075

Values are mean±SD; C, group of animals killed immediately after the 10 min of spontaneous ventilation; SV, PPMV, NP$_{WB}$MV and NP$_{TO}$MV, groups of animals which subsequently underwent 4 h of spontaneous ventilation, positive pressure and whole-body or thorax-only negative pressure ventilation, respectively; V_T, tidal volume; T_I and T_E, inspiratory and expiratory duration; E_L and R_L, lung dynamic elastance and resistance.

Table 2. Respiratory mechanics assessed during the short, final period of positive-pressure mechanical ventilation

Group	n	$Est_{,L}$ $cmH_2O \cdot ml^{-1}$	$Est_{,w}$ $cmH_2O \cdot ml^{-1}$	$Rint_{,L}$ $cmH_2O \cdot s \cdot ml^{-1}$	$Rvisc_{,rs}$ $cmH_2O \cdot s \cdot ml^{-}_{1}$	$\tau visc_{,rs}$ s
SV	8	1.39±0.25	0.60±0.17	0.096±0.020	0.48±0.14	1.33±0.18
PPMV	8	1.41±.0.39	0.58±0.12	0.077±0.021	0.52±0.13	1.60±0.42
$NP_{WB}MV$	8	1.28±0.42	0.60±0.12	0.094±0.016	0.40±0.10	1.10±0.42
$NP_{TO}MV$	8	1.44±0.25	0.53±0.16	0.078±0.017	0.52±0.06	1.37±0.28
P		0.793	0.720	0.086	0.160	0.054

Values are mean±SD; SV, PPMV, $NP_{WB}MV$ and $NP_{TO}MV$, groups of animals which underwent 4 h of spontaneous ventilation, positive pressure and whole-body or thorax-only negative pressure ventilation, respectively; $Est_{,L}$ and Est,w, quasi-static lung and chest wall elastance; $Rint_{,L}$, lung interrupter resistance, $Rvisc_{,rs}$ and $\tau visc_{,rs}$, respiratory system viscoelastic resistance and time constant.

Table 3. Indexes of parenchymal, airway, and vascular damage

Group	n	Lm μm	CV_{Lm} %	A-A %	D μm	IS	PAC %	PMN mm^{-1}
C	8	69 (60-73)	16 (13-22)	3 (2-6)	51 (41-58)	6.1 (5-8)	5 (0-14)	0.4*(0.4-0.7)
SV	8	67 (63-67)	16 (14-19)	4 (2-4)	51 (42-52)	6.6 (5-8)	5 (2-7)	0.8 (0.5-1.3)
PPMV	8	65 (64-70)	16 (13-18)	4 (3-5)	50 (41-57)	6.2 (5-9)	2 (0-30)	1.2 (0.5-1.3)
$NP_{WB}MV$	8	70 (61-75)	16 (13-19)	3 (2-5)	50 (44-56)	6.5 (5-8)	3 (0-10)	1.2 (0.7-1.5)
$NP_{TO}MV$	8	61 (54-76)	19 (16-22)	5 (2-6)	52 (50-57)	6.3 (5-9)	0 (0-15)	3.0*(1.8-3.4)
P		0.585	0.299	0.585	0.610	0.984	0.650	<0.001

Values are medians with range in parentheses; C, group of animals killed immediately after the 10 min of spontaneous ventilation; SV, PPMV, $NP_{WB}MV$ and $NP_{TO}MV$, groups of animals which underwent 4 h of spontaneous ventilation, positive pressure and whole-body or thorax-only negative pressure ventilation; Lm, mean linear intercept; CV_{Lm}, coefficient of variation of Lm; A-A, percentage of abnormal alveolar-bronchiolar attachments; D, distance between normal alveolar-bronchiolar attachments; IS, bronchiolar injury score; PAC, peri-arteriolar cuffs; PMN, polymorphonuclear leukocytes in the alveolar septa per mm of alveolar wall length; *significantly lower (P=0.004 or less) or higher (P<0.001) than any other group.

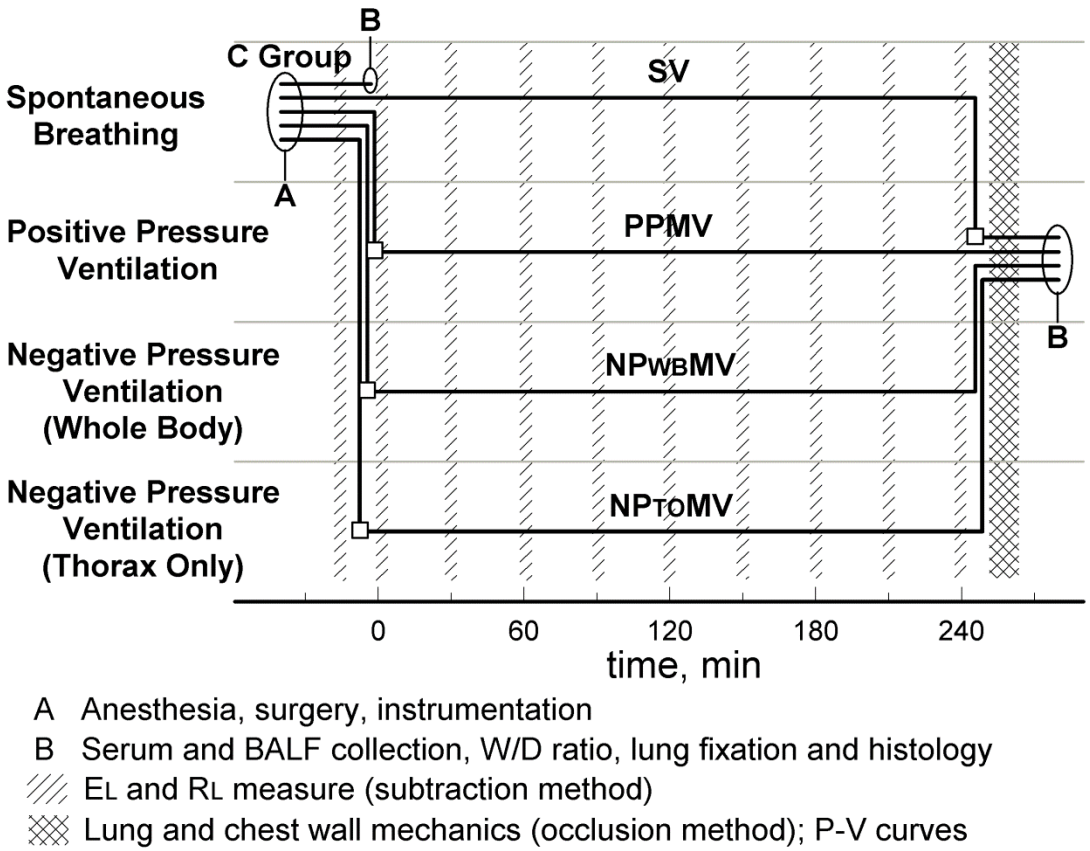
B
C Group
SV
Spontaneous Breathing
A
Positive Pressure Ventilation
PPMV
B
Negative Pressure Ventilation (Whole Body)
NPWBMV
Negative Pressure Ventilation (Thorax Only)
NPTOMV
0
60
120
180
240
time, min
A Anesthesia, surgery, instrumentation
B Serum and BALF collection, W/D ratio, lung fixation and histology
EL and RL measure (subtraction method)
Lung and chest wall mechanics (occlusion method); P-V curves

Figure 1

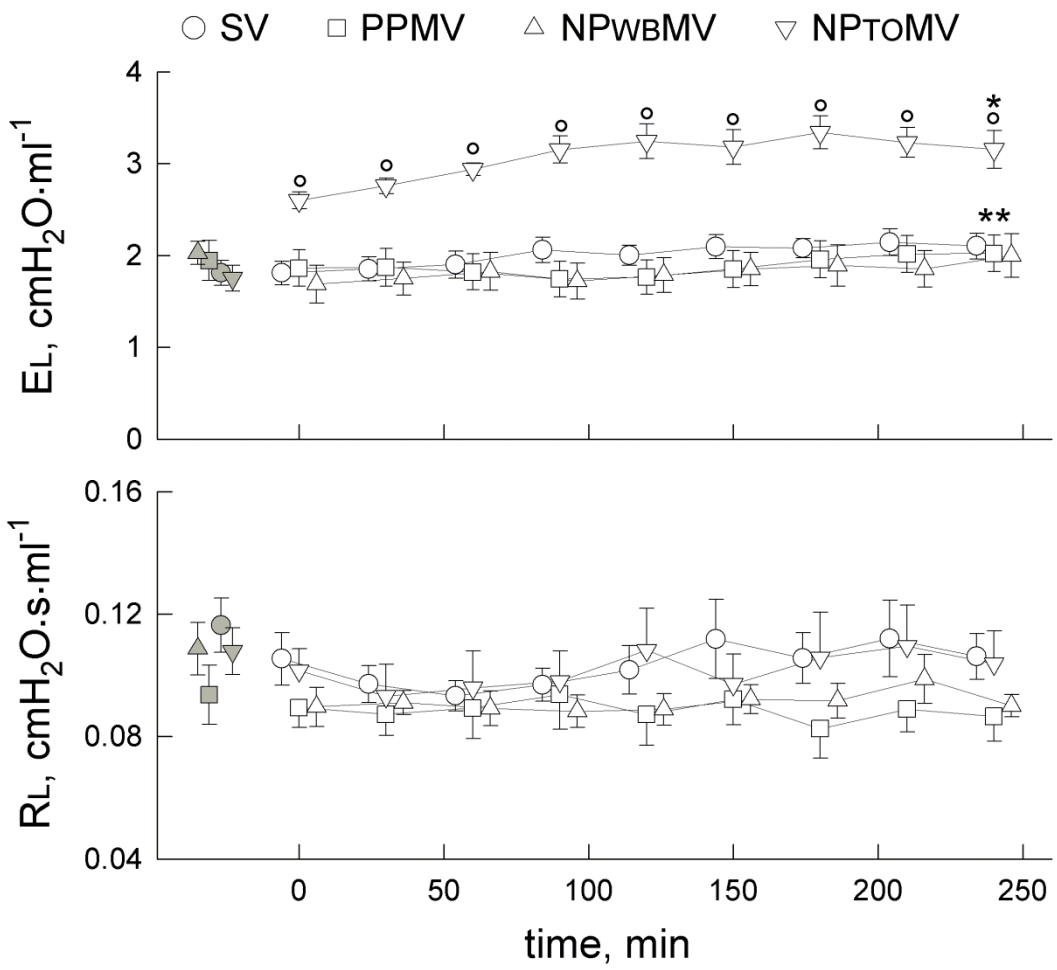
SV
PPMV
NPWBMV
NPTOMV
EL, cmH2O·ml-1
RL, cmH2O·s·ml-1
time, min
0
50
100
150
200
250
0.04
0.08
0.12
0.16
1
2
3
4
*
**

Figure 2

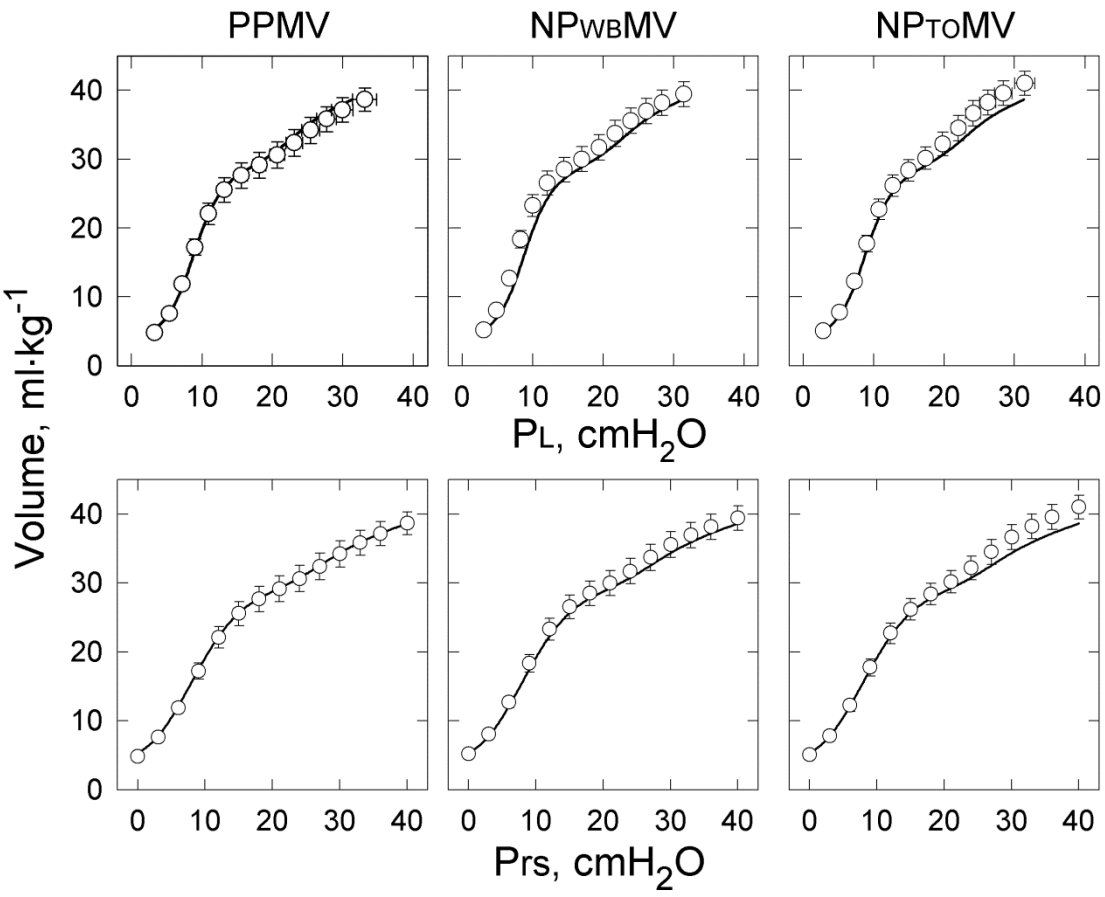
PPMV
NPWBMV
NPTOMV
Volume, ml·kg-1
PL, cmH2O
Prs, cmH2O
0
10
20
30
40

Figure 3

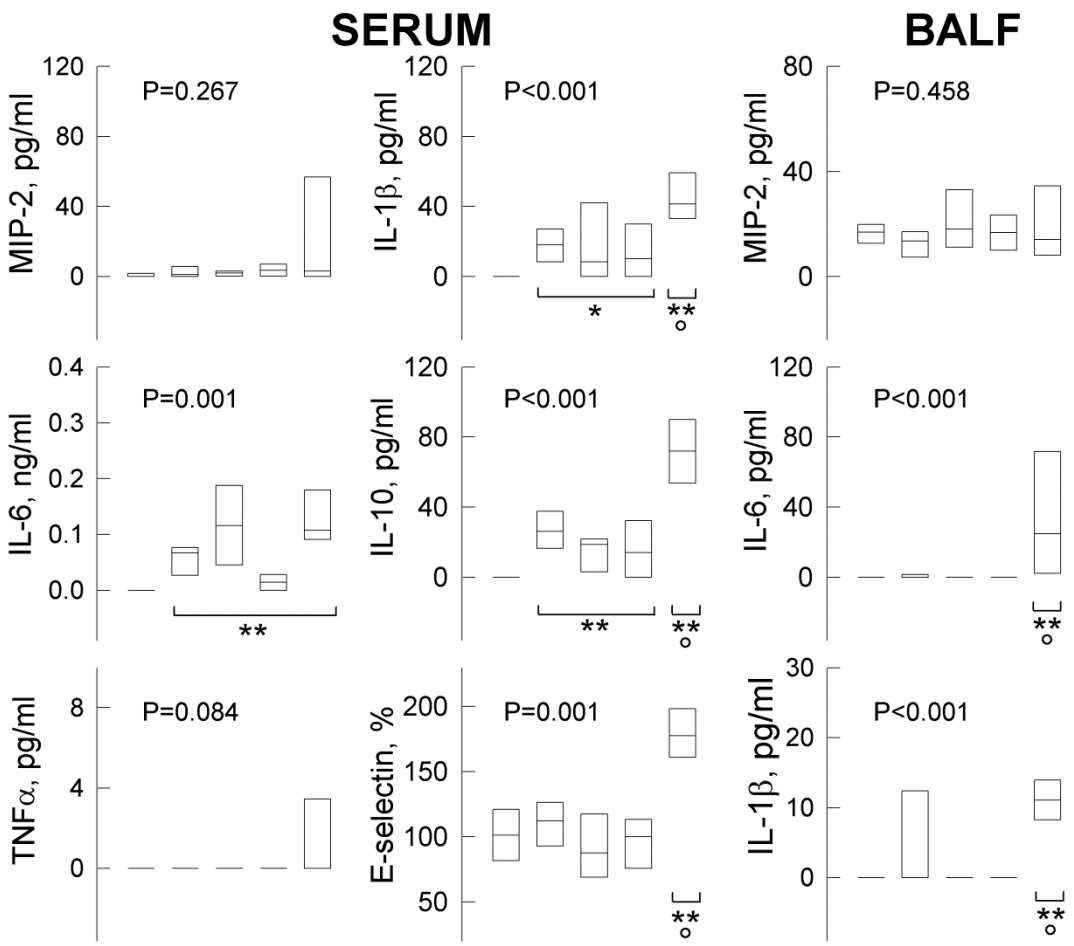
SERUM
BALF
MIP-2, pg/ml
P=0.267
IL-1β, pg/ml
P<0.001
MIP-2, pg/ml
P=0.458
IL-6, ng/ml
P=0.001
IL-10, pg/ml
P<0.001
IL-6, pg/ml
P<0.001
TNFα, pg/ml
P=0.084
E-selectin, %
P=0.001
IL-1β, pg/ml
P<0.001

Figure 4