Dependence of lung injury on surface tension during low-volume ventilation in normal open-chest rabbits

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ABSTRACT

In order to evaluate the role of pulmonary surfactant in the prevention of lung injury caused by mechanical ventilation (MV) at low end-expiratory volumes, lung mechanics and morphometry were assessed in three groups of 8 normal, open-chest rabbits ventilated for 3-4 h at zero end-expiratory pressure (ZEEP) with physiologic tidal volumes (V_T=10 ml·kg^{-1}). One group was left untreated (*group A*), the other two received surfactant intratracheally (*group B*) or aerosolized dioctylsodiumsulfosuccinate (*group C*) before MV on ZEEP. Relative to initial MV on PEEP (2.3 cmH_2O), quasi-static elastance (Est) and airway (Rint) and viscoelastic resistance (Rvisc) increased on ZEEP in all groups. After restoration of PEEP, only Rint (124%) remained elevated in *group A*, only Est (36%) was significantly increased in *group B*, whereas in *group C* Est, Rint and Rvisc were all markedly augmented (274, 253, and 343%). In contrast, prolonged MV on PEEP had no effect on lung mechanics of 8 open-chest rabbits (*group D*). Lung edema developed in *group C* (wet-to-dry ratio=7.1), but not in the other groups. Relative to *group D*, both *group A* and *C*, but not *B*, showed histological indices of bronchiolar injury, while all groups exhibited an increased number of polymorphonuclear leukocytes in alveolar septa, which was significantly greater in *group C*. In conclusion, administration of exogenous surfactant largely prevents the histological and functional damage of prolonged MV at low lung volumes, whereas surfactant dysfunction worsen the functional alterations, also because of edema formation and, possibly, increased inflammatory response.

keywords: lung mechanics, viscoelasticity, recruitment-derecruitment of lung units, airway-parenchymal coupling, parenchymal inflammation

In normal, anaesthetized, open-chest rabbits, prolonged mechanical ventilation with physiological tidal volumes and zero end-expiratory pressure (ZEEP) induces histological evidence of peripheral airway and parenchymal injury with a concomitant increase in airway resistance which persists after restoration of physiological end-expiratory lung volume (9,10). In contrast, no mechanical and morphological alterations occur during prolonged mechanical ventilation at physiological end-expiratory lung volumes maintained by means of a positive end-expiratory pressure (PEEP). Peripheral airway injury consists of epithelial necrosis and sloughing in the membranous and respiratory bronchioles and rupture of the alveolar-bronchiolar attachments, while parenchymal inflammation is reflected by an increased number of polymorphonuclear leukocytes in the alveolar septa. Both types of injury can be responsible for the persistent increase of airway resistance with prolonged mechanical ventilation at low lung volumes, altering the bronchiolar lumen, disrupting the normal alveolar-bronchiolar coupling, and releasing inflammatory mediators with bronchoconstrictor effects. It has been suggested that these morpho-functional alterations are the consequence of abnormal stresses that develop locally both at the level of the bronchiolar epithelium and in the parenchyma, mainly at the alveolar-bronchiolar junctions, because of cyclic opening and closing of peripheral airways with tidal ventilation at low lung volumes (9,10). Such stresses should be enhanced in the presence of increased surface tension due to surfactant depletion or inactivation, which should take place during ventilation at low lung volumes (32).

The present study was aimed at verifying the above hypothesis. Accordingly, the effects of prolonged mechanical ventilation at low volumes on lung mechanics and histological indices of lung injury and inflammation were assessed in normal, open-chest rabbits in which the surface tension had been reduced by means of exogenous surfactant or increased by means of the aerosolized detergent dyoctylsodiumsulfosuccinate. These effects were compared to those observed in untreated rabbits subjected to prolonged mechanical ventilation at low volumes, while the values of the mechanical and histological parameters obtained in untreated rabbits subjected to prolonged mechanical ventilation with physiological end-expiratory lung volumes served as control.

METHODS

Forty New Zealand White rabbits (weight range 2-2.6 kg) were anesthetized with an intravenous injection of a mixture of pentobarbital sodium (20 $mg \cdot kg^{-1}$) and urethane (0.5 $mg \cdot kg^{-1}$). A brass cannula and a polyethylene catheter were inserted into the trachea and carotid artery, respectively. The animals were paralyzed with pancuronium bromide (0.1 $mg \cdot kg^{-1}$) and mechanically ventilated (respirator 660; Harvard Apparatus, Holliston, MA) with a pattern similar to that during spontaneous breathing. Anesthesia and complete muscle relaxation were maintained

with additional doses of the anesthetic mixture and pancuronium bromide. Adequacy of anesthesia was judged from the absence of mydriasis, sudden increase in heart rate and/or systemic blood pressure. The chest was opened via a median sternotomy and a coronal cut made just above the costal arch, while application of PEEP prevented lung collapse.

Airflow ($\dot{V}$) was measured with a heated Fleisch pneumotachograph no.00 (HS Electronics, March-Hugstetten, Germany) connected to the tracheal cannula and a differential pressure transducer (Validyne MP45, ±2 cmH_2O; Northridge, CA). The response of the pneumotachograph was linear over the experimental flow range. Tracheal pressure (Ptr) and systemic blood pressure were measured with pressure transducers (model 1290A; Hewlett-Packard, Palo Alto, CA) connected to the side arm of the tracheal cannula and carotid catheter, respectively. There was no appreciable shift in the signal or alteration in amplitude up to 20 Hz. The signals from the transducers were amplified (model RS3800; Gould Electronics, Valley View, OH), sampled at 200 Hz by a 14-bit A/D converter, and stored on a desk computer. Volume changes (ΔV) were obtained by numerical integration of the digitized airflow signal. Arterial blood P_{O_2}, P_{CO_2} and pH were measured by means of a blood gas analyzer (IL 1620; Instrumentation Laboratory, Milan, Italy) on samples drawn at the beginning and at the end of each test session.

After completion of the surgical procedure, the rabbits were ventilated with a specially designed, computer-controlled ventilator (9), delivering water-saturated air from a high pressure source (4 atm) at constant flow of different selected magnitudes and durations, while Ringer-bicarbonate was continuously infused intravenously at a rate of 4 $ml \cdot kg^{-1} \cdot h^{-1}$, and epinephrine occasionally administered to keep normal arterial blood pressure. A three way stopcock allowed the connection of the expiratory valve of the ventilator either to the ambient or to a drum in which the pressure was set at 2-2.5 cmH_2O by means of a flow-through system. The baseline ventilator setting was kept constant throughout the experiment, and consisted of a fixed tidal volume (V_T) of 10 $ml \cdot kg^{-1}$, inspiratory and expiratory duration of 0.25 and 1.8 s, respectively, and end-inspiratory pause of 0.45 s. No intrinsic PEEP was present under any experimental condition, as evidenced by an end-expiratory pause (zero flow) and absence of Ptr changes with airway occlusion at end-expiration. During measurements, the ribs on the two sides and the diaphragm were pulled widely apart, in order to prevent contact between lung and chest wall, except in their dependent parts.

Curosurf (Chiesi Farmaceutici, Parma, Italy), a modified porcine surfactant containing 99% polar lipids and 1% surfactant-associated proteins SP-B and SP-C with a phospholipid concentration of 80 $mg \cdot ml^{-1}$, was used to lower surface tension (26); 2.5 $ml \cdot kg^{-1}$ of Curosurf were intratracheally injected followed by 20 ml of air. This product can also exhibit antibacterial properties (33), and depress phagocytic function and tumor necrosis factor secretion of human

monocytes (19), but none of these effects is relevant in the present context. A 10% alcoholic solution of dioctylsodiumsulfosuccinate (DOSS), (Aerosol OT, A 6627; Sigma-Aldrich, St. Louis, MO) diluted 1:2 in saline, was used to increase surface tension, this being the only known action of DOSS (19,20) which is relevant to the present study. The aerosolized 5% DOSS solution was delivered by a nebulizer (Ultra-Neb 99; DeVilbiss, Somerset, PA) for 60 consecutive inflations. Rabbits were discarded in which DOSS administration on PEEP caused a marked increase in elastance with grossly inhomogeneous expansion and, occasionally, overt edema.

Procedure and data analysis

Figure 1 provides a time line representation of the procedures performed in the four groups of animals. Eight rabbits underwent mechanical ventilation (MV) for 4-5 h on PEEP only, serving as control, while twenty-four rabbits were subjected to the following sequence of PEEP and ZEEP: *a)* 15 to 25 min of MV with PEEP ($PEEP_1$); *b)* 3-4 h of MV at ZEEP; and *c)* 15 min of MV with PEEP ($PEEP_2$). After assessment of lung mechanics on $PEEP_1$, 8 rabbits were treated with Curosurf, 8 with DOSS, and 8 were left untreated. After Curosurf or DOSS administration the lungs were inflated 3-4 times to Ptr of ~25 cmH_2O, and measurements of lung mechanics were repeated, limitedly to baseline V_T and T_I (see below). The animals were from a single cohort and the various types of experiment were done in random order.

Lung mechanics was assessed with the rapid airway occlusion method (3,7) during the $PEEP_1$ and $PEEP_2$ periods, and after about 10 min ($ZEEP_1$) and at the end of the ZEEP period ($ZEEP_2$). Ten to fifteen min elapsed between measurements on $ZEEP_2$ and $PEEP_2$. Before all measurements on PEEP the lungs were inflated 3-4 times to Ptr of ~25 cmH_2O. Two types of measurements were carried out: *a)* while keeping V_T at baseline values, test breaths were intermittently performed with different $\dot{V}_I$ and T_I in the range 0.25 to 3 s to assess lung mechanics at end-inflation; and *b)* while keeping $\dot{V}_I$ constant, test breaths were intermittently performed with different V_T to obtain quasi-static inflation volume-pressure curves. End-inspiratory occlusions lasting 5 s were made in all test breaths, which were performed in random order and repeated 4-5 times under all experimental conditions. During ventilation at ZEEP, end-inspiratory occlusions were performed only for tidal volumes ≤ baseline V_T. During ventilation with PEEP, the expiratory valve was opened to the ambient for 4-6 expirations in order to measure the difference between the end-expiratory and the resting lung volume (ΔEELV); these breaths were followed by two inflations with Ptr of ~25 cmH_2O.

The end-inspiratory airway occlusions were followed by a rapid initial drop in Ptr (ΔP_1), and by a slow decay (ΔP_2) to an apparent plateau value (Pst). This pressure, computed as the mean pressure recorded during the last 0.5 s of occlusion, was taken to represent the quasi-static

lung recoil pressure. Quasi-static lung elastance (Est) was obtained as (Pst-Pee)/V_T, Pee being the end-expiratory pressure, while ΔP_1 and ΔP_2 divided by $\dot{V}_I$ yielded the interrupter (Rint) and additional resistance (ΔR), respectively.

The quasi-static inflation volume-pressure curves obtained on PEEP were fitted with the function

$$V_o \cdot (1 - e^{-K \cdot Pst}) \quad (1)$$

where V_o is maximum volume above resting lung volume and K=cmH_2O^{-1} is a shape factor, that reflects the overall distensibility of the lung (6,24).

Viscoelastic parameters, Rvisc and τvisc=Rvisc/Evisc, were computed by fitting the values of ΔR and T_I with the function (7)

$$\Delta R = Rvisc\ (1 - e^{-T_I/\tau visc}) \quad (2)$$

After completion of the mechanics measurements, the right lung was removed and immediately weighed, left overnight in an oven at 120°C, and weighed again to compute the wet-to-dry ratio, while the left lung was processed for histological analysis.

Histological analysis

The rabbits were given heparin (355 U·kg^{-1}) and papaverin (5 mg·kg^{-1}) intravenously to prevent bronchospasm. The pericardium was removed, ties were placed around the descending aorta and the hilum of the right lung, and a large needle was inserted through the right ventriculum into the pulmonary artery. After three inflations to 25 cmH_2O, the transpulmonary pressure was kept at 10 cmH_2O, the ties were fastened, the right lung was removed, the right atrium cut, and the left lung perfused with saline until the lobar surfaces became white. Thereafter, lung fixation was obtained by perfusing with 4% formaldehyde, 0.1% glutaraldehyde dissolved in 0.12 M phosphate buffer. Six blocks, ~1 cm thick, involving both subpleural and para-hilar regions, were obtained in each animal. Each block was processed through a graded series of alcohols and embedded in paraffin. From each block, sections of 5 μm thickness were cut and stained with hematoxylin-eosin for light microscopy analysis. Histological evaluation was performed by a single observer in a blind fashion. The following measurements were made: *a)* mean linear intercept (Lm), which is a measure of airspace enlargement, as described by Thurlbeck (31); *b)* indexes of destruction of the alveolar attachments, which are the alveolar walls that extend radially from the outer wall of the non respiratory bronchioles (22); *c)* presence of bronchiolar epithelial necrosis and sloughing, as a measure of bronchiolar injury (17); and *d)* polymorphonuclear leukocytes count in the alveolar walls, which is an index of parenchymal inflammation (23). Morphometry was performed by means

of computer-aided, image analysis system (IMAQ Vision for LabView; National Instruments, Austin, TX).

For Lm measurements, 6 sections from each rabbits was examined at a magnification of 100X, and 10 non-overlapping fields were analyzed on each section. The Lm value was obtained as the ratio between the length in μm of a line passing transversely through each field and the number of alveolar walls intercepting that line, the final result for a given animal being the average Lm of 60 fields.

The number of polymorphonuclear leukocytes within the alveolar wall was computed and the length of the alveolar wall was measured at a magnification of 400X on a total of 60 fields randomly distributed across six slides for each animal.

For alveolar-bronchiolar coupling assessment, alveolar attachments of 50 non-respiratory bronchioles per animal were examined at a magnification of 200X. Any discontinuity of the peribronchiolar alveolar wall qualified that wall as an abnormal attachment. Two indexes were obtained: *a)* percent ratio of abnormal to total (normal and abnormal) attachments; and *b)* distance (μm) between normal attachments computed as the ratio of external circumference to number of normal attachments.

Bronchiolar injury was assessed from the presence of epithelial sloughing, *i.e.* separation of necrotic tissue, in the respiratory and membranous bronchioles. At least 50 bronchioles were examined per animal, and the injury score (IS) was computed as the percent ratio of injured to total respiratory and membranous bronchioles (17).

Statistics.

Results from mechanical studies are presented as means±SE. The least-square regression method was used to assess the parameters in *Eqs. 1* and *2*. Comparisons among experimental conditions were performed using one-way analysis of variance (ANOVA); when significant differences were found, the Bonferroni test was performed to determine significant differences between different experimental conditions. Results from histological studies are expressed as median and range, and the statistical analysis was performed using the Mann-Whitney test. The level for statistical significance was taken at $P \leq 0.05$.

Preliminary experiments

In an attempt to infer the distribution of Curosurf, 2.5 $ml \cdot kg^{-1}$ of Evans blue-dyed saline were instilled into the trachea of 8 open-chest rabbits. The lungs were immediately removed, connected to a source of compressed air, fixed dry at a distending pressure of 20 cm H_2O, and finally cut perpendicularly to their major axis to obtain a total of 8-10 slices. Although the

distribution of the dye differed among animals and lobes, most of the parenchyma was stained: on average, the dyed parenchyma amounted to 79±5 and 84±6% in the right and left lung, respectively.

RESULTS

In each animal, the values of Pa_{O_2}, Pa_{CO_2}, and pHa obtained at the beginning and at the end of each test session did not differ significantly and were thus averaged. During $PEEP_1$, the mean values of these parameters were similar for all groups of rabbits (Fig. 2). With $PEEP_2$, pHa was significantly reduced in all groups of animals, while Pa_{O_2} and Pa_{CO_2} were significantly decreased and, respectively, increased only in animals treated with DOSS. Relative to $PEEP_1$, with $ZEEP_1$ there was a similar increase of Pa_{CO_2} and decrease of Pa_{O_2} and pHa in all groups of rabbits. No further changes took place on $ZEEP_2$, except in animals treated with DOSS in which there was a significant increase of Pa_{CO_2} and decrease of Pa_{O_2} and pHa.

Mechanics

In all animals, the inflation V-P curve on PEEP was closely fitted (r>0.988) by *Eq. 1*, while a unique function in the form of *Eq. 2* adequately described (r>0.975) the experimental ΔR-T_I data under all conditions, allowing computation of Rvisc and τvisc. During $PEEP_1$, no significant differences occurred among the various groups for any of the mechanical parameters in *Eq. 1* (Table 1 and Fig. 3), as well as Rint, Est, Rvisc and τvisc (Fig. 4). Administration of Curosurf caused an immediate and marked increase of Est, Rint, and ΔR, while with DOSS administration, the corresponding changes were smaller and non significant (Fig. 4).

Static inflation V-P relationships. Ptp at end-expiration was similar during $PEEP_1$ and $PEEP_2$, averaging 2.3 cm H_2O in all groups. In untreated animals, ΔEELV was also similar on $PEEP_1$ and $PEEP_2$, whilst it was significantly reduced on $PEEP_2$ both in Curosurf and, markedly more, in DOSS treated rabbits (Table 1).

In untreated animals, the inflation V-P curve was similar on $PEEP_1$ and $PEEP_2$, independent of ventilation on ZEEP, whereas in Curosurf or DOSS treated rabbits, the V-P curve shifted downwards with $PEEP_2$ (Fig. 3). As a consequence, V_o and K in *Eq. 1* remained unchanged in untreated animals, V_o decreased significantly with $PEEP_2$ both in rabbits receiving Curosurf and DOSS, whilst K changed significantly only in animals treated with DOSS (Table 1). The lung wet-to-dry ratio was similar in control and untreated animals ventilated on ZEEP (Table 1), and similar to that of freshly excised lungs (9). In animals treated with Curosurf, the wet-to-dry ratio was slightly (8%) but significantly increased from control, while it was markedly increased in rabbits treated with DOSS (52%).

On ZEEP, the quasi-static inflation V-P curve shifted downwards in all groups of rabbits (Fig. 3); that of untreated and Curosurf treated rabbits became s-shaped, while that of animals receiving DOSS became markedly concave towards the volume axis. With $ZEEP_2$, the V-P curve shifted rightwards in untreated and, more markedly, in DOSS treated rabbits, while the opposite occurred in animals treated with Curosurf.

Elastance. On the basis of the V_o and $\Delta EELV$ values in Table 1, tidal ventilation occurred in the range 30-60 and 0-30%V_o on PEEP and ZEEP, respectively.

Relative to $PEEP_1$, no significant changes of Est occurred on $PEEP_2$ in control animals and untreated rabbits ventilated on ZEEP, whereas Est was increased significantly in animals treated with DOSS and, to a much lesser extent, Curosurf (Fig. 4). On the other hand, relative to corresponding post-treatment values on $PEEP_1$, Est on $PEEP_2$ decreased in rabbits treated with Curosurf (-40±8%; $P<0.001$), and increased in animals treated with DOSS (196±50%; $P<0.01$).

On ZEEP, Est increased significantly in all groups of rabbits (Fig. 4). Relative to $ZEEP_1$, Est increased with $ZEEP_2$ in untreated and DOSS treated rabbits (42±8 and 33±9%; $P<0.01$), whereas it decreased in Curosurf treated rabbits (-19±7%; $P<0.05$).

Rint. At the end-inspiratory volume of baseline ventilation, Rint did not change systematically with flow: hence the values of Rint obtained in each rabbit and condition were averaged.

Relative to $PEEP_1$, no significant changes of Rint occurred on $PEEP_2$ in control animals and in Curosurf treated animals (Fig. 4). In contrast, Rint was significantly increased in untreated rabbits ventilated on ZEEP (124±13%) and in DOSS treated animals (253±41%), in which it was also increased (181±26%; $P<0.001$) relative to corresponding post-treatment values on $PEEP_1$.

On ZEEP, Rint increased significantly in all groups of animals (Fig. 4). Relative to $ZEEP_1$, Rint increased with $ZEEP_2$ only in untreated and DOSS treated animals (117±31 and 52±13%; $P<0.01$).

Viscoelastic properties. The group mean relationships of ΔR to T_I are depicted in Figure 5, while the group mean values of Rvisc and τvisc are shown in Figure 4. Relative to $PEEP_1$, no significant changes of Rvisc and τvisc occurred on $PEEP_2$ in control animals, untreated rabbits ventilated on ZEEP, and Curosurf treated animals, while both parameters increased in rabbits treated with DOSS (Fig. 4).

On ZEEP, Rvisc increased significantly in all groups of animals, while τvisc increased signficantly only in Curosurf and DOSS treated animals (Fig. 4).

Histology

No focal alveolar collapse or epithelial desquamation, alveolar and peribronchial edema, damage of large airway (> 1mm) epithelium, and hemorrhages were observed, except in DOSS treated rabbits in which edema and scattered areas of alveolar collapse were present.

The results of measurements of airspace enlargement (Lm), peribronchiolar alveolar wall destruction (% abnormal attachments, distance between normal attachments), bronchiolar epithelial injury (IS), and trapping of polymorphonuclear leukocytes in the alveolar wall (cells) are shown in Table 2 for all groups of rabbits. The values of Lm did not differ significantly among the various groups of animals, whereas the percentage of abnormal attachments, the distance between normal attachments, and IS were significantly larger in untreated animals ventilated on ZEEP and in rabbits receiving DOSS than in untreated animals ventilated on PEEP only and in rabbits treated with Curosurf, in which all these values were similar. The number of polymorphonuclear leukocytes within the alveolar wall was significantly larger in all groups of animals ventilated on ZEEP than in rabbits ventilated on PEEP only. On the other hand, the cell count was markedly larger ($P<0.01$) in animals treated with DOSS than in untreated and Curosurf treated animals, in which cell counts were almost the same.

DISCUSSION

In line with previous results (9,10), prolonged mechanical ventilation at low lung volumes with physiologic tidal volumes of normal, open-chest rabbits caused histological damage of small airways, characterized by epithelial sloughing and rupture of alveolar-bronchiolar attachments, with a concurrent increase in airway resistance that persisted after restoration of physiological end-expiratory lung volume, while no mechanical alterations or signs of histological injury were observed when the end-expiratory lung volumes were kept within the physiological range with PEEP. The main finding of the present study is that administration of exogenous surfactant largely prevents both the mechanical alterations and the histological damage caused by ventilation at low lung volumes.

Immediate mechanical effects of Curosurf and DOSS administration. Intratracheal injection of exogenous surfactant in animals ventilated on PEEP caused an immediate, marked increase of Est, Rint (Fig. 4) and tissue viescoelasticity, as reflected by greater ΔR values at control inspiratory duration. These changes should be ascribed to the relatively large volume of injected fluid occluding a substantial amount of peripheral airways, because even greater mechanical effects were observed in 4 additional, open-chest rabbits after intratracheal instillation of 2.5 $ml \cdot kg^{-1}$ of saline. In this circumstance, the injected fluid reached most of the lung, as shown by the pulmonary distribution of dyed saline injected intratracheally, but that of exogenous surfactant might have been even more

uniform (1). In contrast, DOSS had no significant immediate mechanical effects during ventilation on PEEP (Fig. 4), in line with previous observations (30), the occasional elevation of Est and Rint being likely due to increased surface tension and bronchomotor tone with aerosol administration.

Immediate mechanical effects of ventilation on ZEEP. The pattern of the changes in lung mechanics with ventilation at low volumes differed among the various groups of animals. In untreated rabbits, Ptr tracings on $PEEP_1$ and of the first inflation on ZEEP superimposed during the end-inspiratory pause (Fig. 6A, right): because tissue elastic and viscoelastic properties remained unchanged, no changes in surface tension and dependent airway closure should have occurred during the first inflation on ZEEP. Although this is consistent with the observation that in general airway closure during deflation from large volumes occurs at negative transmural pressures (21), compression of the film lining the bronchiolar walls should have eventually resulted in film rupture and surfactant inactivation on repeated reexpansion (34), leading to airway closure with progressive increase of static and dynamic elastance and airway resistance (Fig. 6A, left). On the other hand, tissue elastic and viscoelastic properties and interrupter resistance increased since the first inflation on ZEEP in DOSS treated rabbits and the progressive increase of static and dynamic elastance and airway resistance was more pronounced than in untreated animals (Fig. 6B), this being consistent with the surfactant dysfunction caused by DOSS. An immediate increase of tissue elastic and viscoelastic properties and interrupter resistance occurred also in animals receiving exogenous surfactant (Fig. 6C, right), which should be, however, due to the injected fluid occluding a larger amount of small airways as their dimensions decreased with lung deflation. Indeed, at variance with untreated and DOSS treated rabbits, Curosurf treated animals did not exhibit a progressive increase of static and dynamic elastance (Fig. 6C, left).

Effects of prolonged ventilation at low volumes. On ZEEP, relative to $PEEP_1$, there was a significant increase of Est, Rint, and Rvisc in all animal groups (Fig. 4). This should be due to increased surface forces leading to greater lung stiffness and, in combination with reduced dimensions, to airway closure, gas trapping, microatelectasis, and hence decrease of ventilated tissue. Indeed, an increase of surface tension at low end-expiratory transpulmonary pressure and lung volume has been advocated to explain the changes of lung compliance in the absence of detectable airway closure (32,34). It could also explain the increase in Rvisc, since most of tissue viscoelasticity should reside in the air-liquid interface (2), while the concomitant reduction in ventilated tissue should cause proportional changes of Est and Rvisc leaving τvisc unaffected. Indeed, this occurred in untreated and Curosurf treated animals (Fig. 4), in which small airway closure and dependent gas trapping and microatelectasis should have been evenly distributed throughout the lung because, on visual inspection, lung expansion was apparently as uniform on ZEEP as on PEEP. Based on theoretical considerations,

diffuse alveolar collapse has been predicted to take place at low lung volumes (28,29), but visible areas of atelectasis and grossly inhomogeneous expansion occurred only in DOSS treated rabbits, thus explaining the significant changes of τvisc (Fig. 4). Accordingly, increased surface tension, dependent small airway closure and reduction of ventilated lung units should represent the main mechanisms leading to the mechanical modifications occurring with ventilation on ZEEP, with the important addition of the effects of lung edema in animals treated with DOSS, as suggested by the increased wet-to-dry ratio (Table 2).

In untreated and DOSS treated animals, all mechanical variables increased progressively during ventilation on ZEEP (Fig. 4). A progressive increase of dynamic lung elastance during mechanical ventilation at low lung volumes has been previously reported in normal open-chest rabbits and dogs (12,30). In contrast, Est and Rvisc decreased from $ZEEP_1$ to $ZEEP_2$ in Curosurf treated rabbits, reflecting the ongoing absorption of the injected fluid.

The increase of Rint on ZEEP (Fig. 4) could not be ascribed to a decrease in lung recoil (16), because at baseline V_T (i.e. the volume at which Rint was assessed) it was larger during ZEEP than PEEP (Fig. 3), nor to the changes in arterial blood gases or pH (Fig. 2), because hypercapnia and acidosis should exert a bronchodilating action (8) and a protective effect on ventilator induced lung injury (5). The increase in Rint should be instead related to *a)* reduction of ventilated tissue due to small airway closure (see above); *b)* uncoupling between peripheral airways and lung parenchyma, as suggested by the occurrence of a large number of abnormal alveolar attachments and increased distance between attachments, such that the airway caliber was reduced in spite of increased lung recoil; and *c)* increased bronchomotor tone due to release of inflammatory mediators, as suggested by the presence of polymorphonuclear leukocytes in the alveolar walls (Table 2). Mechanism *b* probably explains a substantial part of the changes of airway resistance in untreated and DOSS treated rabbits, because on $ZEEP_2$ the largest increase in Rint (~60 $cmH_2O \cdot s \cdot l^{-1}$ relative to $PEEP_1$ post-treatment; Fig. 4) occurred in these groups of animals, which exhibited a similar and greater increase of abnormal alveolar-bronchiolar attachments (Table 2). Mechanism *c* was likely not operating in untreated and Curosurf treated animals, because *a)* the number of polymorphonuclear leukocytes per unit length of alveolar septa was similar whilst the increase in Rint was markedly different, and *b)* no significant cytokine release has been shown to occur in untreated rabbits ventilated on ZEEP, at least as evaluated from TNF-α concentration in serum and broncho-alveolar lavage fluid (11). This mechanism might have, however, contributed in DOSS treated animals, in which the number of polymorphonuclear leukocytes was markedly greater than in the other two groups of rabbits ventilated on ZEEP (Table 2).

Persistent effects of prolonged ventilation at low volumes. After return to PEEP ($PEEP_2$), Est and Rvisc of untreated animal reversed to the initial ($PEEP_1$) values (Fig. 4), and the quasi-static inflation V-P curves superimposed (Fig. 3). A substantial reversal to pre-treatment conditions occurred also in Curosurf treated animals; the shape factor *K* of the quasi-static inflation V-P curve was in fact the same on $PEEP_1$ and $PEEP_2$, while the slight decrease of Vo and ΔEELV (Table 1) and increase in Est (Fig. 4) can be attributed to a small amount of the injected fluid still excluding some lung units, as indicated by the very modest increase of the wet-to-dry ratio (Table 1). In contrast, Est and Rvisc were markedly increased in DOSS treated animals, while Vo, ΔEELV, and *K* were markedly decreased. These changes should have been mostly due to lung edema, as indexed by the increased wet-to-dry ratio, and demonstrated on lung dissection by the presence of foam in peripheral airways, that developed during the period of ventilation at low volume because of the higher surface tension and alveolar collapsing forces caused by DOSS (19,20). Interestingly, in addition to increased Rint and small airway injury like in open-chest animals, lung edema and increased static elastance on $PEEP_2$ have been observed also in normal, closed-chest rabbits after 3-4 h of mechanical ventilation with an end-expiratory pressure of –7.7 cmH_2O (11), as well as in DOSS treated, open chest rabbits ventilated on NEEP (-2 to – 4 cmH_2O) but not on PEEP (30). Moreover, edema was likely the main cause of the reduced lung diffusing capacity in DOSS treated animals, because only in this group of rabbits hypoxia and hypercapnia were still marked on $PEEP_2$ (Fig. 2).

After return to PEEP, Rint remained markedly elevated in untreated rabbits, as well as in DOSS treated animals, both relative to pre- and post-treatment values on $PEEP_1$ (Fig. 4). In contrast, Rint of animals treated with exogenous surfactant did not differ significantly from baseline values, like in animals ventilated on PEEP only. The different behavior of Rint among the various groups paralleled that in histogical injury scores. Thus, indices of bronchiolar epithelial damage and destruction of alveolar-bronchiolar attachments were high in untreated or DOSS treated rabbits, but similar in control and Curosurf treated animals (Table 2). Both the histological damage and the concomitant increase of Rint have been attributed to cyclic opening and closing of peripheral airways (9-11). In all groups of animals, there was no evidence of airway closure during ventilation with PEEP, the static inflation V-P curve being concave to the pressure axis (Fig. 3), and the ratio between Est with the lowest inflation volume (~4 $ml \cdot kg^{-1}$) and Est with baseline V_T being less than unity (0.85±0.02). In contrast, the initial part of the inflation V-P curve on ZEEP became convex to the pressure axis (Fig. 3), reflecting progressive reopening of small airway (<1 mm; 15). Taking that ratio as an estimate of airway involvement in cyclic opening and closing, more airways should have been involved on $ZEEP_2$ in DOSS (3.16±0.44) than in untreated (1.34±0.09) and Curosurf treated rabbits (1.18±0.09). Hence, cyclic airway opening and closing on ZEEP should have been more marked in

DOSS treated and untreated animals, the only groups in which significant histological alterations of small airways were found (Table 2). Accordingly, it appears that *a)* the histological damage of small airways occurring with cyclic opening and closing during prolonged mechanical ventilation of normal lungs at low volume is the cause of the increase in airway resistance that persisted after restoration of physiological end-expiratory volumes; and *b)* these histological alterations in both normal and surfactant deficient lungs are due to high surface forces, as they were prevented with the administration of exogenous surfactant. This is consistent with the conclusions from theoretical model studies showing the primary role played by the surface tension in reducing airway opening pressure and limiting the stresses and deformation applied on reopening to airway epithelium and walls (13,14,18), as well as the results of a physical model study showing that the injury caused to pulmonary epithelial cells lining the bottom of a channel through which a bubble was made to progress was completely abated by the presence of adequate amounts of surfactant (4). Moreover, the "anti-glue" action which has been attributed to lung surfactant (26), could represent another mechanism preventing epithelial injury with repeated small airway reopening.

In conclusion, the present study has shown that administration of exogenous surfactant is capable to prevent the histological and functional damage caused in normal, open-chest rabbits by prolonged mechanical ventilation at low lung volumes with physiologic tidal volumes. It is shown for the first time that small airway injury with cyclic opening and closing during prolonged mechanical ventilation of normal lungs at low volume is the cause of the increase in airway resistance that persists after restoration of physiological end-expiratory volumes, and that the histological alterations of the small airways are due to the progressive increase of surface tension with ventilation at low lung volumes. Artificially induced surfactant dysfunction worsen the functional alterations caused by ventilation at low lung volumes, mainly because of edema formation, and, possibly, inflammatory response.

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Table 1. Values of Vo and *K* computed according to Eq.1 and ΔEELV during ventilation on PEEP and wet-to-dry ratio of the lung in control, untreated, Curosurf or DOSS treated rabbits

		Vo ml	*K* cm H_2O^{-1}	ΔEELV ml	wet/dry
Control	$PEEP_1$	78.2±3.4	0.186±0.006	21.9±0.6	
	$PEEP_2$	77.1±4.1	0.187±0.006	21.8±0.9	4.60±0.06
Untreated	$PEEP_1$	76.8±3.8	0.185±0.008	26.7±2.2	
	$PEEP_2$	76.3±4.2	0.180±0.011	26.8±2.4	4.73±0.11
Curosurf	$PEEP_1$	68.4±5.9	0.212±0.010	29.5±3.8	
	$PEEP_2$	62.7±6.4*	0.205±0.015	26.0±4.3†	4.98±0.11#
DOSS	$PEEP_1$	64.8±4.9	0.201±0.012	25.5±2.7	
	$PEEP_2$	42.9±3.8‡	0.121±0.019†	12.2±2.0‡	7.12±0.17§

Values are means±SE. Vo, maximum volume above resting lung volume; *K*, shape factor; ΔEELV, difference between end-expiratory and resting volume. Significantly different from corresponding values on $PEEP_1$: *P<0.05; †P<0.01; ‡P<0.001; significantly different from control: #P<0.05; §P<0.0001.

Table 2. Indices of emphysematous and inflammatory lesions and of bronchiolar injury in lungs after 3-4 h of ventilation on ZEEP or PEEP in control, untreated, Curosurf or DOSS treated rabbits

	Lm μ	IS %	A-A %	D μ	Cells mm^{-1}
Control	81 (76-102)	7.9 (3.2-14.8)	14.1 (9.9-19)	51 (44-71)	0.26 (0.18-0.33)
Untreated	84 (69-89)	32.6* (20.7-68.4)	31.5* (26.8-35.6)	87* (62-120)	1.23* (0.82-2.1)
Curosurf	88 (53-92)	15.8 (10-34.5)	17.8 (15.2-20.2)	51 (35-70)	1.30* (0.39-2.45)
DOSS	72 (49-100)	35.5* (30.6-55.3)	31.3* (27.2-56.3)	95* (77-136)	4.55* (3.22-6.84)

Values are medians with range in parentheses. Lm, mean linear intercept; A-A, percentage of ruptured alveolar-bronchiolar attachments; D, distance between normal alveolar-bronchiolar attachments; IS, bronchiolar injury score; Cells, number of polymorphonuclear leukocytes per unit length of alveolar septa. *Significantly different from corresponding values of control rabbits ($P<0.01$).

LEGENDS

Fig. 1. Time line representation of the procedure used in the four groups of animals. Dots indicate Curosurf and DOSS administration.

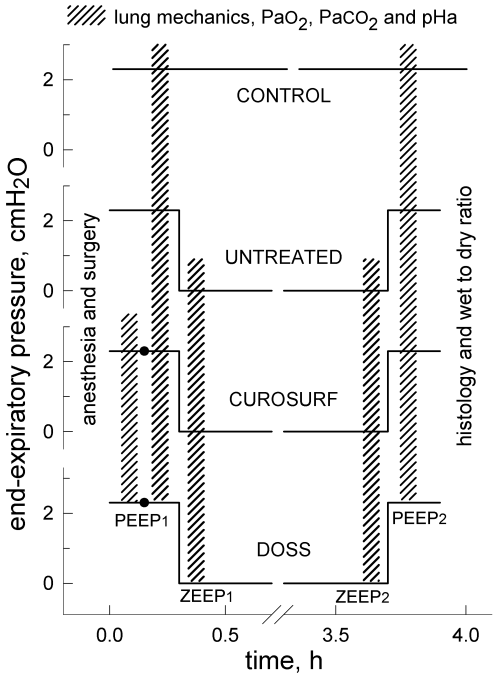
Fig. 2. Mean values of arterial P_{O_2}, P_{CO_2}, and pH in control, untreated, Curosurf or DOSS treated rabbits during mechanical ventilation on PEEP and ZEEP. Bars: SE. Values significantly different from corresponding ones on $PEEP_1$: *P<0.05; †P<0.01; ‡ P<0.001.

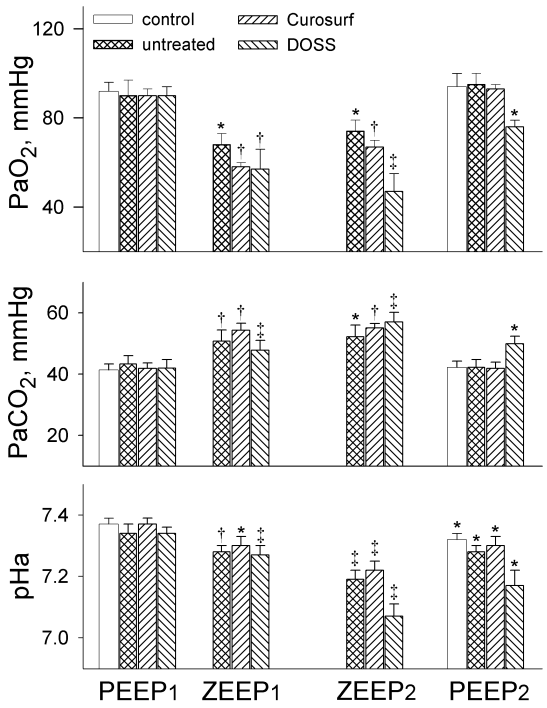
Fig. 3. Average relationship between volume above resting lung volume (ΔV) and quasi-static transpulmonary pressure obtained during ventilation with PEEP of 2.3 cmH_2O before ($PEEP_1$) and after 3-4 h of ventilation on ZEEP ($PEEP_2$), and during the initial ($ZEEP_1$) and final period ($ZEEP_2$) of ventilation on ZEEP (see key to symbols) in 8 untreated (**B**), Curosurf (**C**), and DOSS treated, open-chest rabbits (**D**), and in 8 open-chest rabbits (**A**) before ($PEEP_1$) and after 3-4 h of ventilation on PEEP only ($PEEP_2$). Bars: SE. In Curosurf and DOSS treated animals, data on $PEEP_1$ refer to pre-treatment condition. On PEEP, the data fit a monoexponential function.

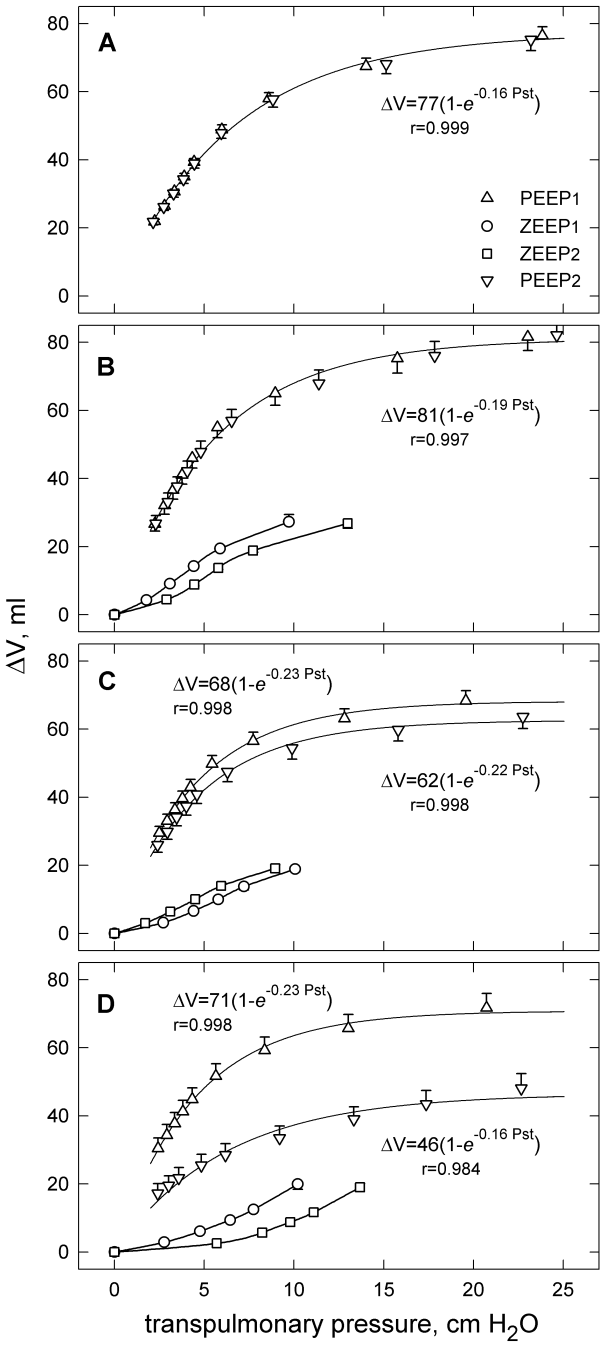
Fig. 4. Mean values of interrupter resistance (Rint), quasi-static elastance (Est), and viscoelastic resistance (Rvisc) and time constant (τvisc) in control, untreated, Curosurf or DOSS treated rabbits during mechanical ventilation on PEEP and ZEEP. Arrows indicate Curosurf or DOSS administration on $PEEP_1$. Bars: SE. Values significantly different from corresponding ones on $PEEP_1$: *P<0.05; †P<0.01; ‡ P=0.001.

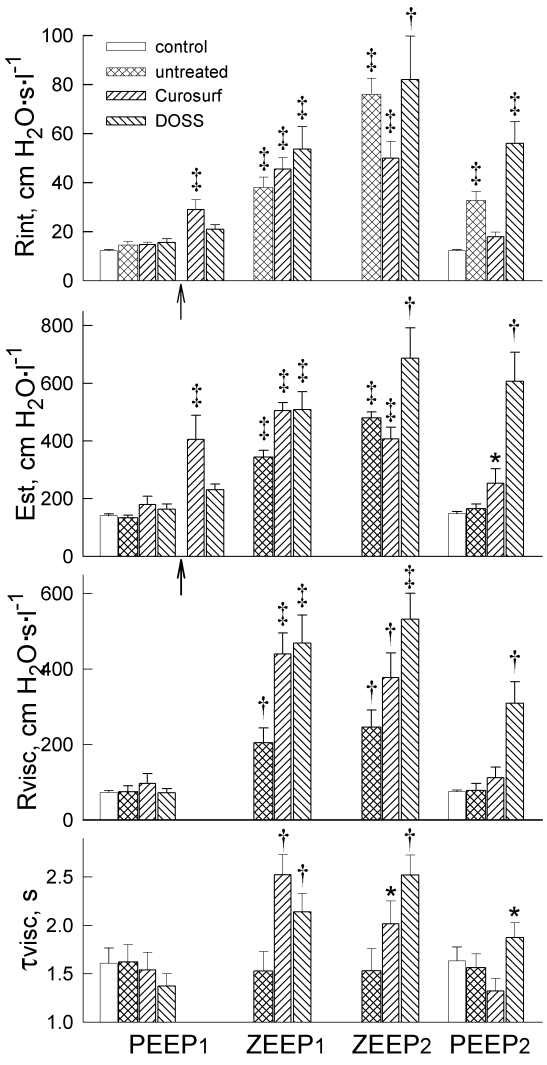
Fig. 5. Relationships of additional lung resistance (ΔR) to duration of inflation obtained during ventilation with PEEP of 2.3 cmH_2O before ($PEEP_1$) and after 3-4 h of ventilation on ZEEP ($PEEP_2$), at the beginning ($ZEEP_1$) and end of the 3-4 h period ($ZEEP_2$) of ventilation on ZEEP (see key to symbols) in 8 untreated (**B**), Curosurf (**C**), and DOSS treated, open-chest rabbits (**D**), and in 8 open-chest rabbits (**A**) before ($PEEP_1$) and after 3-4 h of ventilation on PEEP only ($PEEP_2$). Bars: SE. In Curosurf and DOSS treated animals, data on $PEEP_1$ refer to pre-treatment condition. Under all conditions, the data fit a monoexponential function.

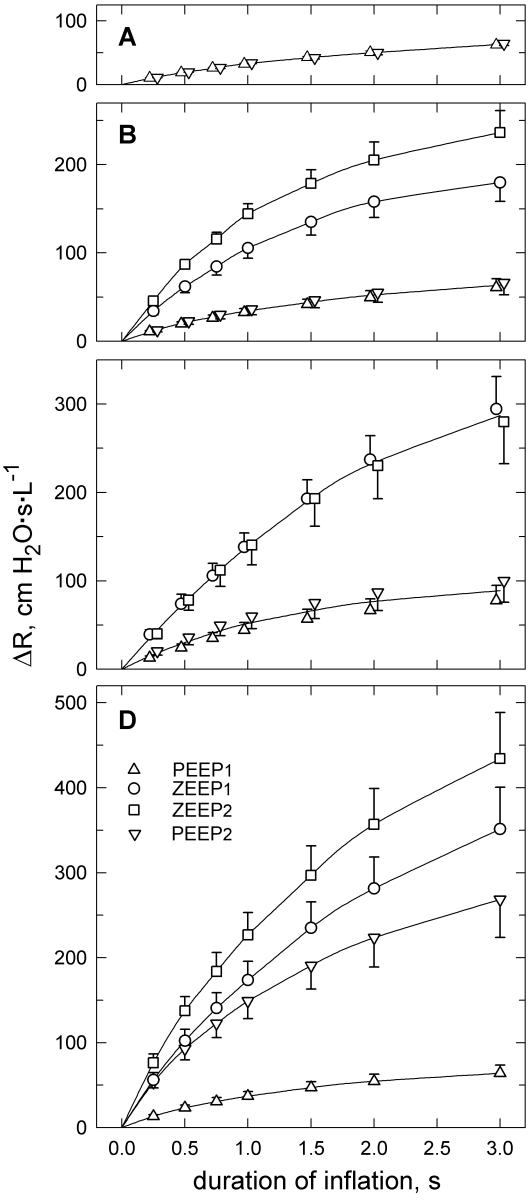
Fig. 6. Left: ensemble average of records of volume changes (ΔV) and tracheal pressure (Ptr) during baseline ventilation in 8 untreated (**A**), DOSS (**B**), and Curosurf treated, open-chest rabbits (**C**) at the transition from PEEP to ZEEP. Right: average records of tidal volume and tracheal pressure changes of the last inflation on $PEEP_1$ (continuous line) and the first inflation on $ZEEP_1$ (dotted line).

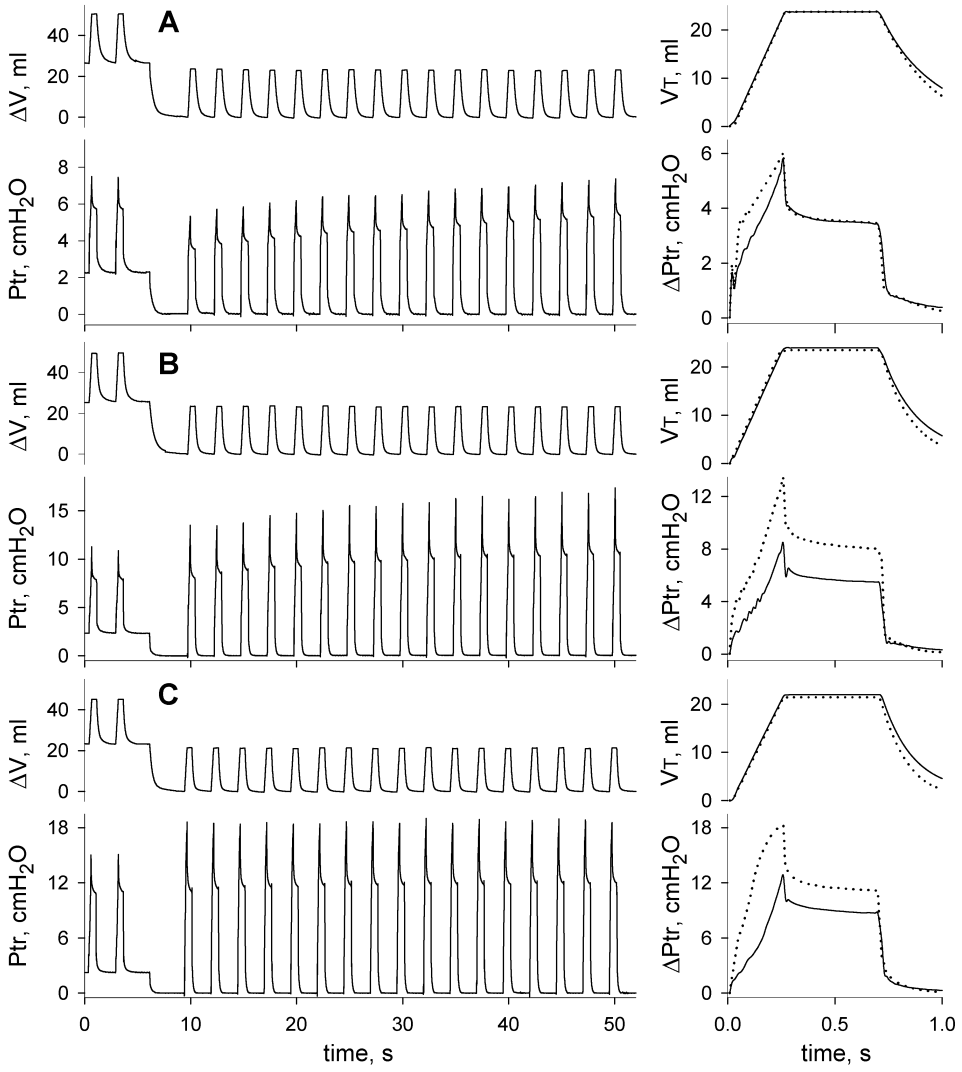

lung mechanics, PaO2, PaCO2 and pHa
CONTROL
UNTREATED
CUROSURF
DOSS
PEEP1
PEEP2
ZEEP1
ZEEP2
anesthesia and surgery
histology and wet to dry ratio
end-expiratory pressure, cmH2O
time, h
0.0
0.5
3.5
4.0


control
untreated
Curosurf
DOSS
120
80
40
PaO2, mmHg
60
40
20
PaCO2, mmHg
7.4
7.2
7.0
pHa
PEEP1
ZEEP1
ZEEP2
PEEP2


A
ΔV=77(1-e^-0.16 Pst)
r=0.999
PEEP1
ZEEP1
ZEEP2
PEEP2
B
ΔV=81(1-e^-0.19 Pst)
r=0.997
C
ΔV=68(1-e^-0.23 Pst)
r=0.998
ΔV=62(1-e^-0.22 Pst)
r=0.998
D
ΔV=71(1-e^-0.23 Pst)
r=0.998
ΔV=46(1-e^-0.16 Pst)
r=0.984
ΔV, ml
transpulmonary pressure, cm H2O


control
untreated
Curosurf
DOSS
Rint, cm H2O·s·l-1
Est, cm H2O·l-1
Rvisc, cm H2O·s·l-1
τvisc, s
PEEP1
ZEEP1
ZEEP2
PEEP2


A
B
D
PEEP1
ZEEP1
ZEEP2
PEEP2
ΔR, cm H2O·s·L-1
duration of inflation, s
0
100
200
300
400
500
0.0
0.5
1.0
1.5
2.0
2.5
3.0


A
B
C
ΔV, ml
Ptr, cmH2O
VT, ml
ΔPtr, cmH2O
time, s
time, s