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

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D’Angelo E, Pecchiari M, Gentile G. Dependence of lung injury on surface tension during low-volume ventilation in normal open-chest rabbits. J Appl Physiol 102: 174–182, 2007. First published September 7, 2006; doi:10.1152/japplphysiol.00405.2006.—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 eight normal, open-chest rabbits ventilated for 3–4 h at zero end-expiratory pressure (ZEEP) with physiological tidal volumes (VT = 10 ml/kg). 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 positive end-expiratory pressure (PEEP; 2.3 cmH2O), 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 eight 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 groups A and C, but not B, showed histological indexes of bronchiolar injury, whereas all groups exhibited an increased number of polymorphonuclear leukocytes in alveolar septa, which was significantly greater in group C. In conclusion, administration of surfactant largely prevents the histological and functional damage of prolonged MV at low lung volumes, whereas surfactant dysfunction worsens the functional alterations, also because of edema formation and, possibly, increased inflammatory response.

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/kg) and urethane (0.5 mg/kg). 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/kg) 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 was made just above the costal arch, while application of PEEP prevented lung collapse.

Airflow (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 cmH2O; 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. Arterial blood gases and blood pH were determined with a pH meter and a blood gas analyzer (model 2700; Radiometer, Copenhagen, Denmark).

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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 RS8300; Gould Electronics, Valley View, OH), sampled at 200 Hz by a 14-bit analog-to-digital converter, and stored on a desktop computer. Volume changes (ΔV) were obtained by numerical integration of the digitized airflow signal. Arterial blood Po2 (PaO2), PCO2 (PaCO2), and pH (pHa) 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 the surgical procedure was completed, 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, whereas Ringer bicarbonate was continuously infused intravenously at a rate of 4 ml·kg−1·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 cmH2O by means of a flow-through system. The baseline ventilator setting was kept constant throughout the experiment and consisted of a fixed tidal volume (Vt) of 10 ml/kg, 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-inspiratory pause (zero flow) and absence of P

Lung mechanics were assessed with the rapid airway occlusion (9), delivering water-saturated air from a high-pressure source (4 atm) at a rate of 4 ml·kg−1·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 cmH2O by means of a flow-through system. The baseline ventilator setting was kept constant throughout the experiment and consisted of a fixed tidal volume (Vt) of 10 ml/kg, 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-inspiratory pause (zero flow) and absence of P

Figure 1 provides a time line representation of the procedure used in the 4 groups of animals. Dots indicate Curosurf and dioctylsodiumsulfosuccinate (DOSS) administration. PaO2 and PaCO2, arterial Po2 and Po2, respectively; PEEP and ZEEP, positive and zero end-expiratory pressure, respectively (subscripts 1 and 2 refer to the periods of PEEP and ZEEP); pHa, arterial pH.
120°C, and weighed again to compute the wet-to-dry ratio, whereas the left lung was processed for histological analysis.

**Histological analysis.** The rabbits were given heparin (355 U/kg) and papaverine (5 mg/kg) 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 ventricle into the pulmonary artery. After three inflations to 25 cmH2O, the transpulmonary pressure was kept at 10 cmH2O, the ties were fastened, the right lung was removed, the right atrium cut, and the left lung was 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: 1) mean linear intercept (Lm), which is a measure of airspace enlargement, as described by Thurlbeck (31); 2) indexes of destruction of the alveolar attachments, which are the alveolar walls that extend radially from the outer wall of the nonrespiratory bronchioles (22); 3) presence of bronchiolar epithelial necrosis and sloughing, as a measure of bronchiolar injury (17); and 4) 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, six sections from each rabbit were examined at a magnification of ×100, and 10 nonoverlapping fields were analyzed on each section. The Lm value was obtained as the ratio between the length in micrometers 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 ×400 on a total of 60 fields randomly distributed across six slides for each animal.

For alveolar-bronchiolar coupling assessment, alveolar attachments of 50 nonrespiratory bronchioles per animal were examined at a magnification of ×200. Any discontinuity of the peribronchiolar alveolar wall qualified that wall as an abnormal attachment. Two indexes were obtained: 1) percent ratio of abnormal to total (normal and abnormal) attachments and 2) 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 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/kg of Evans blue-dyed saline was instilled into the trachea of eight open-chest rabbits. The lungs were immediately removed, connected to a source of compressed air, fixed dry at a distending pressure of 20 cmH2O, and finally cut perpendicularly to their major axis to obtain a total of eight to ten 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 \( P_{aO_2} \), \( P_{aCO_2} \), and pH obtained at the beginning and at the end of each test session did not differ significantly and were thus averaged. During PEEP1, the mean values of these parameters were similar for all groups of rabbits (Fig. 2). With PEEP2, pH was significantly reduced in all groups of animals, whereas \( P_{aO_2} \) and \( P_{aCO_2} \) were significantly decreased and, respectively, increased only in animals treated with DOSS. Relative to PEEP1, with ZEEP1 there was a similar increase of \( P_{aCO_2} \) and decrease of \( P_{aO_2} \) and pH in all groups of rabbits. No further changes took place on ZEEP2, except in animals treated with DOSS in which there was a significant increase of \( P_{aCO_2} \) and decrease of \( P_{aO_2} \) and pH.

**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 \( \Delta P - Ti \) data under all conditions, allowing computation of \( R_{visc} \) and \( r_{visc} \). During PEEP1, 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 \( R_{int} \), Est, \( R_{visc} \), and \( r_{visc} \) (Fig. 4). Administration of Curosurf caused an immediate and marked increase of Est, Rint, and \( \Delta R \), while with DOSS administration, the corresponding changes were smaller and nonsignificant (Fig. 4).

**Static inflation V-P relationships.** Ptp at end expiration was similar during PEEP1 and PEEP2, averaging 2.3 cm H2O in all groups. In untreated animals, \( \Delta EELV \) was also similar on PEEP1 and PEEP2, while it was significantly reduced on
PEEP2 both in Curosurf- and, markedly more, in DOSS-treated rabbits (Table 1).

In untreated animals, the inflation V-P curve was similar on PEEP1 and PEEP2, independent of ventilation on ZEEP, whereas in Curosurf- or DOSS-treated rabbits, the V-P curve shifted downward with PEEP2 (Fig. 3). As a consequence, Vo and K in Eq. 1 remained unchanged in untreated animals, Vo decreased significantly with PEEP2 in rabbits receiving both Curosurf and DOSS, whereas K changed significantly in animals treated only 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, whereas it was markedly increased in rabbits treated with DOSS (52%).

On ZEEP, the quasi-static inflation V-P curve shifted downward in all groups of rabbits (Fig. 3); that of untreated and Curosurf-treated rabbits became S shaped, whereas that of animals receiving DOSS became markedly concave toward the volume axis. With ZEEP2, the V-P curve shifted rightward in all groups of rabbits (Fig. 3); that of untreated and, more markedly, in DOSS-treated rabbits, whereas the opposite occurred in animals treated with Curosurf.

Elastance. On the basis of V0 and ΔEELV values in Table 1, tidal ventilation occurred in the range 30 – 60 and 0 – 30% VO on PEEP and ZEEP, respectively.

Relative to PEEP1, no significant changes of Est occurred on PEEP2 in control animals and in Curosurf-treated animals (Fig. 4). In contrast, Est 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 posttreatment values on PEEP1.

On ZEEP, Est increased significantly in all groups of animals (Fig. 4). Relative to ZEEP1, Est increased with ZEEP2 and similar to that of freshly excised lungs (9).

### 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

<table>
<thead>
<tr>
<th></th>
<th>ΔVo, ml</th>
<th>K, cmH2O l⁻¹</th>
<th>ΔEELV, ml</th>
<th>Wet/Dry</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control PEEP1</td>
<td>78.2 ± 3.4</td>
<td>1.08 ± 0.006</td>
<td>21.9 ± 0.6</td>
<td>0.015</td>
</tr>
<tr>
<td>Untreated PEEP1</td>
<td>77.1 ± 4.1</td>
<td>1.08 ± 0.006</td>
<td>21.8 ± 0.9</td>
<td>0.008</td>
</tr>
<tr>
<td>Curosurf PEEP1</td>
<td>76.8 ± 3.8</td>
<td>1.05 ± 0.008</td>
<td>26.7 ± 2.2</td>
<td>0.012</td>
</tr>
<tr>
<td>DOSS PEEP1</td>
<td>68.4 ± 5.9</td>
<td>0.21 ± 0.010</td>
<td>29.5 ± 3.8</td>
<td>0.010</td>
</tr>
<tr>
<td>Untreated PEEP2</td>
<td>62.7 ± 6.4</td>
<td>0.20 ± 0.015</td>
<td>26.0 ± 4.4</td>
<td>0.012</td>
</tr>
<tr>
<td>Curosurf PEEP2</td>
<td>64.8 ± 4.9</td>
<td>0.20 ± 0.012</td>
<td>25.5 ± 2.7</td>
<td>0.012</td>
</tr>
<tr>
<td>DOSS PEEP2</td>
<td>42.9 ± 3.8</td>
<td>0.12 ± 0.019†</td>
<td>12.2 ± 2.0</td>
<td>0.012</td>
</tr>
</tbody>
</table>

Values are means ± SE. Vo, maximum volume above resting lung volume; K, shape factor; ΔEELV, difference between end-expiratory and resting volume; DOSS, diocytlsodiumsulfosuccinate. Significantly different from corresponding values on positive end-expiratory pressure. (PEEP1); *P < 0.05; †P < 0.01; ‡P < 0.001; significantly different from control: *P < 0.05; †P < 0.0001.

Fig. 3. Average relationship between volume above resting lung volume (ΔV) and quasi-static transpulmonary pressure obtained during ventilation with PEEP of 2.3 cmH2O before (PEEP1) and after 3 – 4 h of ventilation on ZEEP (PEEP2), and during the initial (ZEEP1) and final period (ZEEP2) of ventilation on ZEEP (see key to symbols) in 8 untreated (A), Curosurf (C), and DOSS-treated, open-chest rabbits (D), and in 8 open-chest rabbits (A) before (PEEP1) and after 3 – 4 h of ventilation on PEEP only (PEEP2). Bars, SE. In Curosurf- and DOSS-treated animals, data on PEEP refer to pretreatment condition. On PEEP, the data fit a monoexponential function. Pst, quasi-static pressure.
ZEEP only in untreated and DOSS-treated animals (117 ± 31 and 52 ± 13%; \( P < 0.01 \)).

**Viscoelastic properties.** The group mean relationships of \( R \) to \( T \) are depicted in Fig. 5, whereas the group mean values of \( R_{visc} \) and \( \tau_{visc} \) are shown in Fig. 4. Relative to PEEP, no significant changes of \( R_{visc} \) and \( \tau_{visc} \) occurred on PEEP in control animals, untreated rabbits ventilated on ZEEP, and Curosurf-treated animals, whereas both parameters increased in rabbits treated with DOSS (Fig. 4).

On ZEEP, \( R_{visc} \) increased significantly in all groups of animals, whereas \( \tau_{visc} \) increased significantly 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 (>1 mm) 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 physiological 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, whereas 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 viscoelasticity, as reflected by greater AR 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 four additional, open-chest rabbits after intratracheal instillation of 2.5 ml/kg 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, and of the first inflation on ZEEP were 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, 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, because most of tissue viscoelasticity should reside in the air-liquid interface (2), whereas the concomitant reduction in ventilated tissue should cause proportional changes of Est and Rvisc, leaving $\gamma_{visc}$ 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 $\gamma_{visc}$ (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).

Table 2. Indexes 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

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

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, ZEEP, zero end-expiratory pressure. *Significantly different from corresponding values of control rabbits ($P < 0.01$).
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 to ZEEP2 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 VT (i.e., the volume at which Rint was assessed) it was larger during ZEEP than PEEP (Fig. 3), nor could the increase be ascribed 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 1) reduction of ventilated tissue due to small airway closure (see above); 2) 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 3) increased bronchomotor tone due to release of inflammatory mediators, as suggested by the presence of polymorphonuclear leukocytes in the alveolar walls (Table 2). Mechanism 2 probably explains a substantial part of the changes of airway resistance in untreated and DOSS-treated rabbits, because on ZEEP2 the largest increase in Rint (~60 cmH2O·s−1·l−1 relative to PEEP1 posttreatment; Fig. 4) occurred in these groups of animals, which exhibited a similar and greater increase of abnormal alveolar-bronchiolar attachments (Table 2). Mechanism 3 was likely not operating in untreated and Curosurf-treated animals, because 1) the number of polymorphonuclear leukocytes per unit length of alveolar septa was similar whereas the increase in Rint was markedly different, and 2) no significant cytokine release has been shown to occur in untreated rabbits ventilated on ZEEP, at least as evaluated from tumor necrosis factor-α concentration in serum and bronchoalveolar 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 (PEEP2), Est and Rvisc of untreated animals reversed to the initial (PEEP1) values (Fig. 4) and the quasi-static inflation V-P curves were superimposed (Fig. 3). A substantial reversal to pretreatment 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 PEEP1 and PEEP2, whereas the slight decrease of $V_{o}$ and $\Delta EELV$ (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 Rs were markedly increased in DOSS-treated animals, whereas $V_o$, $\Delta EELV$, 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$_2$O (11), as well as in DOSS-treated, open-chest rabbits ventilated on negative ($-3$ cmH$_2$O) but not on positive (2 cmH$_2$O) end-expiratory pressure (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 were hypoxia and hypercapnia 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 posttreatment 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 histological injury scores. Thus indexes 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/kg) and Est with baseline $V_r$ 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; Ref. 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 1) 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 2) 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 that 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 of preventing the histological and functional damage caused in normal, open-chest rabbits by prolonged mechanical ventilation at low lung volumes with physiological 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 worsens the functional alterations caused by ventilation at low lung volumes, mainly because of edema formation, and, possibly, inflammatory response.

REFERENCES


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