Effect of surfactant on regional lung function in an experimental model of respiratory distress syndrome in rabbit

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Bayat S, Porra L, Broche L, Albu G, Malaspinas I, Doras C, Strengell S, Peták F, Habre W. Effect of surfactant on regional lung function in an experimental model of respiratory distress syndrome in rabbit. J Appl Physiol 119: 290–298, 2015. First published May 21, 2015; doi:10.1152/japplphysiol.00047.2015.—We assessed the changes in regional lung function following instillation of surfactant in a model of respiratory distress syndrome (RDS) induced by whole lung lavage and mechanical ventilation in eight anaesthetized, paralyzed, and mechanically ventilated New Zealand White rabbits. Regional specific ventilation (sV˙) was measured by K-edge subtraction synchrotron computed tomography during xenon washin. Lung regions were classified as poorly aerated (PA), normally aerated (NA), or hyperinflated (HI) based on regional density. A functional category was defined within each class based on sV˙ distribution (High, Normal, and Low). Airway resistance (Raw), respiratory tissue damping (G), and elasstance (H) were measured by forced oscillation technique at low frequencies before and after whole lung saline lavage-induced (100 ml/kg) RDS, and 5 and 45 min after intratracheal instillation of beractant (75 mg/kg). Surfactant instillation improved Raw, G, and H (P < 0.05 each), and gas exchange and decreased atelectasis (P < 0.001). It also significantly improved lung aeration and ventilation in atelectatic lung regions. However, in regions that had remained normally aerated after lavage, it decreased regional aeration and increased sV˙ (P < 0.001) and sV heterogeneity. Although surfactant treatment improved both central airway and tissue mechanics and improved regional lung function of initially poorly aerated and atelectatic lung, it deteriorated regional lung function when local aeration was normal prior to administration. Local mechanical and functional heterogeneity can potentially contribute to the worsening of RDS and gas exchange. These data underscore the need for reassessing the benefits of routine prophylactic vs. continuous positive airway pressure and early “rescue” surfactant therapy in very immature infants.

respiratory distress syndrome; synchrotrons; respiratory mechanics; pulmonary surfactants; xenon; tomography; X-ray computed

SURFACTANT ADMINISTRATION is recognized as an effective therapy for immature infants with respiratory distress syndrome (RDS) due to surfactant deficiency (23). Exogenous surfactant has been studied either as a rescue treatment of infants with established RDS or as a prophylactic measure at birth. Several randomized, controlled trials have shown that rescue therapy by surfactant replacement in immature infants with RDS reduces the incidence of pneumothoraces and emphysema, and decreases mortality (10, 14, 17, 28, 32). Although the preventive administration of exogenous surfactant was initially shown to have beneficial effects on morbidity and mortality in infants at high risk of developing RDS, these beneficial effects were no longer demonstrated with the advent of routine application of continuous positive airway pressure (CPAP). Actually, a higher incidence of death has been observed in infants preventively treated with surfactant, compared with those stabilized on CPAP alone (23, 26, 29). These findings raise the question of the mechanisms through which exogenous surfactant can positively or potentially adversely affect lung function.

Histological studies of the lungs of infants dying of RDS have shown areas of atelectasis and others of expansion, with a remarkably heterogeneous spatial distribution (7, 16). We previously found that, in a surfactant depletion model of RDS in rabbit, the ventilation mechanics of the lung periphery is also highly heterogeneous, ranging from complete atelectasis and cyclic recruitment to gas trapping (2). Mechanical ventilation can potentially worsen this condition by further injuring the lung because of the concentration of mechanical stresses (5), particularly at the boundary between open and collapsed alveoli (19, 21). This adverse effect of mechanical ventilation could be involved in the development of bronchopulmonary dysplasia (BPD) in preterm infants surviving RDS (30). The acute beneficial effects of surfactant on lung mechanics and gas exchange have been shown to be due to a rapid increase in FRC (9) and reduced shunt (33). Currently, it is not known how the administration of surfactant might differentially affect the regional ventilation and mechanical behavior of peripheral lung units in RDS. This is an important question for at least two reasons: 1) it is not known to what extent the increase in FRC is due to the recruitment of atelectatic regions, or to the distension of already aerated lung zones by an improved regional compliance. The description of these mechanisms is important, because they determine how mechanical stresses are distributed within the lung tissue during mechanical ventilation; 2) since the tracheally instilled viscous solution of exogenous surfactant is distributed through the branching airways among both atelectatic and aerated peripheral pulmonary regions, it is not known how exogenous surfactant might affect the function of aerated and ventilated regions within the lung.

We previously found that by using K-edge subtraction CT imaging (KES), a technique that uses synchrotron-generated monochromatic X-rays, the regional functional behavior of the lung periphery could be characterized by combined measure-

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ments of aeration and regional ventilation. This is because with this technique, both the lung structure and regional ventilation can be assessed within the same CT images. Furthermore, the combination of this method with global measurements of the lung airway and tissue mechanics with forced oscillatory impedance (FOT) gives further insight into the mechanical behavior of the respiratory system (33).

The goal of the present study was to assess the short-term effects of exogenous surfactant administration on regional lung aeration and the distribution of regional ventilation by KES imaging, in a model of RDS induced by surfactant depletion by whole lung lavage, followed by mechanical ventilation in anesthetized rabbits. The measurements were confronted with the forced oscillatory mechanics of the respiratory system in normal lung and after RDS induction.

MATERIALS AND METHODS

Animal preparation. The procedures for the animal care and the experiments were in accordance with the Directive 2010/63/EU of the European Parliament on the protection of animals used for scientific purposes (6) and were approved by the Internal Evaluation Committee for Animal Welfare in Research of the European Synchrotron Radiation Facility (Grenoble, France). Eight male New Zealand rabbits were involved in the experiments (2.9 ± 0.1 kg). Anesthesia was induced by intravenous (iv) injection of sodium thiopental (25 mg/kg) via a catheter (22 G) introduced into the marginal ear vein under local anesthesia (5% topical lidocaine). The animals were tracheotomized with a No. 3 Portex tube (Smiths medical, Kent, United Kingdom) and were mechanically ventilated by a commercial neonatal ventilator (Servo-I, Maquet Critical Care, Solna, Sweden) with an electronic modification that allowed synchronizing mechanical ventilation with the image acquisition. The initial ventilator settings were as follows: tidal volume: 7 ml/kg; respiratory rate: 40 l/min; positive end-expiratory pressure (PEEP): 3 cmH2O; inspired oxygen fraction (FiO2): 0.5. These ventilator settings resulted in an end-tidal CO2 (ETCO2) of 3.5-6.5 kPa.

The left carotid artery and jugular vein were catheterized for blood gas measurements and for drug delivery. Anesthesia was then maintained with 0.1 mg kg⁻¹ h⁻¹ iv midazolam and analgesia was ensured by iv administration of fentanyl (50 µg·kg⁻¹·h⁻¹). After ensuring adequate anesthesia from the hemodynamic parameters, continuous iv infusion of atracurium (1.0 mg·kg⁻¹·h⁻¹) was started. The animal was immobilized in the vertical position in a cylindrical polynyl chloride custom-made holder for imaging.

Synchrotron radiation computed tomography imaging. The experiments were performed at the Biomedical Beamline of the European Synchrotron Radiation Facility (Grenoble, France). The K-edge subtraction (KES) imaging technique allows quantitative measurements of regional specific ventilation (sV) as well as lung tissue density. A detailed description of the methodology and instrumental setup has been extensively discussed in previous studies (1, 3, 20, 24). This imaging technique uses two X-ray beams at slightly different energies above and below the Xe K-edge energy (34.56 keV). X-rays from a synchrotron radiation source are required since, as opposed to standard X-ray sources, they allow the selection of monochromatic beams from the full X-ray spectrum while conserving enough intensity for imaging with sufficient temporal resolution. Two computed tomography images are thus simultaneously acquired and subtracted during the inhalation of stable Xe (20%) in air. The density due to tissue and to Xe can be separately calculated in each image voxel by a specifically developed computer algorithm (1) explained in detail elsewhere (25).

The “Xe density” image allows the direct quantitative measurement of this gas within the airways and that of the regional gas volume. Dynamic KES imaging during Xe washin or washout allows quantitative measurement of the regional lung aeration (2).

Image analysis. Images were processed with the MATLAB programming package (Mathworks, Natick, MA), as described previously (2). Lung tissue was selected within the tissue density computed tomography images by region-growing segmentation. The local specific ventilation, defined as ventilation normalized by the gas volume within the voxel (sV), was calculated from the time constant of the Xe washin by a single compartment model fit of Xe concentration vs. time (24). A 5 × 5 pixel moving average window was applied to the Xe density images prior to the model fit. In each sV image, the histogram of sV was calculated and fit with a log-normal function. The median (μ) and standard deviation (σ) of the distribution were extracted from the fit. Normal, high, and low sV were defined with reference to the median value of each slice at baseline. First, the lung tissue density (D) in grams per milliliter was converted to Hounsfield units (15). The area of lung comprised within the images was computed and totaled over the four axial image slices to calculate the total lung area. A density below −900 Hounsfield units was used to determine the area of lung zones where hyperinflation was most likely to occur (34). Lung regions with a density of −900 to 0 Hounsfield units were qualified as normally aerated (NA), regions with density of −500 to −100 as poorly aerated (PA), and atelectasis was defined as lung regions with a density from −100 to 0 Hounsfield units, based on previous studies in the literature (8, 34). To characterize the functional behavior of normally aerated, poorly aerated, and hyperinflated lung regions, the area of lung within each category was further divided into subcategories as follows: no ventilation: sV < 0.5 min⁻¹; low sV: 0.5 min⁻¹ < sV < (μ − 2σ); normal sV: sV = μ ± 2σ; high sV: (μ + 2σ) < sV. Trapping was defined as aerated areas with no sV. Comparison of lung aeration and sV was performed pixel by pixel, and each subcategory was expressed as percentage of the total lung area within the image slice.

Measurement of respiratory mechanics. The airway and respiratory tissue parameters were assessed by the forced oscillation technique at low frequencies. These measurements were achieved by introducing a loudspeaker-generated small-amplitude (1 cmH2O peak to peak) pressure forcing signal (0.5–21 Hz) into the trachea via a polyethylene tube (100 cm length, 0.375 cm ID) while the mechanical ventilation was paused at end-expiration. The input impedance of the respiratory system (Zrs) was computed as described previously (22). Three to five Zrs spectra were ensemble averaged under each experimental condition. A model that includes airway resistance (Raw), inertances (Iaw) in series with constant-phase tissue compartments incorporating tissue damping (G), and elastance (H) was fitted to the averaged Zrs data (13).

Study protocol. A recruitment maneuver (RM) was performed by inflating the respiratory system to a peak pressure of 30 cmH2O to standardize volume history. Ten minutes were allowed for stabilization of the physiological parameters, a set of Zrs recordings were collected during short end-expiratory pauses, followed by acquisition of 12 subsequent KES images during Xe washin at four approximately equidistant axial positions from the apical (nondependent) to the caudal (dependent) lung. The axial positions were standardized based on the apex-diaphragm distance, measured on a projection image. After baseline measurements, whole lung lavages were performed by instilling 0.9% saline at 37°C into the endotracheal cannula, and removed by gentle manual suctioning. The ventilation was resumed for 2 min and the procedure was repeated. A total volume of 100 ml/kg was instilled over five sequential lavages. After lavage, an RM was performed and all data acquisitions were repeated, after a stabilization interval of 10 min. At the end of data acquisition, beractant (Survanta, 200 mg/8 ml) was instilled in two 4-ml aliquots, through a 1-mm-internal-diameter polyethylene catheter introduced in the tracheal cannula, in supine position. After the first instillation, the ventilation was reconnected for 10 to 15 s. The second instillation was followed by three manual sighs, up to 30 cmH2O. Respiratory me-
Mechanical and imaging data acquisitions were repeated at 5 min (Surfactant-1) and 45 min (Surfactant-2) after surfactant. To verify the effect of surfactant instillation in lung regions with normal aeration and sV, Zrs and KES image data were obtained following the same protocol as above, in one control animal at baseline and once after surfactant was instilled.

Statistical analysis. The scatters in the parameters were expressed by the SEM values, except for blood gas data where scatter was expressed as interquartile range. The Shapiro-Wilk test was used to test data for normality. Respiratory mechanical parameters were tested by the Friedman repeated measures analysis of variance on ranks. Two-way repeated measures ANOVA was applied to evaluate the changes in imaging parameters with gravitational level (1: nondependent; 2, 3, and 4: dependent) and experimental condition (baseline; lavage; surfactant1; surfactant2) as within-subject variables. Blood gas data were analyzed by one-way repeated measures ANOVA. Pairwise comparisons were performed by Student-Newman-Keuls multiple comparison procedures. The statistical analyses were conducted with SigmaPlot (version 11.0, Systat Software, Chicago, IL). All statistical tests were carried out with a significance level of $P < 0.05$.

RESULTS

Respiratory mechanics and gas exchange. The airway and respiratory tissue mechanical parameters are shown in Fig. 1. Whole lung lavage led to a significant deterioration in tissue elastance, with a significant increase in H ($P = 0.001$) and a smaller rise in tissue damping, G ($P = 0.074$). These changes were associated with a significant increase in Raw ($P < 0.05$). Lavage also led to a significant decrease in arterial blood oxygenation (Table 1). Administration of surfactant significantly improved oxygenation ($P = 0.0013$). Surfactant significantly improved H by 45 min after administration (Fig. 1).

Regional lung function. Figure 2 shows sample KES “tissue density” images, ventilation images depicting the distribution of sV, and spatial distribution of the categories defined based on aeration and sV in a representative rabbit. Whole lung lavage led to the appearance of patchy lung regions with varying degrees of poor aeration down to large areas of complete atelectasis. This heterogeneous deterioration of the regional ventilation resulted in a redistribution of ventilation to the remaining normally aerated lung regions, which were then unevenly hyperventilated. Lavage-induced injury caused a significant increase in regional ventilation heterogeneity, as measured by the coefficient of variation (CV) of sV (Fig. 3).

The relative area of lung regions in each of the categories defined based on aeration and sV are summarized in Fig. 4. Following lavage, the area of atelectatic and poorly aerated areas increased. Part of the poorly aerated regions had significantly faster specific ventilation due to the reduction in gas volume in these compartments. However, the majority of these regions had normal or slow sV. A significant subset of the injured lung regions that were normally aerated had a high sV, suggesting ventilation redistribution from atelectatic and poorly ventilated regions.

Figure 5 shows the effect of gravity on the changes in regional lung function in the different experimental conditions. The distribution in selected functional categories are shown separately in each of the four axial image levels, where Level 1 is the least, and Level 4 is most dependent with respect to gravity. Small areas with gas trapping appeared after surfactant instillation, particularly in the nondependent lung regions (Level 1). Atelectasis was strongly gravity dependent and occurred significantly more in the caudal lung images. Conversely, the redistribution of ventilation resulted in a significant increase in areas with normal aeration and high sV in the least dependent lung region. Poor aeration was equally distributed between the axial image levels in the injured lung, but showed significant gravity dependence after surfactant instillation.

Surfactant had a different effect on regional lung function depending on the functional behavior prior to administration. In the lavage-injured lung, surfactant converted a significant fraction of completely atelectatic lung to poorly aerated regions (Fig. 2). Surprisingly, after surfactant instillation, some areas of the injured lung that were normally aerated became poorly aerated (Fig. 2). These phenomena are quantitatively depicted in Figs. 4 and 6.

The increase in the amount of poorly aerated regions after surfactant was not entirely accounted for by the reduction in atelectasis [from 10.5 to 3.1% of the total imaged lung regions of interest (ROI)], and was concomitant with an average drop in the area of normally aerated regions (~9.7%) and an
increase in trapping (+1.8%, NS). To verify the effect of surfactant instillation in spatially defined lung regions categorized based on aeration (Fig. 4), individual ROIs corresponding to each aeration category (NA, PA, HI, or atelectasis) were studied. The same topographic ROI was then reanalyzed separately in each experimental condition. The results of this analysis are presented in Table 2 and further detailed based on regional sV˙ in Fig. 6. Surfactant instillation was effective in reducing the area of poorly aerated regions after lavage; 48% of these regions became normally aerated, while a small but significant fraction (4%) became atelectatic. Also, ventilation heterogeneity within these regions, which was significantly higher than that of the normally aerated zones, was improved by surfactant instillation (PA and Atelectasis in Table 2). Similarly, a large fraction of atelectatic regions were converted to poorly aerated and

Table 1. Arterial blood gases

<table>
<thead>
<tr>
<th></th>
<th>Baseline</th>
<th>Lavage</th>
<th>Surfactant</th>
</tr>
</thead>
<tbody>
<tr>
<td>PaO₂, mmHg</td>
<td>303.4 (26)</td>
<td>103.1 (49.5)*</td>
<td>240.6 (179.5)†</td>
</tr>
<tr>
<td>pH</td>
<td>7.37 (0)</td>
<td>7.35 (0.1)</td>
<td>7.32 (0.1)</td>
</tr>
<tr>
<td>PaCO₂, mmHg</td>
<td>36 (2)</td>
<td>42.7 (9.5)</td>
<td>45.6 (4)</td>
</tr>
<tr>
<td>HCO₃⁻, mmol/l</td>
<td>20.3 (3.1)</td>
<td>23.2 (6.7)</td>
<td>23 (3.1)</td>
</tr>
</tbody>
</table>

Data are means (interquartile range). *P < 0.05 versus baseline; †P < 0.005 versus lavage.

Fig. 2. Sample composite images showing the distribution of specific ventilation (sV˙) (top row) and tissue density (middle row) in a single image slice in one representative animal at baseline, after whole lung lavage-induced RDS, and at 5 and 45 min after surfactant instillation (Surfactant-1 and Surfactant-2, Respectively) Bottom row: topographic distribution of lung regions defined as atelectatic, trapped, poorly aerated (PA) normally aerated (NA), or hyperinflated (HI). Color scale indicates subregions with high, normal, or low specific ventilation (sV˙) within the PA, NA, and HI categories. Note that aeration improved in atelectatic regions (red) after surfactant, while it was reduced in normally aerated regions (green), particularly in the left lung.

Fig. 3. Overall ventilation heterogeneity assessed as the coefficient of variation (CV) of sV˙. §P < 0.05 vs. baseline.

Fig. 6. Overall ventilation heterogeneity assessed as the coefficient of variation (CV) of sV˙. §P < 0.05 vs. baseline.
but also resulted in a spectrum of different sV˙ values within each category. Color scale indicates subregions with high, normal, or low sV within each category. *P < 0.05 vs. baseline; **P < 0.001 vs. baseline; #P < 0.001 vs. Lavage; § P < 0.05 vs. baseline; # P < 0.05 vs. Level 4. ROI, regions of interest.

normally aerated regions after surfactant. However, these positive outcomes went along with a decreased aeration in 32% of the lung regions that were normally aerated after lavage (NA in Table 2), with a small fraction of these becoming atelectatic. In the NA regions, ventilation heterogeneity (CV of sV) increased after surfactant administration. Figure 6 shows that surfactant not only modified the distribution of aeration within each aeration category ROI, but also resulted in a spectrum of different sV values within these regions. The differences in the relative areas of NA, PA, and atelectatic regions did not significantly change with time after the instillation of surfactant (Surfactant-1 vs. Surfactant-2).

To verify the effect of surfactant in normally aerated and ventilation lung regions, one control rabbit with healthy lungs was studied at baseline and after beractant instillation to determine the percentage of the ROI area that became normally aerated (NA), poorly aerated (PA), atelectatic (atelectasis), or did not change. CV, coefficient of variation. *P < 0.05 versus Lavage; † P < 0.001 vs. Lavage; §P < 0.001 vs. NA.

Table 2. Quantitative analysis of the effect of surfactant in spatially defined ROIs where aeration was either normal, poor, or absent after lavage-induced lung injury

<table>
<thead>
<tr>
<th>% ROI Area</th>
<th>Lavage</th>
<th>Surfactant-1</th>
<th>Surfactant-2</th>
</tr>
</thead>
<tbody>
<tr>
<td>NA → HI</td>
<td>0</td>
<td>1.3 ± 0.27†</td>
<td>1.5 ± 0.25†</td>
</tr>
<tr>
<td>NA → NA</td>
<td>100</td>
<td>65.1 ± 2.94‡</td>
<td>67.6 ± 3.43‡</td>
</tr>
<tr>
<td>NA → PA</td>
<td>0</td>
<td>31.5 ± 2.72‡</td>
<td>29.1 ± 3.30‡</td>
</tr>
<tr>
<td>NA → atelectasis</td>
<td>0</td>
<td>2.1 ± 0.49*</td>
<td>1.8 ± 0.34*</td>
</tr>
</tbody>
</table>

Within-ROI CV of sV

| NA   | 0.24 ± 0.08 | 0.36 ± 0.23* | 0.33 ± 0.18 |
| PA   | 0           | 0.4 ± 0.09*   | 0.4 ± 0.17* |
| PA → NA | 0     | 48.0 ± 3.36† | 51.6 ± 3.71† |
| PA → PA | 100   | 47.5 ± 3.41† | 44.8 ± 3.78† |
| PA → atelectasis | 0 | 4.1 ± 0.99* | 3.2 ± 0.67* |

Within-ROI CV of sV

| NA   | 0.45 ± 0.19§ | 0.38 ± 0.21 | 0.34 ± 0.18* |
| PA   | 0.08 0.2   | 0.2 ± 0.08   | 0.2 ± 0.081 |
| PA → NA | 0    | 25.8 ± 4.64* | 33.7 ± 5.32* |
| PA → PA | 0    | 66.1 ± 6.49* | 60.9 ± 17.2* |
| PA → atelectasis | 100 | 7.9 ± 10.7* | 5.2 ± 8.18* |

Within-ROI CV of sV

| NA   | 0.74 ± 0.16§ | 0.40 ± 0.19* | 0.34 ± 0.20* |

Data are means for all 4 image slices (SE); n = 8. The same regions of interest (ROIs) were assessed 5 min (Surfactant-1) and 45 min (Surfactant-2) after surfactant instillation to determine the percentage of the ROI area that became normally aerated (NA), poorly aerated (PA), atelectatic (atelectasis), hyperinflated (HI), or did not change. CV, coefficient of variation. *P < 0.05 versus Lavage; †P < 0.001 vs. Lavage; §P < 0.001 vs. NA.
lung aeration, thereby inducing increases in the mean lung density (0.51 ± 0.08 vs. 0.34 ± 0.03 g/ml at baseline), in mean sV (7.7 ± 0.2 vs. 7.1 ± 0.2 min⁻¹), and in ventilation heterogeneity (CV of sV: 0.37 vs. 0.21) of the four axial image slices, while the total volume of imaged lung decreased (3.19 ± 0.59 vs. 3.54 ± 0.59 ml). The apparent tissue mass contained in the four image slices, including the instilled surfactant, slightly increased (1.75 ± 0.36 vs. 1.25 ± 0.23 g). These changes went along with an increase in airway resistance and in lung tissue elastance (Fig. 1, dashed lines).

DISCUSSION

The goal of this study was to assess the effect of surfactant administration on regional lung ventilation as well as airway and lung tissue mechanics in whole lung lavage-induced injury, a model of RDS. The main finding of this study is that the regional effect of surfactant was remarkably different depending on the function of lung units prior to surfactant administration. Although surfactant significantly reduced atelectasis, improved overall lung tissue elastance, and gas exchange, it reduced aeration in previously normally aerated lung regions, where Xe washin became significantly more heterogeneous (Fig. 4 and Table 2). To verify this finding, we administered surfactant to one healthy animal with normal regional lung aeration and specific ventilation distribution at baseline. Our data show that following surfactant instillation, widespread areas of increased regional lung density were observed. The sV within these poorly aerated regions was heterogeneous, as demonstrated by the increases in within-ROI CV of sV (Table 2).

After whole lung lavage followed by mechanical ventilation, significant areas of the lung became either poorly aerated or atelectatic. The regional distribution of these regions was strongly gravity dependent. As a result of reduced aeration, therefore, the reduced size of the gas compartment within each image voxel sV is expected to be accelerated, which was the case in part of these regions. However, in the majority of the poorly aerated regions, sV was normal or slow, suggesting increased resistances of peripheral airways subtending these lung regions. Conversely, sV was faster in a fraction of the normally aerated lung regions because of ventilation redistribution from the poorly aerated dependent lung regions. These were located in the least gravity dependent lung regions that were imaged (Fig. 5). The redistribution of ventilation causes a fraction of the normally aerated lung regions to receive a larger relative share of tidal ventilation, increasing the risk of exaggerated stretch in these zones. The above findings are in agreement with our previous data in this model (2). To our knowledge, this is the first study to directly visualize the effect of surfactant on the regional lung ventilation behavior using functional lung imaging in a model of RDS with widespread heterogeneities in regional lung function.

One of the limitations of this study was that the stationary, horizontal X-ray beam used for functional imaging in the

![Fig. 6. Quantitative analysis of the effect of surfactant in spatially defined ROIs where aeration was either NA, PA, or absent (Atelectasis) after lavage-induced lung injury. Data are means for all four image slices (SE); n = 8. Color scale indicates subregions with high, normal, or low sV within each category. The same ROIs were assessed 5 min (Surfactant1) and 45 min (Surfactant2) after surfactant instillation, to determine the percentage of the ROI area that became NA, PA, atelectatic, HI, or did not change. Statistical significance for the changes in aeration following surfactant administration is shown in Table 2 for clarity. Note that within the same aeration category, a range of different sV values were found both before and after surfactant administration.](http://jap.physiology.org/)
present study required that the animal be positioned upright. We previously performed a methodological investigation in a separate group of rabbits \( (n = 7) \) to assess the effect of position (3). The upright body position was associated with systematic decreases in the respiratory mechanical parameters relative to those obtained with the animals in the supine position. However, since these effects were similar throughout the experiments, we were able to assess the effect of gravity on regional lung function within each experimental condition. Also, we did not include a separate group of control animals undergoing whole lung lavage without surfactant treatment. In a previous study in a very similar experimental model, we performed imaging and FOT mechanics measurements successively at a PEEP of 3, 9, and again 3 cmH\(_2\)O at baseline and after whole lung lavage (2). We found that despite time (~3 h) and PEEP elevation, imaging, and respiratory mechanical and inert gas washout, measurements (lung clearance index) tended to worsen with time as the PEEP level was reduced back to 3 cmH\(_2\)O. It is therefore very unlikely that the short-term beneficial effects of surfactant on respiratory mechanics and gas exchange observed in this study were due to spontaneous recovery.

Tracheal instillation of surfactant results in the formation of liquid plugs that transiently obstruct the central airways over the first few branching generations (12). Previous theoretical studies have suggested that precisely because of their excellent surface active properties, natural surfactants tend to spread out to a thin layer, coating the airways beyond the first branching generations out to the small airways and alveoli, where the transport of surfactant material is dominated by surface tension gradients causing Marangoni flows (12). The transport and spreading of liquid plugs depend on their viscosity, density, surface tension, airway branching geometry, airflow shear effects, and gravity. When and where the viscous surfactant plugs rupture and spread out to thin layers, in addition to the above parameters, depends on the bolus volume relative to the surface area of the branching airways, which rapidly increases with branching generations toward the lung acini (11). The results of the present study show that the spread of surfactant to high surface tension lung regions that were either completely collapsed or poorly aerated significantly improved local aeration and ventilation. The improvement in regional aeration was concomitant to a reduction in ventilation heterogeneity, as shown by the decrease in within-ROI CV of sV\( \dot{\gamma} \) (Table 2). However, in regions with normal aeration and regional ventilation, the administered amount of surfactant (75 ml/kg) may have been in excess and have caused the compression of existing surfactant, producing thicker surface layers. Close examination of the effect of exogenous surfactant in an additional rabbit with normal lungs (Fig. 7) showed reduced re-

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**Fig. 7.** Sample composite images showing the distribution of sV (top row) and tissue density (middle row) in a single image slice in one healthy animal at baseline and 5 min after surfactant instillation. Bottom row: topographic distribution of lung regions defined as atelectatic, trapped, PA, NA, or HI. Color scale indicates subregions with high, normal, or low sV within the PA, NA, and HI categories.
gional lung aeration, manifested as increases in lung tissue density, mean specific ventilation due to faster Xe washin, and lung tissue elastance, while the volume of lung included in the four axial image slices decreased. In addition to reduced aeration, the increase in lung tissue density may have been in part due to the exogenous surfactant itself.

Poor aeration following surfactant instillation in initially normally aerated lung regions is a significant phenomenon since it leads to remarkable regional heterogeneities in specific ventilation within these areas (Table 2 and Fig. 6). The resulting heterogeneities in the distribution of mechanical stresses in these regions could potentially promote inflammatory processes leading to additional lung injury (18, 19, 21). Moreover, the clearance of surfactant molecules is a slow process. Exogenous surfactant clearance is significantly slower in premature infants (35). A disappearance half-life of 96 h has been measured following tracheal 13C-labeled phophatidyl choline-palmitate administration in preterm infants with RDS (4). Correspondingly, we did not observe significant changes in the regional patterns of lung function within the 45-min observation period following surfactant instillation in the present study. The local alterations in regional lung function within previously aerated and ventilated lung regions are therefore likely to persist for several hours following exogenous surfactant instillation, enough to trigger inflammatory processes that may potentially lead to further lung injury in these initially spared regions.

Another consequence of reduced aeration and increased ventilation heterogeneity in initially NA regions are an increased shunt flow, and ventilation/perfusion (V\(_{A}/Q\)) mismatch, which may counteract to a significant extent the positive effects of surfactant in collapsed and poorly aerated zones. Previously, Schermuly et al. (27) found that instillation of surfactant in isolated normal pig lungs significantly increased shunt, the perfusion of low V\(_{A}/Q\) areas, and promoted ventilation heterogeneity as assessed by the multiple inert gas elimination technique. Although rescue instillation of surfactant in lungs with lavage-induced RDS significantly reduced shunt flow, the perfusion of low V\(_{A}/Q\) areas increased instead of decreased, and ventilation-perfusion matching in the mid-range V\(_{A}/Q\) regions was not improved (27). These results are in full agreement with those of the present study, with the difference that using functional imaging, the behavior of spatially identical regions could be directly visualized and studied quantitatively in intact lungs. The study by Schermuly et al. suggested that the nebulized rather than instilled route of surfactant administration should be preferred, since none of the adverse effects of surfactant instillation on V\(_{A}/Q\) matching were observed with the former route. However, in the setting of a functionally heterogeneous lung, such as the RDS model used in this study, nebulized surfactant particles which are transported by the gas flow are expected to deposit mainly in well-ventilated lung regions. This represents a major limitation in utilizing this route of administration.

A possible approach to limit the occurrence of poor heterogeneous ventilation following surfactant instillation in the initially spared lung regions in RDS is to optimize the dose of instilled surfactant. Currently, the dosage of surfactant is based on the manufacturer’s instructions and is often modelled after research protocols (23). Theoretical studies suggest that the lack of rupture and spread of viscous liquid plugs into thin films may be due to the excessive amount of surfactant in proportion to the area of lung regions where surface tension is abnormally high (11). Further study with direct visualization of regional lung function in this RDS model is needed to address this question.

In summary, we directly observed the effect of surfactant on regional lung function in a whole lung saline lavage model of RDS. Our findings show that although rescue surfactant instillation significantly reduced atelectasis and improved overall lung tissue elastance and gas exchange, it reduced aeration in previously normally ventilated lung regions, where specific ventilation became significantly accelerated and heterogeneous. These findings are significant, since local mechanical heterogeneity can potentially contribute to the worsening of lung injury and heterogeneous regional ventilation adversely affects gas exchange, counteracting the beneficial effects of surfactant on initially atelectatic and poorly aerated lung regions. Our data underscore the need for reassessing the benefits of routine prophylactic vs. CPAP and early rescue surfactant therapy (31) in very immature infants.

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AUTHOR CONTRIBUTIONS


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