0031-3998/11/7001-0050
PEDIATRIC RESEARCH

Vol. 70, No. 1, 2011
Printed in U.S.A.

Surfactant Increases the Uniformity of Lung Aeration at Birth in Ventilated Preterm Rabbits

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ABSTRACT: Surfactant deficiency is a major cause of respiratory failure in newborns. We have investigated the roles of surfactant and positive end-expiratory pressure (PEEP) in the development of a functional residual capacity (FRC) and the distribution of ventilation at birth. Preterm rabbit pups (28 d GA) were delivered and received either saline or surfactant and then ventilated with (3PEEP) or without (0PEEP) 3 cm H_2O PEEP (groups: saline/0PEEP, surfactant/0PEEP, saline/3PEEP, surfactant/3PEEP). Lung gas volumes were measured using plethysmography, and the uniformity of ventilation was analyzed using phase contrast (PC) x-ray imaging. Surfactant/0PEEP pups had greater FRCs and the lungs were more uniformly ventilated than saline/0PEEP pups; FRC at inflation 19–21 was 2.46 ± 0.52 mL/kg *versus* 0.91 ± 0.95 mL/kg ($p < 0.05$). Saline/3PEEP pups developed an FRC of 7.54 ± 1.68 mL/kg at inflation 19–21 ($p < 0.05$), but the distribution of ventilation was initially nonuniform. Surfactant/3PEEP pups had an FRC of 8.50 ± 0.80 mL/kg (at inflation 19–21), and the distribution of ventilation was more uniform than with saline/3PEEP ($p < 0.05$). In ventilated preterm newborn rabbits, PEEP has a greater effect on FRC than surfactant, although the two are additive. Surfactant, administered at birth, markedly improved the uniformity of ventilation irrespective of whether PEEP was applied. **(*Pediatr Res* 70: 50–55, 2011)**

At birth, the liquid that fills the airways during fetal life must be cleared to allow the entry of air and the onset of pulmonary gas exchange (1). With the entry of air, an air-liquid interface forms, which creates surface tension within the lung and increases lung recoil. Surfactant, which is secreted by type-II alveolar epithelial cells (AECs) greatly reduces surface tension and thereby reduces the work of breathing and stabilizes the lung at end expiration (2). However, as mature type-II AECs only develop late in gestation (3), infants born extremely preterm (23–28 wk GA) are born with few surfactant-producing type-II AECs.

Surfactant deficiency, which is common in very preterm infants, can lead to inadequate aeration and small airway collapse at end expiration if no positive end-expiratory pressure (PEEP) is applied. This restricts the development of a functional residual capacity (FRC), reduces gas exchange, and increases the risk of lung injury (4). Nonuniform lung aeration, where regions of the lung remain liquid filled, reduces lung gas volumes, restricts gas exchange to aerated regions, and exacerbates the risk of lung injury. Restricting ventilation to aerated regions when using tidal volumes (V_t) intended for the entire lung can cause overdistension and injury in those regions (5,6). Thus, in a very preterm infant, initiating pulmonary ventilation at birth without causing lung injury is a multifactorial challenge that attempts to promote uniform lung aeration and maintain FRC. However, the most effective ways of achieving these aims are unclear.

It has not previously been possible to assess the spatial distribution and temporal nature of ventilation within the lung as it aerates after birth. However, using phase contrast (PC) x-ray imaging we are now able to visualize and measure the volume of air entering different regions of the lung on a breath-by-breath basis from birth (7–9). Using this technique, we can measure the temporal and spatial pattern (uniformity) of lung aeration and the regional distribution of ventilation within the lung (10–12). We have shown that PEEP facilitates FRC development and prevents lung collapse at end expiration in ventilated very preterm rabbits (12). However, despite improving FRC recruitment, aeration of the lung was initially heterogeneous and the distribution of ventilation very regional (11,12).

During lung aeration, the air-liquid interface moves distally through the airways because of the transpulmonary pressures generated by each inspiration (7,13). Nonuniform aeration and ventilation arises when air enters one airway branch, but not another, as the air-liquid interface moves distally (7,14). Although the mechanisms are unknown, the failure of air to pass down both airway branches after a branching point may result

Received September 17, 2010; accepted January 25, 2011.

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Supported by the Australian Research Council and the Australian National Health and Medical Research Council. Financial support from the Access to Major Research Facilities Programme was provided by the Australian Government.

Supplemental digital content is available for this article. Direct URL citations appear in the printed text and are provided in the HTML and PDF versions of this article on the journal's Web site (www.pedresearch.org).

Abbreviations: AEC, alveolar epithelial cell; C_{RS}, respiratory system compliance; **CV,** coefficient of variation; **ET,** endotracheal; **FRC,** functional residual capacity; **PC,** phase contrast; **PEEP,** positive end-expiratory pressure; V_{peak}, peak inflation volume; V_t, tidal volume

from high surface tension within the airway-liquid and/or from differences in diameter of the subtending airway branches.

We hypothesized that the addition of surfactant to the air-liquid interface would enhance the probability of air moving down both airway branches at each branching point and improve the uniformity of aeration and subsequent ventilation. In this experiment, we examined the effect of early surfactant administration on the uniformity of ventilation in mechanically ventilated very preterm rabbits. As the surface tension lowering ability of surfactant is thought to enhance recruitment and stabilization of alveoli at end expiration (2), we have also compared the effects of PEEP and surfactant on the recruitment of FRC from birth.

MATERIALS AND METHODS

Experimental procedure. All animal procedures were approved by the SPring-8 Animal Care and Monash University's School of Biomedical Science's Animal Ethics Committees. All studies were conducted in experimental hutch 3 of beamline 20B2, in the Biomedical Imaging Centre at the SPring-8 synchrotron in Japan.

Newborn rabbits at 28 d GA (term = 32 d) were used because they are unable to develop an FRC without PEEP (11,12) and are surfactant deficient at this age (15). Pregnant New Zealand White rabbits were anesthetized using Rapinovet (i.v.; 12 mg/kg bolus; propofol, Schering-Plough Animal Health), intubated and anesthesia was maintained by isoflourane inhalation (1.5–4%; Isoflurane, Delvet Pty. Ltd., Australia). Pups were delivered one at a time by cesarean section, as needed, and sedated (0.1 mg, i.p.; Nembutal, Abbott Laboratories). Before intubation, pups were randomly allocated into two groups in which the endotracheal (ET) tube (18 G) was preloaded with either 0.05 mL saline or 0.05 mL surfactant (Curosurf; Chiesi Pharmaceuticals, Italy; ~100 mg/kg). The ET tube was occluded to prevent lung aeration caused by the spontaneous inspiratory efforts of the pup before connecting to the ventilator.

After delivery, the pup was immediately placed, head out, in a warmed (40°C) water-filled plethysmograph (custom-made) located within the experimental imaging hutch as previously described (7,12). The ET tube was connected to a purpose-built, time-cycled, pressure-controlled ventilator (16) and simultaneous ventilation and image acquisition began as soon as possible.

Pups in both surfactant and saline-treated groups were further randomized into two groups and ventilated with either a PEEP of 3 cm H_2O (3PEEP) or no PEEP (0PEEP); pups from a single litter were equally divided among the four groups. Pups were ventilated with air at a rate of 24 inflations/min, beginning with a peak inspiratory pressure of 35 cm H_2O that was subsequently adjusted to maintain a V_t of 10 mL/kg. Ventilation continued for 7 min during which airway pressures and lung gas volumes (from the plethysmograph) were digitally recorded (Powerlab; ADInstruments, Sydney, Australia). A 1-mL calibration performed before the experiment was used to convert, in real-time, pressure changes in the plethysmograph into lung gas volumes. All animals were humanely killed at the conclusion of the experiment with an overdose of Nembutal (100 mg/kg) administered i.v. (doe) or i.p. (pups).

PC x-ray imaging. The imaging procedures were identical to those reported previously (7–9). In brief, we used an x-ray energy of 24 keV, and the pups were positioned 2.0 m upstream of the detector (EM-CCD C9100-02), which had an effective pixel size of 32.3 μm and an active field of view of 32.3 (W) × 32.3 (H) mm^2. At the onset of each inflation, the ventilator triggered a train of seven images acquired 250 ms apart; four images were acquired during inflation, one during deflation, and two images at end expiration. Each exposure was limited to 50 ms, and a 1-s inflation time was used to minimize motion blur.

Plethysmograph analysis. All lung gas volumes, measured using plethysmography, were adjusted for the pup's body weight. FRC, respiratory system compliance (C_{RS}; V_t/Δ airway pressure), and peak inflation volume (V_{peak}; FRC + V_t) were obtained as an average over 3 inflations and measured at inflation 5 ± 1 (*i.e.* average of inflations 4–6), inflation 20 ± 1, and at every 20 ± 1 inflations thereafter for up to 120 inflations.

Image analysis. Image analysis was used to determine regional gas volumes within the lung from which the uniformity of lung aeration and ventilation was determined; the details of this analysis have been reported previously (10,11). Briefly, phase retrieval algorithms are applied to the four quadrants of the PC x-ray image to calculate the volume of gas in that quadrant relative to the first image of that sequence (11). The coefficient of variation (CV) of gas volumes between the four quadrants was determined at both FRC and peak inflation (10, 11). A high CV indicated much variation between the four quadrants and is indicative of nonuniform aeration/ventilation. A low CV indicated that all four quadrants were similarly aerated and is indicative of a more uniform pattern of aeration. The results are presented as an average of 2 inflations from the start of ventilation for 30 inflations (*i.e.* average of inflations 1–2, 3–4, etc.); the average FRC after each inflation is also presented for comparison.

PC x-ray movies. PC x-ray movies were generated by combining the PC x-ray image frames acquired sequentially at 5 frames/s (~1.5× normal speed). Movie 1 (see supplemental digital content, available at: http://links.lww.com/PDR/A72) is of a saline-treated pup, ventilated without surfactant or PEEP. Movie 2 (see supplemental digital content, available at: http://links.lww.com/PDR/A73) is of a surfactant-treated pup without PEEP. Movie 3 (see supplemental digital content, available at: http://links.lww.com/PDR/A74) is of a pup ventilated with 3 cm H_2O PEEP but without surfactant. Movie 4 (see supplemental digital content, available at: http://links.lww.com/PDR/A75) is of a rabbit pup treated with surfactant and ventilated with 3 cm H_2O PEEP.

Statistical analysis. Results were presented as means ± SEM. A p value of <0.05 was considered statistically significant. Changes in FRC, C_{RS}, V_{peak}, and the CV were analyzed using a two-way repeated measures ANOVA with a Fishers least significant difference (LSD) post hoc test. L_n or square root transformations were applied to the data set if equal variance was not found in that data set.

RESULTS

Animal data. Twenty-six preterm rabbit pups (from a total of 7 does) were ventilated from birth and used for simultaneous plethysmography and PC x-ray imaging analysis. Six pups were ventilated in the saline/0PEEP (control) and surfactant/0PEEP groups, whereas seven pups were ventilated in the saline/3PEEP and surfactant/3PEEP groups. However, two pups from the saline/3PEEP group were excluded from the plethysmograph analysis because of leaks in the plethysmograph. As the PC x-ray images from these pups were not affected, they were still used in the image analysis. There were no significant differences in mean pup weight between any of the groups ($p < 0.001$).

Functional residual capacity. All pups in the saline/0PEEP group were unable to develop an FRC that was significantly above zero at least until inflation 120 (Fig. 1). From the start of ventilation, the average FRC of pups ventilated with sur-

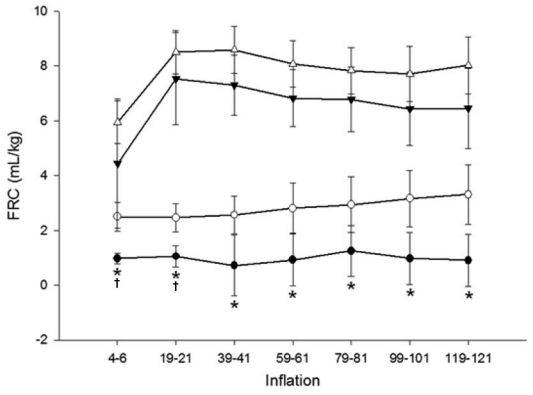

Figure 1. FRC (mean ± SEM) measured in preterm rabbit pups pretreated with/without surfactant and with/without 3PEEP from birth. Saline/0PEEP (●), surfactant/0PEEP (○), saline/3PEEP (▼), and surfactant/3PEEP (△). (*) indicate values are significantly different from saline/3PEEP and surfactant/3PEEP. (†) indicate values are significantly different from surfactant/0PEEP.

factant/0PEEP was greater than those ventilated with saline/0PEEP; however, FRC was significantly greater only until inflation 19–21 (2.5 ± 0.5 mL/kg *versus* 1.1 ± 0.4 mL/kg, respectively; $p < 0.05$). The saline/3PEEP group had FRCs that were ~3 times greater than those of the surfactant/0PEEP group (Fig. 1; 7.5 ± 1.7 mL/kg *versus* 2.5 ± 0.5 mL/kg at inflation 19–21, respectively; $p < 0.05$). FRCs in the surfactant/3PEEP group tended to be ~1 mL/kg greater at all inflations than those in the saline/3PEEP group, but this difference was not statistically significant.

Respiratory system compliance. At the onset of ventilation (inflations, 4–6), pups that received surfactant (surfactant/0PEEP and surfactant/3PEEP) had C_{RS} ~3 times greater than the two groups that did not receive surfactant (saline/0PEEP and saline/3PEEP; Fig. 2). Pups of the saline/3PEEP group had significantly improved C_{RS} compared with pups in the saline/0PEEP group (inflation 39–41: 0.50 ± 0.08 mL/cm H_2O/kg *versus* 0.26 ± 0.02 mL/cm H_2O/kg, respectively; $p < 0.05$) and the C_{RS} of the saline/3PEEP group closely mimicked the shape of the increase in C_{RS} in the surfactant/3PEEP group (Fig. 2). The surfactant/3PEEP group had a C_{RS} that was significantly greater compared with the surfactant/0PEEP group (inflation 39–41: 0.60 ± 0.07 mL/cm H_2O/kg *versus* 0.37 ± 0.05 mL/cm H_2O/kg, respectively; $p < 0.05$). However, by inflation 99–101, pups that received either surfactant, 3PEEP, or both had similar C_{RS} ($p > 0.05$) that were significantly greater than pups receiving saline/0PEEP ($p < 0.05$).

Uniformity of lung aeration at FRC. At FRC, treatment with both surfactant and PEEP (surfactant/3PEEP) markedly improved the uniformity of lung aeration during the initial stages of ventilation (Fig. 3, *top graph*). From inflations 1–2, aeration of the lung remained remarkably uniform across all four quadrants in this group as indicated by a low CV. This occurred despite the fact that the lung was initially only partially aerated (Fig. 3, *bottom graph*). Indeed, the CV remained low and stable, indicating that uniformity of lung aeration was maintained throughout the measurement period (inflation 1–2, 23.9 ± 3.1% *versus* inflation 29–30, 21.6 ± 3.2%; $p > 0.05$; Fig. 3, *top graph*), whereas the FRC doubled

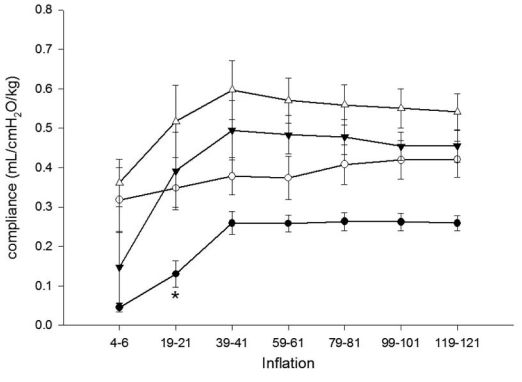

Figure 2. C_{RS} (mean ± SEM) in preterm rabbit pups pretreated with/without surfactant and with/without 3PEEP from birth. Saline/0PEEP (●), surfactant/0PEEP (○), saline/3PEEP (▼), and surfactant/3PEEP (△). (*) indicate values are significantly different from surfactant/0PEEP, saline/3PEEP, and surfactant/3PEEP.

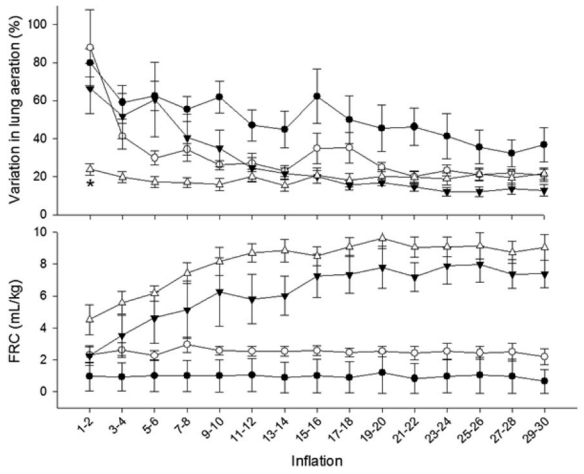

Figure 3. FRC (mean ± SEM; *bottom graph*) and the uniformity of lung aeration at FRC, measured as the CV of lung aeration analyzed across four lung quadrants (*top graph*; see Methods). Saline/0PEEP (●), surfactant/0PEEP (○), saline/3PEEP (▼), and surfactant/3PEEP (△). Preterm rabbit pups were pretreated with/without surfactant and with/without 3PEEP from birth. *Values are significantly different from saline/0PEEP, surfactant/0PEEP, saline/3PEEP.

over this time (Fig. 3; *bottom graph*). In contrast, lung aeration was not uniform in all other groups during the initial stages of aeration and had CVs between lung quadrants that were ~4 times greater than the surfactant/3PEEP group ($p < 0.05$; Fig. 3, *top graph*).

In all pups, except for those in the surfactant/3PEEP group, the uniformity of lung aeration at FRC improved with increasing inflation number, despite the fact that FRC increased only in the saline/3PEEP group. The uniformity of lung aeration at FRC improved rapidly in pups in the saline/3PEEP and surfactant/0PEEP groups, so that at inflation 11–12, the uniformity was similar to the surfactant/3PEEP group. In contrast, in pups ventilated with saline and 0PEEP, although the CV of lung aeration decreased to approximately half of its starting value by inflation 25–26 (inflation 1–2, 79.8 ± 7.4% *versus* inflation 25–26, 35.6 ± 8.9%), it remained higher than the other three groups (Fig. 3, *top graph*).

Uniformity of lung aeration at peak inflation. Measured at peak inflation, the uniformity of lung aeration was similar in both groups ventilated with surfactant (surfactant/0PEEP or surfactant/3PEEP) throughout the measurement period (Fig. 4, *top graph*). Furthermore, surfactant treatment resulted in uniform lung aeration at peak inflation essentially from the first inflation. Pups ventilated with surfactant achieved volumes at peak inflation that were ~2 times greater than pups ventilated without surfactant (surfactant/3PEEP, 13.2 ± 2.2 mL/kg *versus* saline/3PEEP 6.7 ± 1.5 mL/kg at inflations 1–2; $p < 0.05$).

In contrast to surfactant-treated pups, the CV in lung gas volume at peak inflation in saline-treated pups was initially high but gradually decreased with increasing inflation number. However, the rate of decrease in CV (increase in uniformity) was faster in pups ventilated with PEEP compared with pups ventilated without PEEP. At 119–121 inflations, pups ventilated without surfactant or PEEP continued to have a relatively high CV in lung gas volume at peak inflation (17.0 ±

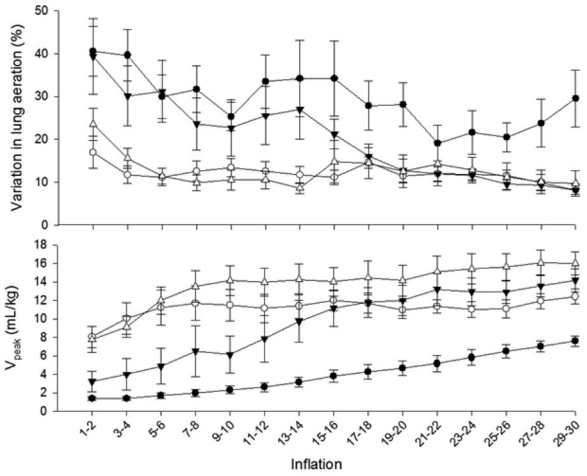

Figure 4. V_{peak} (mean ± SEM; *bottom graph*) and the uniformity of lung aeration at peak inflation, measured as the CV of lung aeration analyzed across four lung quadrants (*top graph*; see Methods). Saline/0PEEP (●), surfactant/0PEEP (○), saline/3PEEP (▼), and surfactant/3PEEP (△). Preterm rabbit pups were pretreated with/without surfactant and with/without 3PEEP from birth.

3.5%), whereas all other groups demonstrated a low CV that was <10% (data not shown).

Observations from PC x-ray movies. Without surfactant and PEEP, the lungs failed to accumulate an FRC or achieve large gas volumes at peak inflation, which is demonstrated in Movie 1 and Figure 5*A* and *E*. The lungs retained some air within the major airways for the first few inflations but quickly lost most of their FRC and seemed to collapse or refill with liquid at end expiration. As a result, each inflation reinflated many of the airways from a collapsed or liquid-filled state.

Ventilation with surfactant and no PEEP facilitated lung inflation virtually from the first inflation (Movie 2). Within the next several inflations, aeration reached the peripheral airways, and the lungs can be clearly seen at peak inflation (Movie 2). After the first few inflations, gas was retained in the apical lobes of the lungs at end expiration and with subsequent inflations; the FRC, although small, was evenly distributed throughout the lung (see Movie 2, Fig. 5*B* and compare Fig. 3, *top graph* with Fig. 3, *bottom graph*).

In pups ventilated with saline and 3PEEP, the first inflations aerated the lungs down to the level of the alveoli (Movie 3). FRC initially accumulated in the upper lobes but was uniformly distributed by the ninth inflation. The overall intensity of the lungs in the image was much brighter in pups ventilated with saline/3PEEP than in pups ventilated without PEEP (compare Movies 1 and 3). The lungs were so well aerated that the brightness and abundance of speckle, which indicates aeration of the distal airways (9), obstructed the view of the distal bronchi, particularly at peak inflation (Fig. 5*C* and *G*, respectively).

Movie 4 shows a pup ventilated with surfactant/3PEEP. The lungs aerated down to the level of the alveoli within the first few inflations, especially in regions localized adjacent to the ends of the bronchi. At both FRC and peak inflation, aeration of the distal basal lobes occurred before the distal apical lobes. By the seventh inflation, both the FRC and gas volumes at peak inflation were maximal and did not seem to increase further during the measurement period. Lungs were so well aerated that the margins of the lung were clearly seen at FRC and peak inflation (also see Fig. 5*D* and *H*).

DISCUSSION

Our results demonstrate that both surfactant, when given before the first inflation, and PEEP can facilitate uniform lung aeration and that their effects on FRC accumulation are additive. Both PEEP and surfactant increase FRC, although the effects of PEEP are significantly better than surfactant alone. However, surfactant markedly improved the uniformity of

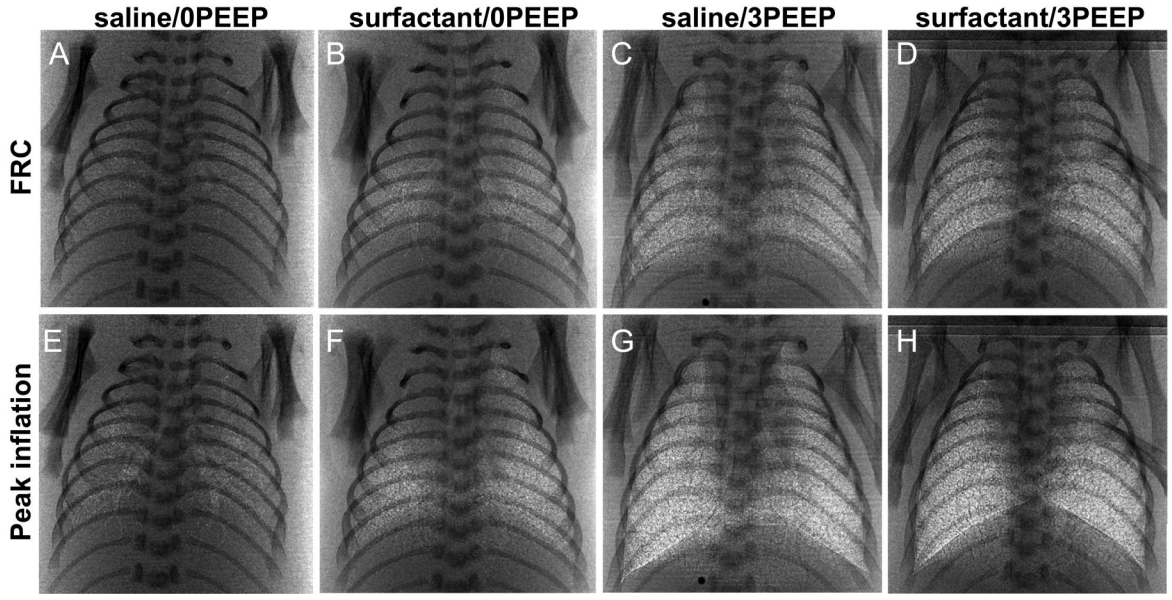

Figure 5. PC x-ray images of preterm rabbit pups pretreated with/without surfactant and with/without 3PEEP from birth. Images were collected at the 20th inflation at FRC (*top row*) and at V_{peak} (*bottom row*) in all four groups (saline/0PEEP, surfactant/0PEEP, saline/3PEEP, and surfactant/3PEEP). Saline/0PEEP (●), surfactant/0PEEP (○), saline/3PEEP (▼), and surfactant/3PEEP (△).

lung aeration at FRC and the distribution of ventilation at peak inflation. Thus, the combination of surfactant and PEEP allowed the lungs to quickly develop a large FRC that was uniformly distributed across the lung during lung inflations (see Movie 4). Indeed, surfactant was able to promote uniform gas distribution within the lung at both FRC and at peak inflation from the first inflation (Fig. 5*D* and *H*). The marked improvement in the uniformity of lung aeration and C_{RS} caused by surfactant indicates that surface tension plays a significant role in determining the resistance and pathways that the air-liquid interface progresses down during the process of lung aeration.

In the absence of surfactant and PEEP, ventilated preterm rabbit pups not only failed to recruit an FRC, the little volume that they did accumulate was unevenly distributed throughout the lungs (Fig. 3). The movies clearly demonstrate that initially even the largest airways (major bronchi) collapsed or refilled with liquid at end expiration (see Movie 1), and so it is not surprising that aeration at FRC was nonuniform. Although the uniformity of lung aeration gradually improved with time, FRC did not increase (Fig. 3), indicating that the improvement in uniformity at FRC resulted from redistribution of air between lung regions (see Movie 1). Similarly, without surfactant and PEEP, the distribution of air within the lung at peak inflation was nonuniform, and although the uniformity improved with increasing inflation number, it remained significantly worse than in all other groups. Initially, PEEP without surfactant had no effect on the uniformity of lung aeration at peak inflation, but with increasing inflation number, PEEP markedly improved the volume and uniform distribution of gas within the lungs at peak inflation. This indicates that PEEP not only enhances FRC but also improves the tidal distribution of gas within the lung during ventilation.

Although surfactant in the absence of PEEP only marginally improved FRC, it caused an immediate and marked improvement in the uniformity of lung aeration (Fig. 3; *top graph*) at both FRC and at peak inflation. In addition, the trachea and the bronchi rarely collapsed at end expiration, which likely explains the small increase in FRC (see Movie 2). In spontaneously breathing newborns, the primary factors regulating the spatial pattern of lung aeration relates to dependent *versus* nondependent lobes and position of lung regions in relation to the diaphragm (12,17). Our findings demonstrate that the differential spatial pattern of lung aeration is lost after surfactant pretreatment, possibly indicating that surface tension plays a major role in the spatial distribution of aeration and tidal ventilation immediately after birth; this is in addition to the effect of gravity and chest wall dynamics in response to diaphragmatic contraction (7). Thus, although surfactant may not have a major influence on FRC recruitment, it plays a major role in promoting uniform lung aeration at FRC and ensuring that air is evenly distributed throughout the lung during tidal inflation. This effect may be the reason why surfactant is lung protective and limits albumin leak into the airways in the absence of PEEP (18).

Pups ventilated with surfactant and 3PEEP developed the largest FRC and, although this was statistically not different from the saline/3PEEP group, it indicates that surfactant and PEEP may have an additive effect on FRC; unfortunately two pups in the saline/3PEEP group could not be analyzed because of leaks in the plethysmograph, thus reducing the power of the statistical analysis. Movie 4 demonstrated the lungs were fully aerated and possibly too distended. However, results from Rider *et al*., suggest that lung injury is very limited during ventilation with PEEP and surfactant. We deliberately used a low PEEP level (3 cm H_2O) in case higher PEEP levels masked any potential additive effects of surfactant and PEEP on FRC. We have previously shown that preterm rabbit pups ventilated with 5 cm H_2O of PEEP developed an FRC of ~18 mL/kg (12), which is greater than the FRC accumulated by pups ventilated with surfactant and 3 cm H_2O PEEP. This suggests that FRC recruitment is very sensitive to PEEP level as even slightly higher PEEPs are able to markedly improve FRC, compared with surfactant treatment. Although it is possible that the pups were slightly more mature at birth in our previous study, it is likely that a PEEP of 5 cm H_2O in this study would have obscured any additive effects of surfactant and PEEP.

Pups ventilated with surfactant initially developed a higher C_{RS} than pups ventilated without surfactant, irrespective of whether they received PEEP. This indicates that during the early stages of lung aeration, surfactant has a greater ability to improve lung compliance than PEEP. Because C_{RS} continued to increase in surfactant-treated pups ventilated with PEEP, but not in surfactant-treated pups ventilated without PEEP, these findings also indicate that surfactant and PEEP have an additive effect on lung compliance (Fig. 2). These findings are consistent with previous studies demonstrating that PEEP potentiates the effect of surfactant on lung compliance (19).

At 120 inflations, C_{RS} was similar in all pups (Fig. 2) except for pups ventilated without both surfactant and PEEP, because these pups had significantly lower compliances throughout the ventilation period. PEEP and surfactant are considered to increase C_{RS} by different means. Surfactant reduces lung recoil by lowering surface tension and therefore alters the pressure volume relationship to favor higher volumes at lower pressures. However, PEEP is thought to increase FRC and shift the resting lung volume into a more compliant region of the pressure-volume curve (20,21). It is not surprising, therefore, that in the saline/3PEEP group, C_{RS} increased with increasing FRC (Figs. 1 and 2). Initially (inflations 4–6), pups in the surfactant/0PEEP group had higher C_{RS} than pups in the saline/3PEEP group, despite having a lower FRC. This indicates that surfactant has a greater influence than PEEP on C_{RS} during the initial lung aeration process. As high resistance to fluid movement through the airways is a major contributor to low C_{RS} during lung aeration (14), it is possible that surface tension greatly contributes to this resistance, which is reduced by the presence of surfactant at the air-liquid interface. Indeed, Movie 2 shows that lungs treated with surfactant easily displace fluid and allow aeration during lung inflation.

This study illustrates the separate and combined benefits of PEEP and surfactant in aerating the newborn lung immediately after birth. Mechanical ventilation with PEEP greatly facilitated the recruitment of FRC from the first inflation and enhanced the distribution of ventilation, presumably as a result of FRC recruitment. Although surfactant had much less influence on FRC

recruitment, it increased C_{RS} and markedly improved the uniformity of lung aeration at FRC and the distribution of ventilation at peak inflation. An improved understanding of how different ventilation techniques and treatments influence lung mechanics and the distribution of ventilation immediately after birth will help to improve pulmonary ventilation in very preterm infants and reduce the risk of lung injury.

Acknowledgments. The authors thank the infrastructure support provided by the SPring-8 synchrotron facility (Japan), which was provided by the SPring-8 Program Review Committee (proposal nos. 2007B0002). In addition, we thank Chiesi Pharmaceuticals who generously provided the surfactant (Curosurf) for these studies.

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