Positive End-Expiratory Pressure during KL₄ Surfactant Instillation Enhances Intrapulmonary Distribution in a Simian Model of Respiratory Distress Syndrome

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ABSTRACT

Intrapulmonary distribution of a peptide-phospholipid (KL₄) surfactant administered through an adapter permitting maintenance of positive end-expiratory pressure was compared with distribution by instillation with disconnection from mechanical ventilation in 10 surfactant-deficient *Macaca mullata* preterm infants. Animals received KL₄ surfactant (200 mg/kg) when the arterial to alveolar (oxygen ratio) (a/A_{O_2}) was ≤ 0.22 (approximately 50 min after birth) on mechanical ventilation. Six rhesus infants received bolus instillation of two half doses of KL₄ surfactant through an endotracheal tube adapter over 10–15 s while maintaining positive end-expiratory pressure (0.4 kPa) accompanied by turning to the right and left lateral positions for 60 s. In four rhesus premature infants KL₄ surfactant was injected as two half-dose boluses through the endotracheal tube with disconnection from mechanical ventilation while positioning the infant rhesus monkey in the right and left lateral positions for 30 s of mechanical ventilation between instillation. Acute effects on oxygen saturation were monitored, and physiologic measures of a/A_{O_2} , mean airway pressure, and the ventilatory efficiency index were monitored over the 12-h study. Intrapulmonary distribution of KL₄ surfactant was determined using dye-labeled microspheres or [³H]dipalmitoylphosphatidylcholine-labeled KL₄ surfactant, measured by colorimetry or by scintillation counting. Lungs of each monkey were processed into 50 ± 5 pieces to determine distribution of radiolabel or

microspheres and for scanning electron microscopy. The drop in oxygen saturation was greater among monkey infants disconnected from the ventilator for surfactant instillation. Surfactant distribution in lung pieces inside a distribution interval 0.6–1.4 of the mean was greater among infants having instillation accompanied by positive end-expiratory pressure ($p < 0.04$), whereas the proportion of lung pieces receiving $\leq 10\%$ of the normalized mean number of microspheres/mg of lung tissue was significantly greater in the group removed from the ventilator for surfactant instillation. The a/A_{O_2} , lung compliance, and mean airway pressures improved in both treatment groups; however, the ventilatory efficiency index increased more above pretreatment values among infants treated using the endotracheal adapter with positive end-expiratory pressure ($p < 0.04$). More homogeneous distribution of KL₄ surfactant results when this surfactant is administered while maintaining positive end-expiratory pressures during surfactant instillation. (*Pediatr Res* 38: 211–217, 1995)

Abbreviations

RDS, respiratory distress syndrome
DPPC, dipalmitoylphosphatidylcholine
Fio₂, fraction of inspired oxygen
 a/A_{O_2} , arterial to alveolar (oxygen ratio)

Instillation of exogenous surfactant intratracheally is routine in the management of RDS infants. Several surfactant formulations using differing methods of administration of the surfactant material into the airways (1) have been demonstrated to be efficacious in treatment of RDS. However, concerns have been raised regarding the frequency of adverse occurrences includ-

ing arterial oxygen desaturation, transient fall in blood pressure, and hypercarbia during administration and the possible association of these events with intraventricular hemorrhage (2). Techniques recommended for the administration of these surfactants have varied according to the preparation chosen for treatment, and it has been difficult to distinguish between specific surfactant characteristics and/or those associated with the methods of instillation in the randomized, controlled trials (3). Because immediate arterial oxygen desaturation and bradycardia can also result from the manipulation of the head, neck, and airways, presumably due to vagal stimulation, and acute airway obstruction may result from instillation of approx-

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imately 4.0–5.0 mL/kg surfactant suspension administered via the endotracheal tube (4, 5), many clinicians are modifying treatment techniques (6). These modifications have generally involved lengthening the duration of administration by infusion of surfactant, and/or altering the recommended positioning of the infant's head, neck, and airways during the instillation process. The distribution characteristics in the airways and lung using these modifications of surfactant administration have not been well studied in premature animal models of RDS.

Studies in the preterm animal have found that a more uniform distribution of the instilled surfactant will improve the clinical response, and among animals without lung injury, distribution of the surfactant is enhanced as the volume of the surfactant suspension is increased (7, 8). In studies with oleic acid injury to the adult rabbit lung, distribution of exogenous surfactant was less dependent on the volume of surfactant than on the regions of injury (9), with less surfactant reaching the atelectatic regions. Davis *et al.* (9) using radioscinigraphy found the rapid spreading of a 3.3 mL/kg calf lung surfactant extract, given as a single bolus throughout the lungs of piglets, that were injured by saline lavage. Segerer *et al.* (10) found uneven surfactant distribution in the lung after a 45-min infusion of surfactant contrasted to bolus injection of Curosurf into the trachea, and Jobe (11) reported nonhomogenous distribution of surfactant in preterm lambs, after infusion over 30 min using an infusion pump, and selective distribution of subsequent doses to areas initially treated with the first dose of surfactant.

Zola *et al.* (12) compared the variations in administration technique using Survanta in human infants. These investigators compared instillation of two half doses (rather than the recommended four quarter doses) with the infant being removed from the ventilator with an interval of at least 30 s of mechanical ventilation between fractional dosing to a procedure in which the surfactant was instilled through a device that permitted continuous ventilation during continuous dosing. Using the latter technique the infant was positioned to the right and left approximately 45° as in the first procedure. These two techniques were compared with the standard dosing protocol used in the randomized clinical trials of administration of quarter doses with interruption of mechanical ventilation and positioning of the infant for each quarter dose. Although the magnitude of decline in oxygen saturation was reduced by the use of the endotracheal tube adapter device for the initial dose of Survanta, no changes were noted in the initial 72-h course of RDS. This clinical study could not address differences in distribution resulting from these differing instillation techniques.

We studied the distribution of a peptide-phospholipid surfactant (KL₄) in ventilated preterm rhesus monkey infants (13) soon after developing severe RDS to determine whether instillation is associated both with a reduction in immediate adverse effects, as well as the distribution of this surfactant throughout the airways. We further sought to determine whether the instillation technique altered the physiologic response to this surfactant over the first 12 h of surfactant treatment for simian RDS. Our hypothesis was that instillation of this surfactant combined with positive end-expiratory pressure during admin-

istration would not only reduce the magnitude of oxygen desaturation and bradycardia, but it would also enhance distribution of this surfactant in the lungs and be associated with an improved efficacy as determined by a greater improvement in a/Ao₂, permitting less mean airway pressure during mechanical ventilation.

METHODS

Laboratory animals. All procedures used in this study conformed to the Animal Welfare Act. The California Regional Primate Research Center is fully accredited by the Association of Laboratory Animal Care, and all study protocols were approved before implementation by the Institutional Animal Use and Care Committee of the University of California at Davis. Activities related to animal care and surgery/necropsy were performed according to the Primate Center operating procedures.

Surfactant. A peptide of lysine and leucine in combination with phospholipids and palmitic acid (KL₄ surfactant; The Scripps Research Institute, La Jolla, CA) (14) was used in this study. This surfactant was radiolabeled by adding [³H]DPPC with a sp act of 12 μ Ci/mL in 0.26 mL of diluent to 4.0 mL of surfactant containing 40 mg/mL phospholipid with 3% peptide by weight. To the radiolabeled surfactant, a suspension of 1.5×10^5 yellow dye-labeled microspheres, $15 \pm 0.2 \mu$ m (Triton Tech., San Diego, CA) per mL of surfactant was added in a volume of 200 μ L, and the suspension was mixed by multiple inversions. KL₄ surfactant containing both radiolabel and microspheres was used in two animals to determine the relationship of the distribution of radiolabel and microspheres in lung pieces. In experiments where only microspheres were used, 3×10^5 were added per mL of surfactant. Using a pulsating surfactometer, we demonstrated that addition of this number of microspheres did not alter the minimum surface tension nor equilibrium surface tension characteristics of this surfactant at multiple concentrations of KL₄ surfactant ranging from 1.25–5.0 mg/mL, and with microsphere concentrations from 1.5×10^5 to 1×10^6 microspheres/mL.

Delivery and ventilation of monkeys. Ten time-mated gestations of 127–130 d (~79% term) gestations were monitored ultrasonographically to establish normal fetal growth (femur length and biparietal diameter) for *Macaca mullata*. Dams were immobilized with intramuscular 10 mg/kg ketamine hydrochloride, intubated, and given oxygen-halothane anesthesia while being ventilated during the cesarean section. At cesarean delivery the uterus was exposed, a sample of amniotic fluid was withdrawn for surfactant maturity studies, the fetal head and neck were exposed, and the neck area over the trachea infiltrated with 1% xylocaine. The trachea was isolated, an incision made beneath the second tracheal ring, and a 2.0 French endotracheal tube was advanced to the midtrachea and secured by ligature. The infant was delivered, and the cord was doubly clamped and severed. The infant was weighed and placed under a radiant warmer. Within 3 min of birth the infant was placed on intermittent mandatory ventilation (InfantStar, Infrasonics, San Diego, CA) at peak inspiratory pressures of 4.65 kPa (sufficient to move the anterior chest wall) and 0.4

kPa positive end-expiratory pressure at a rate of 40 breaths/min with an inspiratory time 0.3 s. Electrocardiograph leads, rectal thermister, and an oxygen saturation probe (Nellcor 200, Palo Alto, CA) were placed and a 3.5 French umbilical artery catheter inserted to the region of the fourth lumbar vertebra on radiograph, and the pressure was monitored via a transducer.

Vecuronium (0.1 mg/kg) was administered. Arterial blood gases were frequently monitored (NOVA Biomedical, Waltham, MA), and Fio_2 was adjusted to maintain the blood gases in the following ranges: arterial $\text{Po}_2 = 6.6\text{--}10.6$ kPa (50–80 torr); arterial $\text{PCO}_2 = 4.7\text{--}7.3$ kPa (35–55 torr), and pH = 7.25–7.45.

Chest radiographs were obtained for documentation of diffuse reticulogranular pattern or pulmonary “white-out” soon after instrumentation, and arterial/alveolar oxygen tension ratios were determined by at least hourly blood gases. Dextrose (5%) in water was infused at 150 mL/kg/24 h, and heparinized blood from either the placenta or mother was given for hypotension. Each fetus was randomly assigned before birth to have KL₄ surfactant administration either with endotracheal tube instillation without positive end-expiratory pressure using in two half doses with transient disconnection from the ventilator with a 30-s interval of mechanical ventilation between half doses (12). Alternatively two half doses were administered using an endotracheal tube adapter (Bodai Neo₂-Safe, B&B Medical Technologies Inc., Rancho Cordova, CA) which permitted maintenance of positive end-expiratory pressure (0.4 kPa) using the Infant Star ventilator throughout dosing. Surfactant was given when the arterial/alveolar oxygen tension ratio was <0.22 , indicative of severe respiratory distress. Five milliliters of KL₄ surfactant (200 mg) per kg/body weight was instilled through a 3.5 cm umbilical arterial catheter cut to the length of the endotracheal tube and advanced through this tube at dosing. Changes in heart oxygen saturation, blood pressure, and the reflux of surfactant up the endotracheal tube were recorded by an observer not dosing the infant rhesus monkeys.

Physiologic measurements. Compliance of the respiratory system was measured through a calibrated pneumotachograph (Bicore Technologies, Irvine, CA) 6 h before and 10–12 h after surfactant instillation. Tidal volumes were maintained at 7–9 mL/kg by adjustment of the positive inspiratory pressure and inspiratory time. Blood gases were monitored initially every 15–30 min, and thereafter hourly. Mean airway pressures were monitored using the ventilator integration of pressures and time, and the ventilatory efficiency index (15) and the a/AO_2 ratio were calculated (16).

Lung processing. At the end of each of the study period the rhesus infant was euthanized with pentobarbital, and the endotracheal tube was clamped during peak inspiration at a constant pressure of 3.3 kPa. The animal was weighed, and the heart and lungs were removed *en bloc*, trimmed of mediastinal tissues, and photographed. The right upper lobe was identified, and the mainstem bronchus was ligated. An apical-lateral piece was removed for scanning electron and light microscopy. The remaining lung was frozen by immersion in liquid nitrogen. Each lung was then cut into a mean of 50 ± 5 pieces while thawing, and the lobar location was mapped. Each piece was weighed and digested in 4 N KOH containing 2% Tween 80

with a ratio of tissue to digest solution of 1:10. After vacuum filtration (10 μm) (Poretics, Livermore, CA) of the digest for recovery of the microspheres, the yellow dye was released using dimethylformamide (Sigma, St. Louis, MO) in a proportion equal to the number of microspheres per unit of lung for calorimetric quantitation with a Spectronic 601 spectrophotometer (Bausch & Lomb, Buffalo, NY). Aliquots of this digest were taken for protein determination in the presence of high volumes of phospholipids or detergents using the BCA microtiter method (Pierce, Rockford, IL).

Validation experiment. To determine whether the microspheres distributed in the lung similarly to that of radiolabeled KL₄ surfactant, two animals received simultaneous treatment with KL₄ surfactant containing both [³H]DPPC-labeled KL₄ and microspheres. The lungs of each of these animals were handled identically to the others except that aliquots were taken for lipid extraction which were dried under nitrogen, and [³H]phospholipid-associated radioactivity was measured by liquid scintillation counting. In addition, arterial blood samples were taken at 6 h and added for liquid scintillation counting to determine the amount of [³H]DPPC that may have reached the systemic circulation.

Data analysis. Distributions of surfactant were expressed as normalized values for each piece of lung (17). Normalized values were calculated by dividing the calorimetric quantitation of the number of microspheres (or radioactivity) per mg for each piece by the mean value per mg of all pieces for each lung. The distribution of surfactant was determined by computing the distribution interval of microspheres or radioactivity for each piece or the ratio of the observed number/unit weight and the expected number/unit weight, determined by the total number of microspheres (or radioactivity)/lung divided by the total weight of the lungs. Twenty distribution intervals of 0.1 unit width were calculated. If the surfactant distribution was perfectly uniform, each of the pieces would receive its expected amount of label or microspheres based on the total number of microspheres or counts per minute of the lung; each would have a distribution interval of unity. Distribution intervals greater than 1.95 were added to the 1.9–2.0 distribution interval total. The normality of the distribution of all lung pieces was tested with D’Agostoni’s *D* statistic and found to be normal ($p < 0.05$) (18). To determine the most appropriate range of distribution intervals about unity to compare the groups, we plotted the cumulative sum of the lung pieces from all treatment groups as a function of increasing distribution. The percentage of pieces contained in the interval 0.6–1.4 (the central distribution intervals) was 71% of the total, slightly more than that expected in the range of 1 SD about unity. The number of pieces of lungs with distribution intervals within the range for each method of administration was compared using the Mann-Whitney rank test with expected occurrence rate based on data pooled from all lung pieces. Lobar distribution differences were also compared using the Mann-Whitney ranks between treatment groups by lobe of the lung. Values for a/AO_2 , ventilatory efficiency index, and mean airway pressure are expressed as mean $\pm$ SE, and difference in means between groups were tested for significance by analysis of variance followed by the Newman-Keuls multiple comparison proce-

dure. Slopes of the curves for microspheres or radiolabel were calculated by linear regression using the least squares methods. Multiple comparison analysis of pretreatment and posttreatment values within each group were assessed using analysis of variance for repeated measures using Statview (Abacus Concepts, Berkeley, CA).

RESULTS

Lung maturity. Analysis of the amniotic fluid for surfactant maturity found all fetuses to have a disaturated phosphatidylcholine to sphingomyelin ratio of <1.5 (1.1 ± 0.4), and absence of phosphatidylglycerol on two-dimensional thin layer chromatography was predictive of surfactant deficiency and RDS.

Preliminary studies. Addition of Dye-Trak microspheres, $15.5 \pm 0.2 \mu\text{m}$ (specific weight 1.09 g/ml) at $6 \times 10^6/\text{mL}$ of surfactant diluted to contain 5 mg of KL₄ surfactant/ml did not significantly alter minimum surface tensions achieved at 1 min of pulsations (0.7 mN/m without microspheres and 1.1 mN/m with microspheres), nor the time to reach equilibrium surface tension (8 s, respectively) using the Pulsating Bubble Surfactometer (Electronetics, Amherst, NY) when pulsed at 20 cycles/min at 37°C.

The content of microspheres correlated very well with the content of the labeled DPPC per mg of protein ($R = 0.896$) of lung after instillation, and was similar to the correlation coefficient of 0.92 reported by Segerer *et al.* (10) using Curosurf in adult rabbits undergoing repeated tracheal lavage when comparing the efficacy of radiolabel and microspheres in tracking the instilled surfactant. Given these correlations, we were confident that distribution of dye-labeled microspheres of this dimension was equivalent to distribution of surfactant *per se*. Furthermore, by 6 h after treatment only 0.01% of the total instilled [³H]DPPC was detected in the circulation within 4 h after treatment based upon an estimated blood volume of 80 mL/kg, documenting that circulation within the lung was not responsible for the majority of [³H]DPPC detected in the lung pieces.

Instillation characteristics. Three of the six infants with instillation accompanied by positive end-expiratory pressure had a decline in oxygen saturation of 25.4%, whereas three of four infants having routine instillation had a drop in oxygen saturation of 39.6% when the Fio_2 was maintained at pretreatment values. Reflux of surfactant up the tube was present in one animal in each group.

Distribution studies. Table 1 describes infant characteristics at time of treatment. Infants randomized to instillation without the use of the Bodai device were 1.5 days more advanced in gestation and had a pretreatment a/Ao_2 of 0.18, suggestive of

less severe respiratory distress; however, both groups of animals reached low a/Ao_2 values at a similar time post birth. KL₄ surfactant was administered at 52 min in animals treated without the Bodai adapter and 50.2 min in 5/6 treated with the adapter and positive end-expiratory pressure (one animal had delayed administration at 139 min because of delayed arterial access) or a mean value of 65.4 min for the total group. After the interval of intermittent mandatory ventilation and at the time of sacrifice, the endotracheal tube was clamped during inspiration, and the lungs were removed within 10 min of autopsy. Fifty (± 5) pieces of lung with a mean wet weight of 116 ± 42.5 (range 54–172) mg were obtained from each animal, and the lobar distribution of each piece was mapped for reconstruction of distribution. The total number of lung pieces inside the central distribution interval of 0.6–1.4 (intervals around the mean approximating 1 SD) was significantly greater ($p < 0.04$) when instillation was accompanied by application of continuous distending pressure using the Bodai adapter to the airways contrasted to removal from the ventilator for surfactant treatment (Fig. 1). This result was evident when either lung pieces were considered on the basis of mg of protein or wet weight. A significantly higher percentage of pieces ($62.15 \pm 6.98\%$ with adapter *versus* $45.55 \pm 2.9\%$ without the adapter) was found in this distribution interval. Of interest, a significantly greater amount of instilled surfactant distributed to the right middle lobe and the left lower lobe ($p < 0.03$) when positive pressure was continued during instillation. Furthermore, the percentage of lung pieces that received $\leq 10\%$ of the normalized mean number of microspheres per mg wet lung tissue was significantly higher in the group in which the infants were removed from the ventilator for instillation ($p < 0.05$). Overall, in the Bodai adapter group a more homogeneous distribution of KL₄ surfactant was found throughout the lungs of these premature rhesus infants.

Physiologic studies. There was a significant improvement in a/Ao_2 over the 10–12 h of study ($p < 0.008$) from mean values of 0.14 ± 0.08 to 0.46 ± 0.19 over the duration of the study; however, there was no difference in a/Ao_2 by method of instillation (Fig. 2). Mean airway pressures declined from approximately 0.98 to approximately 0.8 kPa ($p < 0.04$) for both groups over 12 h ($p = \text{NS}$ between treatment groups). Compliance of the respiratory system improved by 12 h in both groups from mean values 0.1 mL/cm H₂O initially in both groups to 0.4 ($p < 0.04$) at the end of the study; but again there was no significant differences according to instillation technique. The change in ventilatory efficiency index increased from $0.11 \text{ mL/kg/mm Hg} \pm 0.03$ to 0.173 ± 0.04 ($p < 0.05$) over the course of the study for animals with KL₄ surfactant administration with the adapter and positive end-expiratory

Table 1. Characteristics of study infant monkeys

Group	Animals (n)	Body weight (g)	Gestational age (d)	Treatment time (min)	Pretreatment a/Ao_2	Pretreatment mean airway pressure (kPa)
With Bodai adapter	6	283.96 ± 13.86	127.66 ± 0.51	50.2 ± 13.7	0.11 ± 0.04	0.98 ± 0.07
Without adapter	4	333.95 ± 25.92	129 ± 0.81	52 ± 14.16	0.18 ± 0.12	0.95 ± 0.06

Values are expressed as mean $\pm$ SD.

Figure 1. Number of lung pieces within distribution intervals around a normalized mean. (A) With Bodai endotracheal tube adapter and positive end-expiratory pressure during KL₄ surfactant instillation. (B) With removal from positive end-expiratory pressure and KL₄ surfactant instillation during the interval of mechanical ventilation.

pressure, while the increase in ventilatory efficiency index was from 0.178 ± 0.13 to 0.193 ± 0.09 ($p = \text{NS}$) in those animals removed from the ventilator for surfactant administration. Although KL₄ surfactant administration was associated with an improvement in a/Ao_2 , compliance, and mean airway pressure, there was no significant differences between instillation techniques by 12 h.

Scanning electron microscopy. In some infants (three in the Bodai adapter groups and two in the standard instillation group) a 50 ± 10 -mg section of the right upper lobe was prepared in Kornovsky's fixative for microscopy to visualize the deposition of microspheres (Fig. 3) according to the technique of Pinkerton *et al.* (19). Microspheres were visualized in the terminal respiratory bronchioles adjacent to the pleura in distal acini and were also localized on ciliated bronchial epithelium of the terminal airways (Fig. 4).

DISCUSSION

For optimal treatment of RDS with exogenous surfactant, the drug must not only reduce surface tension within the airways (20), it must also be distributed homogeneously throughout the lung. Although techniques of instillation have varied between commercial surfactants, there were few studies evaluating the short term acute effects on cardiopulmonary status, compliance, or improvements in arterial/alveolar oxy-

Figure 2. Changes in a/Ao_2 , mean airway pressures, and ventilatory efficiency index during the 10–12 h of study between the two instillation groups.

gen tensions before controlled clinical trials in human infants. Therefore, before phase III clinical trials with KL₄ surfactant in human infants, the administration techniques that provided improved intrapulmonary distribution and reduced adverse effects on cardiopulmonary function during dosing were justified in primate models of RDS to provide clinically relevant information regarding recommendations for the administration technique in human infants.

In these premature rhesus infants with RDS, either technique of administration resulted in similar physiologic improvements in oxygenation, compliance, and ventilatory efficiency index. At autopsy 12 h after birth, the upper lobes of the lungs appeared uniformly expanded, whereas the lower lobes had a rim of atelectasis at the pleural margin and scattered focal atelectasis in some lobes on the anterior and posterior surfaces. The lungs of preterm lambs and rhesus monkeys have been shown to mature cephalad to caudal (21–23). Thus the lung

Figure 3. Scanning electron micrograph ($\times 450$) showing microspheres ($15\text{-}\mu\text{m}$ diameter) within a terminal respiratory bronchiole (arrows). Alveolar crests are seen subdividing the well inflated terminal respiratory bronchiole. This section was taken from the right upper lobe which was fixed at end inspiration.

Figure 4. Scanning electron micrograph ($\times 1500$) demonstrating microspheres within an inflated terminal alveolar duct (large arrows). Slight deformation of microspheres is secondary to the fixative procedure. Within the lung interstitium are capillaries and collagen fibers. Within the alveolus are type II cells (small arrow).

units with the greatest volume during ventilation are in the upper lobes even after surfactant administration. Our data demonstrate that distribution in the right middle and left lower lobes was enhanced by maintaining positive end-expiratory pressure during surfactant instillation. There were no differences in the right or left upper lobe distribution to suggest that differential lobar maturation and/or positioning contributed to the lobar distribution of the surfactant regardless of method of administration. Furthermore, no differences in the lobar distribution of excessive microspheres (≥ 1.3 times the mean) were found in the upper lobes.

Instillation techniques recommended by manufacturers where $4\text{--}5\text{ mL/kg}$ of surfactant are instilled over $3\text{--}5\text{ min}$ via an endotracheal tube adapter or by quarter doses administered via a tube passed through the endotracheal tube with removal from positive pressure ventilation results in the occurrence of transient bradycardia and oxygen desaturation in 11.9 and

9.8%, respectively (24, 25). Some clinicians have elected to infuse surfactant over many minutes to reduce the magnitude of heart rate decelerations and oxygen desaturation, reduce the number of fractional doses administered (6), or use methods such as an endotracheal tube adapter to maintain positive pressure ventilation during dosing. Except for the report by Zola *et al.* (12) on acute responses to administration in human infants, there have been few studies to assess the effect of administration technique on distribution of instilled surfactant into the lungs of premature infant animals with the surfactant deficiency-associated RDS. Segerer *et al.* (10) recently reported in saline lavaged surfactant-depleted adult rabbits that infusion over 45 min led to nonhomogenous distribution and postulated that this poor distribution may be related to the infants in whom the response to surfactant was judged clinically "poor" (26). Their study also found a high correlation of lung distribution of Dye-Trak microspheres as used in our study to the distribution of $[^{14}\text{C}]\text{DPPC}$ ($R = 0.92$) as found in our experiments, thus providing a methodology which offers a nonradioactive alternative to labeling of microspheres or radiolabeling of surfactant components to determine intrapulmonary distribution. Schürch *et al.* (27) using latex polystyrene particles ($6\text{--}11.9\text{ }\mu\text{m}$) instilled into the airways found them coated with surfactant and completely immersed within an osmophilic film soon after instillation. They further found that the degree of immersion of these particles into the aqueous subphase was highly dependent on the low surface tension of the surfactant in which they were suspended. Our findings on scanning electron microscopy in the distal airways adjacent to the pleura support these observations and suggest that the low surface tension of KL_4 surfactant permits the microspheres to attach to the aqueous subphase created by KL_4 surfactant in the acini of these immature primate lungs. We cannot exclude the possibility that some of the microspheres were transported proximal over the course of the 12-h experiment, nor can we exclude that some microsphere may have been removed during the dehydration process or deformed during the critical point drying for microscopy; however, these small pieces of tissue were not used to establish distribution quantitatively.

Davis *et al.* (9) have demonstrated that a single bolus intratracheal injection of calf lung surfactant extract spreads more rapidly and homogeneously than saline, establishing that interfacial properties are critical for distribution. Ueda *et al.* (28) recently reported that treatment of fetal lambs with either four quarter dose boluses or two half dose boluses provided significantly more uniform distribution than infusion of surfactant over 30 min into the trachea; however, in both of the bolus dosing strategies the animals were disconnected from the ventilator and positive end-expiratory pressure. Of interest, the second dose of surfactant in their study distributed similarly to that of the first dose. Neither in the study of Davis *et al.* nor by Heldt *et al.* (17) were there significant effects of either conventional ventilation, high frequency jet ventilation, or oscillatory ventilation on the distribution of protein-containing surfactants in the lungs of animals with surfactant deficiency, although Walther *et al.* (29) suggested that high frequency oscillation superimposed on mechanical ventilation improved distribution over conventional ventilation alone.

Although more uniform distribution of KL₄ surfactant was documented using an instillation technique that maintained positive end-expiratory pressure throughout dosing, we did not demonstrate that this dosing strategy selectively improved lung mechanics or the a/Ao₂ ratio. Using both techniques of administration there were improvements from the pretreatment levels to those observed at 12 h after treatment. We speculate that our present techniques for measuring these acute effects are too insensitive to discriminate between the differences observed in the distribution of KL₄ surfactant in this primate model limited to 10 animals. Nonetheless, we agree with Jobe and Ikegami (20) that intrapulmonary surfactant distribution characteristics are critical for the surfactant to exert all of its potential beneficial effects in infants with RDS.

In addition to the immediate adverse effects of surfactant administration on oxygenation, heart rate, blood pressure, and ventilation during dosing, other long term adverse events may result from insufficient or excessive surfactant distribution within the lungs. Areas depleted of exogenous surfactant may be prone to abnormalities of ventilation and perfusion, whereas those areas with excessive surfactant deposition may be more prone to pulmonary hemorrhage (30), lipid granulomas with a circumscribed region of inflammation (31), and overinflation with subsequent pulmonary edema with the development of inflammatory changes that have been reported to coincide with the development of bronchopulmonary dysplasia (32). With refinements in the use of exogenous surfactants, and particularly in the evaluation of new formulations, it is critical to establish which method(s) of instillation provide for more uniform distribution of these drugs to hopefully maximally benefit the infant with respiratory distress. Alterations in clinical techniques of drug delivery must be based on data taking into account not only immediate observable events during administration, but also the effects of these changes on the distribution of the surfactant preparation within the lung, changes in lung function, and longer term measures of surfactant efficacy. Use of KL₄ surfactant with an adapter that permits maintenance of positive end-expiratory pressure during administration of surfactant provides for improved distribution within the lungs of premature rhesus infants with RDS, and provides supporting data for adoption of this methodology for surfactant administration in human infants with RDS.

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