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Intratracheal atomized surfactant provides similar outcomes as bolus surfactant in preterm lambs with respiratory distress syndrome

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BACKGROUND: Aerosolization of exogenous surfactant remains a challenge. This study is aimed to evaluate the efficacy of atomized poractant alfa (Curosurf) administered with a novel atomizer in preterm lambs with respiratory distress syndrome.

METHODS: Twenty anaesthetized lambs, 127 ± 1 d gestational age, (mean ± SD) were instrumented before birth and randomized to receive either (i) positive pressure ventilation without surfactant (Control group), (ii) 200 mg/kg of bolus instilled surfactant (Bolus group) at 10 min of life or (iii) 200 mg/kg of atomized surfactant (Atomizer group) over 60 min from 10 min of life. All lambs were ventilated for 180 min with a standardized protocol. Lung mechanics, regional lung compliance (electrical impedance tomography), and carotid blood flow (CBF) were measured with arterial blood gas analysis.

RESULTS: Dynamic compliance and oxygenation responses were similar in the Bolus and Atomizer groups, and both better than Control by 180 min (all $P < 0.05$; two-way ANOVA). Both surfactant groups demonstrated more homogeneous regional lung compliance throughout the study period. There were no differences in CBF

CONCLUSION: In a preterm lamb model, atomized surfactant resulted in similar gas exchange and mechanics as bolus administration. This study suggests evaluation of supraglottic atomization with this system when noninvasive support is warranted.

Surfactant replacement therapy is a well-established standard of care for the treatment and prevention of neonatal respiratory distress syndrome (RDS), significantly reducing mortality and morbidity (1). Currently, endotracheal instillation is the only approved method of administration. However, over the last decade, the management of mild to moderate RDS has dramatically changed, endotracheal tube (ETT) intubation is often avoided and infants were managed with noninvasive modalities, such as continuous positive airway pressure whilst spontaneously breathing. In this population, surfactant administration is generally only provided when oxygen requirement increases and intubation is necessary (2,3).

The increase in noninvasive ventilation use has led to less invasive methods of surfactant delivery during continuous positive airway pressure being developed (4,5). All reported methods require technical skills, some degree of invasive intervention, and still involve instillation of liquid surfactant directly into the trachea via a process that is not dissimilar to intubation (6–8). Nebulization of surfactant offers an attractive alternative. Unfortunately, studies of nebulized surfactant have shown inconsistent clinical response and surfactant distribution (9–16). Moreover, most of the studies, even those in which a lung function improvement was observed, reported very low (<10%) fractions of surfactant deposition into the lung (9,12,17–20). Notwithstanding differences in nebulizer design, and thus particle size production, such modest deposition is likely due to the fact that most studies used aerosols generated extracorporeally by nebulizers connected to the ventilator circuit. This leads to high amounts of surfactant loss in the circuit, environment, and upper airway mucosa rather than the intended deep lung deposition.

An alternative approach, using special catheters designed for intracorporeal surfactant nebulization has been proposed (8,21). Intratracheal nebulization of surfactant using this method has been shown to provide equivalent oxygen response as standard bolus therapy (8,21), with high and even surfactant deposition (21), improved histological score, and less cerebral hemodynamics fluctuations in adult rabbits (21) and preterm lambs (8) with severe induced RDS. These two studies have shown the efficacy of intratracheal aerosolization of surfactant in two very severe models of RDS, surfactant-depleted adult rabbits, and preterm lambs with severe surfactant deficiency. Nevertheless, in these studies the animals were managed with intensive mechanical ventilation and paralysis,

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while in clinical practice the babies that may benefit most from surfactant atomization would be those that can be managed with a less intensive respiratory support strategy.

Recently, we developed a novel atomizing system for intracorporal atomization of surfactant into the supraglottic space during continuous positive airway pressure (22). This system consists of a small multilumen catheter whose tip can be placed in the supraglottis region bypassing the upper airways and directly atomizing into the pharynx, avoiding the need for intubation. The device also synchronizes delivery to inspiration, offering more effective delivery.

The aim of this proof of concept study was to evaluate the efficacy of this new device. Specifically, we aimed to compare the effect of surfactant delivered via atomization with standard bolus instillation on gas exchange, lung mechanics, and homogeneity of lung compliance in preterm lambs with a mild–moderate model of RDS.

Table 1. Study group characteristics

	Weight (kg)	F:M	Singleton (*n*)	Cord pH	Lung weight (g/kg)
Atomizer (*n* = 7)	3.84 ± 0.45	4:3	2	7.35 ± 0.06	29.7 ± 3.8
Bolus (*n* = 6)	3.06 ± 0.42	6:0	0	7.35 ± 0.05	32.0 ± 3.5
Control (*n* = 8)[a]	2.94 ± 0.78*	6:1	2	7.36 ± 0.09	30.1 ± 6.1

Data reported as mean ± SD. F:M, number of female vs. male lambs. Lung weight (g/kg) was normalized for body weight.
[a]One lamb excluded due to pneumothorax. *$P < 0.05$ (two-way ANOVA) Atomizer vs. Control.

RESULTS

Table 1 summarizes the baseline characteristics of each group. All lambs completed their assigned protocol without complications, except one lamb (Control) that developed a pneumothorax and was excluded. There were no statistically significant differences in lung weight, fetal pH, fraction of inspired oxygen or PaO_2 at 5 min of life. There was a trend toward more male and heavier lambs in the Atomizer group. The median (interquartile range, IQR) duration of atomization was 53 (48–56) min, being determined by the atomized volume of surfactant (9.60 ± 1.15 ml; mean ± SD). Surfactant delivery was well tolerated without any adverse events.

Pressure swing (ΔP) was similar between all groups at 5 min. Thereafter, all groups showed a time-based improvement (**Figure 1a**). Compared with baseline, the two surfactant groups showed a statistically and clinically relevant reduction in ΔP with time compared with Control (**Figure 1c**, $P < 0.05$ at 45, 75, 90, 150, and 180 min). Dynamic compliance (C_{dyn}) was higher in the two surfactant groups compared with Control at 5 min, but this was not significant (P = NS; Holm-Sidak post-test); **Figure 1b**. All groups showed a

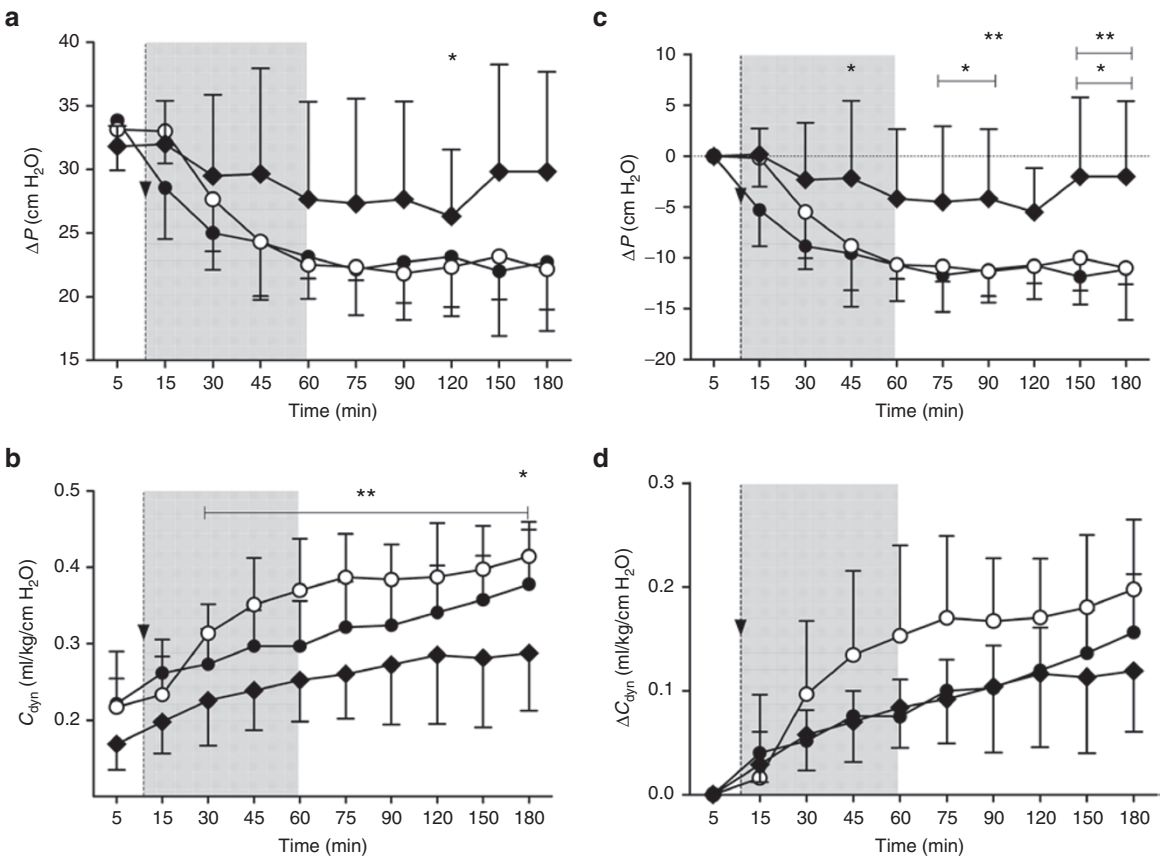

Figure 1. Lung mechanics. Pressure swing (**a**), C_{dyn} (**b**), and change in pressure swing (**c**) and C_{dyn} from initial value (**d**) during the 180-min study period for the Atomizer (closed circles–solid line), Bolus (open circles–solid line), and Control (closed diamonds–solid line) groups. Grey area: surfactant atomization; arrow: bolus instillation. Data mean and SD. *$P < 0.05$ Atomizer vs. Control, **$P < 0.05$ Bolus vs. Control (all P values Holm-Sidak post-tests). C_{dyn}, dynamic compliance.

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similar time-based improvement in C_{dyn} ($P < 0.0001$ two-way ANOVA). Administration of surfactant improved C_{dyn} ($P < 0.01$ at min 180 vs. 5 min) with no significant difference at 180 min between surfactant therapy groups, and with both surfactant groups demonstrating higher C_{dyn} compared with the Control group ($P < 0.0001$, Figure 1b), although there was no significantly difference in the change in C_{dyn} from baseline values between groups at 180 min (Figure 1d).

The oxygenation index (OI) increased with time in the Control group (Figure 2a,d) and was significantly higher than baseline (5 min) values by 90 min ($P < 0.01$, Holm-Sidak post-tests). In contrast, there was no temporal change in OI within the Bolus and Atomizer groups; being significantly lower than Control from 60 min in the Atomizer ($P < 0.05$) and 45 min in the Bolus ($P < 0.01$) groups. There was no difference in both arterial partial pressure of carbon dioxide ($PaCO_2$) and changes in $PaCO_2$ with time between the two surfactant groups (both were lower than Control at all time points, Figure 2c,e). A modified ventilation efficiency index, modified to account for the fact that tidal volume was adjusted to target $PaCO_2$, was significantly lower in the Control group compared with Atomizer, but not in Bolus, from 15 min of life (Figure 2e) with all groups showing similar variations with time (Figure 2f).

There were no differences in heart rate and mean arterial pressure between groups (Table 2). The Bolus group had a transient fall in mean arterial pressure immediately after surfactant delivery (15 min) compared with 5 min of life ($P < 0.05$; Holm-Sidak post-test). Mean arterial pressure was lower in the Atomizer group at 60 and 180 min compared with 5 min ($P < 0.05$). Mean carotid blood flow (CBF) was stable in all groups over time, including during the administration of surfactant.

Figures 3 and 4 show the relative regional gravity-dependent distribution of C_{dyn} at 5 and 180 min of life measured by electrical impedance tomography (EIT). This method provides visual representation of the pattern of dynamic compliance distribution within the lung. Gravity-dependent regional C_{dyn} was uniform across the two study periods for the surfactant-treated groups, demonstrating relatively homogeneous spatiotemporal

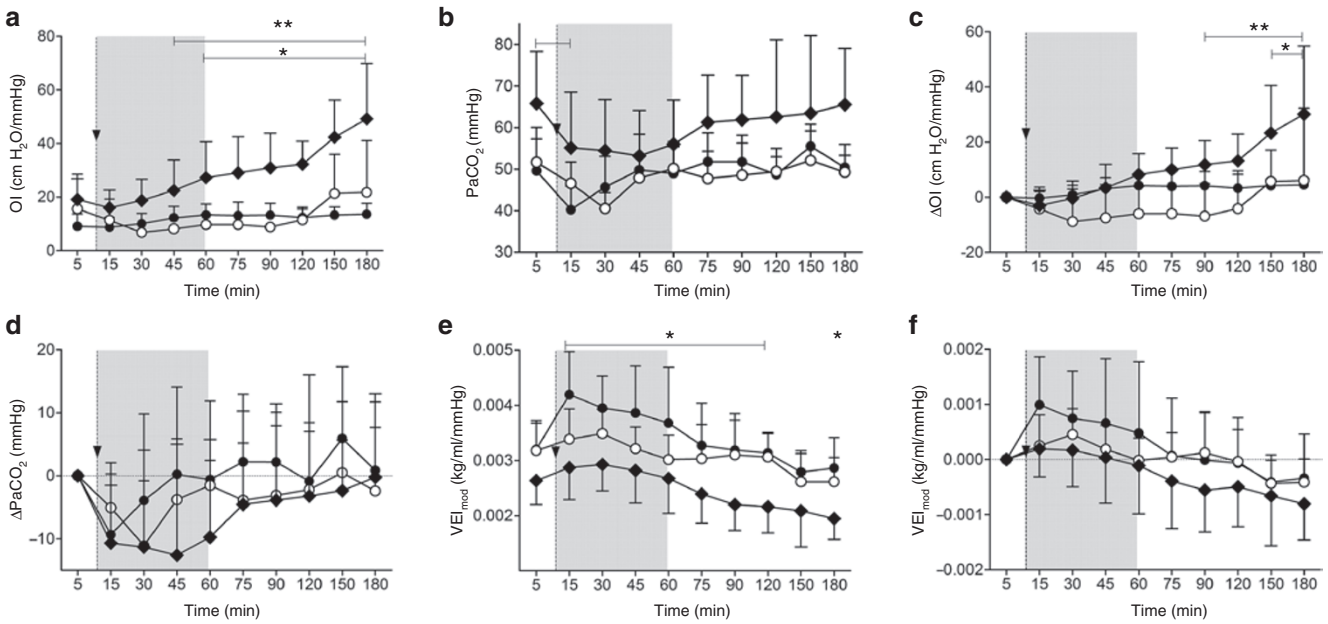

Figure 2. Gas exchange (**a**) OI, (**b**) $PaCO_2$, (**c**) VEI_{mod}, (**d**) ΔOI, (**e**) $\Delta PaCO_2$, (**f**) ΔVEI_{mod}. Data expressed as mean and SD. *$P < 0.05$ Atomizer vs. Control, **$P < 0.05$ Bolus vs. Control (Holm-Sidak post-tests). OI, oxygenation index; VEI_{mod}, modified ventilation efficiency index.

Table 2. Hemodynamic results

Time (min)	HR (bpm)			MAP (mmHg)			Mean CBF (mL/min/kg)		
	Atomizer	Bolus	Control	Atomizer	Bolus	Control	Atomizer	Bolus	Control
5	147 ± 35	131 ± 46	183 ± 46	44 ± 10	44 ± 10	47 ± 8	NA[a]	NA[a]	NA[a]
15	139 ± 14	110 ± 26	149 ± 40*	39 ± 12	29 ± 13[†]	36 ± 13	15.5 ± 9.4	9.9 ± 6.9	15.3 ± 15.6
60	153 ± 20	161 ± 21	160 ± 37	32 ± 3**	37 ± 9	40 ± 12	17.0 ± 9.6	12.0 ± 11.6	12.6 ± 5.8
120	142 ± 14	163 ± 45	158 ± 38	34 ± 5	41 ± 18	40 ± 13	15.8 ± 9.3	14.8 ± 13.0	12.6 ± 7.2
180	137 ± 20	142 ± 60	140 ± 51*	30 ± 8**	33 ± 14	32 ± 8	15.1 ± 9.7	12.7 ± 10.2	10.5 ± 4.8

Data reported as mean ± SD.
CBF, mean carotid blood flow; HR, heart rate; MAP, mean arterial pressure.
[a]Mean CBF at 5 min is not available (NA) because data were not reliable in some lambs due to probe movement during delivery. *$P < 0.05$ vs. 5 min within Control group, **$P < 0.05$ vs. 5 min within Atomizer group, [†]$P < 0.05$ vs. 5 min within Bolus group, Holm-Sidak post-test.

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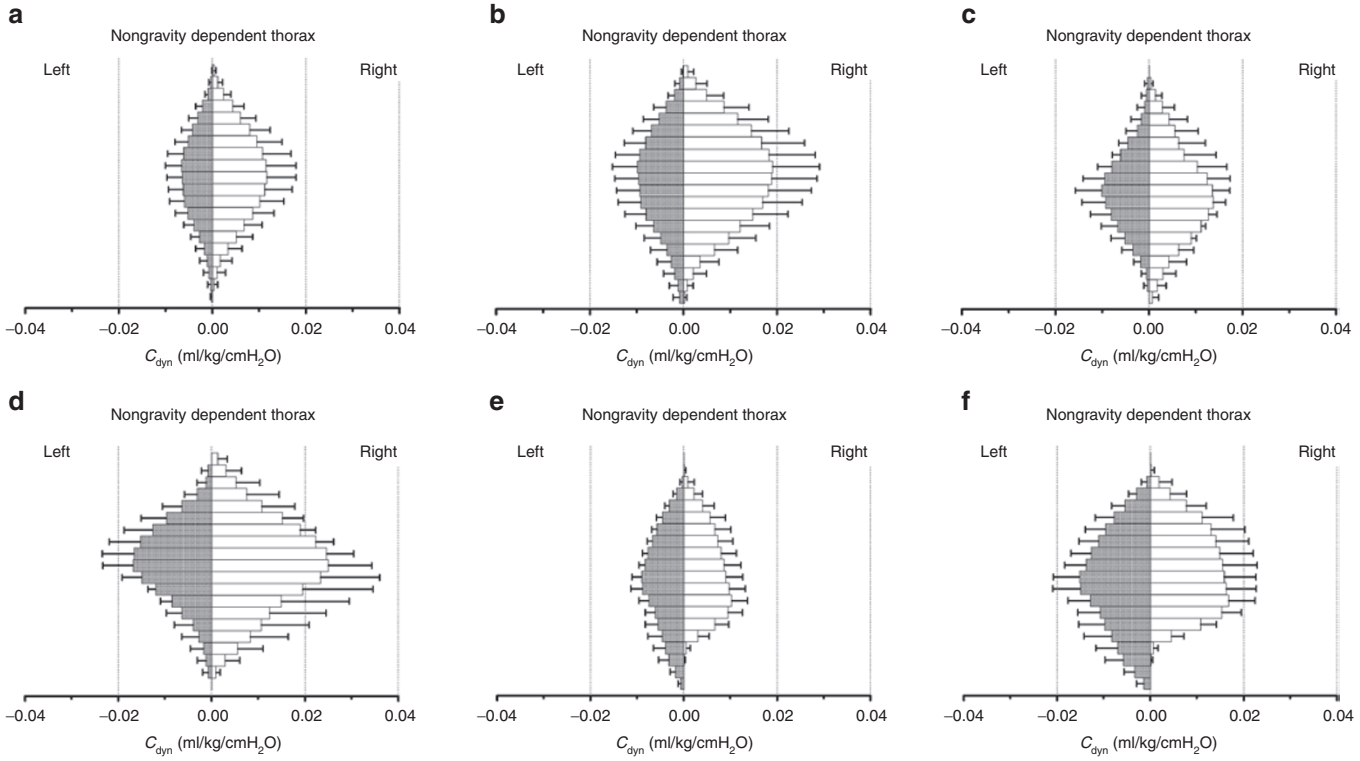

Figure 3. Regional lung compliance. Functional EIT images of the relative regional gravity-dependent distribution of C_{dyn} (expressed in mL/cmH$_2$O/kg) in the Atomizer, Bolus, and Control groups at 5 and 180 min in each of the 20 lung tissue containing slices for the right (white bars) and left (grey bars) hemithoraces. The images are orientated such that the most gravity nondependent (ventral) lung is orientated at the top of each bar graph, and the most dependent (dorsal) regions at the bottom using the method of Frerichs and co-workers (25). Atomizer at 5 min (**a**) and 180 min (**b**), Bolus at 5 min (**c**) and 180 min (**d**), and Control at 5 min (**e**) and 180 min (**f**). C_{dyn}, dynamic compliance; EIT, electrical impedance tomography.

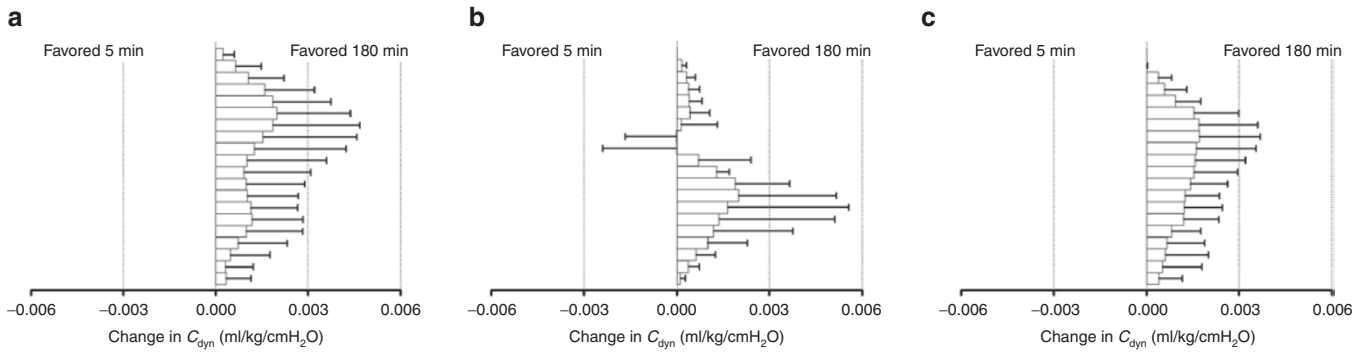

Figure 4. Variation in regional lung compliance. Change in gravity-dependent distribution of C_{dyn} throughout the lung between 5 and 180 min, using subtraction histograms to express the difference in C_{dyn} within each lung region between 5 and 180 min using the same gravity-dependent orientation as **Figure 3**. Atomizer group: 180 min vs. 5 min (**a**), Bolus group: 180 min vs. 5 min (**b**) and Control group: 180 min vs. 5 min (**c**). Data to the right indicates C_{dyn} was better at 180 than at 5 min within that region of interest, and vice versa. All data are mean and SD. C_{dyn}, dynamic compliance.

improvement by 180 min. In contrast, the control group had an inhomogeneous gravity-dependent pattern of C_{dyn} at 5 min, with marked reduction in the right-dependent regions. This spatial inhomogeneity increased during the study period.

DISCUSSION

In our intubated preterm lamb model of moderate RDS, we demonstrated that surfactant delivered via our novel atomization technique had an equivalent effect on lung mechanics and gas exchange as bolus surfactant administration. Importantly, both methods were better than mechanical ventilation without surfactant. These data suggest that atomization of surfactant may be an effective method of surfactant delivery to the lung in spontaneously breathing recipients if a feasible method of supraglottic delivery can be achieved.

Both methods of surfactant administration (bolus and atomization) had a similar positive effect on ΔP and OI. Bolus administration, the current clinical standard, achieved a rapid change in C_{dyn} after administration, whilst this was achieved more gradually with atomization. This pharmacodynamic

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effect is not unexpected due to the longer delivery times. The short study duration after completing the atomization period may not have been sufficient to allow the full post surfactant effect to have occurred, as evident by the ongoing increase in ΔC_{dyn}.

Delivery was dose-related, and lambs have a higher birth weight than preterm human infants, suggesting quicker delivery in the latter population. The slower effect of atomization is not necessarily a limitation of this approach. It may reduce the potential of rapid changes in PaO_2 and $PaCO_2$ (8). In addition, atomized surfactant therapy is intended for infants receiving noninvasive ventilation, who are likely to have a milder form of RDS than intubated infants.

C_{dyn} assesses total lung compliance, but we also used functional EIT to evaluate the effect of different modalities of surfactant administration on ventilation distribution and regional changes in dynamic compliance. Despite the well-described limitations (23), EIT provides the only clinical translatable method of regional lung imaging, and has been used previously to describe the changes in the lung during surfactant administration (24). Importantly, EIT offers the ability to image multiple volume state parameters, including regional ventilation (24) and C_{dyn} (25,26). RDS is a disease characterized by regional heterogeneity of ventilation. Approaches that rectify heterogeneity are essential components of lung protective ventilation strategies (27,28). The ability of bolus surfactant therapy to reduce ventilation heterogeneity has been validated using EIT in animals (24) and humans (29), and our Bolus group data were similar. Both surfactant administration strategies resulted in more homogenous spatial gravity-dependent patterns of regional C_{dyn} over time consistent with the global mechanical and oxygenation outcomes. Although we did not undertake formal deposition analysis, the EIT results suggest similar distribution of surfactant, at least functionally. The Control group had more regional gravity-dependent heterogeneity of C_{dyn} at baseline, and this pattern did not change despite the time-dependent improvement in global C_{dyn}, indeed heterogeneity increased with time.

Interestingly, CBF remained stable in all groups during the study, specifically during and after surfactant treatment. Bolus surfactant therapy has been associated with rapid fluctuations in cerebral blood flow (8). It is possible that the relative respiratory stability of our lamb model and the use of sedatives and analgesia provided resilience to the interruption in mechanical ventilation during bolus delivery. Compared with other studies (8), we did not observe a dramatic change in $PaCO_2$, which may have contributed to stable CBF. The instrumentation of the carotid arteries may also have influenced the CBF data, although any effect would be standardized across all lambs. We also only recorded flow in one of the many vessels supporting cerebral perfusion, and it is possible that total cerebral blood flow was different and other techniques such as near-infrared spectroscopy may yield different results.

Tolerance of the atomizing catheter and process during administration will be major contributors to clinical feasibility. Our lambs were intubated, not exposed to antenatal steroids and had spontaneous breathing effort suppressed, unlikely events for the intended clinical situation, and also confounders in assessing tolerance. These interventions were intentional to ensure that the interaction between modes of surfactant delivery was not influenced by other variables such as site of delivery and potential differences due to spontaneous respiratory effort.

During the atomization period, the Atomizer group did have a nonsignificant trend toward a higher modified ventilation efficiency index from 15 to 60 min of life compared with the Bolus group. This difference was likely associated to the atomization procedure, disappearing on completion. In our opinion, this effect may be related to the washout of the dead space due by the intratracheal injection of the atomizing gas with a mechanism similar to that already reported during high-flow nasal cannulae (30–32).

Our study is not the first to describe intratracheal administration of nebulized surfactant. In surfactant-depleted adult rabbits, Wagner and co-workers (21) demonstrated that a surfactant fog delivered via the ETT improved PaO_2 and $PaCO_2$ similarly to bolus instillation with better distribution and intrapulmonary recovery rates of approximately 86.5%. Rey-Santano and co-workers (8) showed similar gas exchange and lung mechanics to bolus surfactant, with lower lung injury and less cerebral hemodynamics fluctuations, following intratracheal administration of aerosolized surfactant (<5 μm) in preterm lambs with severe RDS in whom noninvasive ventilation would be unlikely. We contend that our lamb model was more representative of most preterm infants with acute RDS in the modern era. To date no clinical devices for the delivery of nebulized surfactant in combination with noninvasive respiratory support have proved to be effective.

The treatment of preterm neonates with nebulized surfactant is challenging, due to the low inspiratory flows, high respiratory rates, and small airways that characterize the preterm lung, and generally high viscosity of most of the surfactants (33). Despite many experimental studies of nebulized surfactant, using various animal models of surfactant deficiency or depletion, the results have been inconsistent and often disappointing. In some studies, there was no benefit from nebulized surfactant (10,12,13), whereas in others a significant improvement in gas exchange and lung mechanics was observed (9,11). A possible explanation of these findings is the low rates of surfactant deposition within the lung. Most previous nebulization methods (9,12,17–20) have been based on a extracorporeal delivery mechanism, often within the ventilator circuit. This will result in significant deposition within the circuit and upper respiratory system. High-yield deposition within the deep lung will be fundamental to the clinical success, and uptake of any nebulization system for surfactant.

Our system was specifically designed to minimize surfactant loss prior to the deep lung, using intracorporeal administration below the upper airways, synchronization to inspiration and generating relatively big particles. Even though the subglottic location of the catheter within our experimental design may have contributed to increased deposition of surfactant

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in the deep lung, we expect that good deposition can also be achieved by placing the catheter above the vocal folds. This would necessitate some changes to the whole system from our surrender system to optimize the placement of the atomizing catheter. Moreover, we used synchronization with inspiration with the rationale of reducing the surfactant dose required and minimizing the risk of surfactant pooling within the pharynx during spontaneous breathing, as the atomized particles are expected to be transported by inspiratory flow in the trachea.

Another important difference in our atomization system from many other studies was particle size generation. We designed our system to deliver relatively large particles (median 91 μm), because we speculated that larger particle size would minimize surfactant loss during expiration and facilitate better distribution to nonventilated lung regions via diffusion gradients, the spreading properties of surfactant, the Marangoni effect, and capillarity compared with small particles. In fact, although many previous studies have suggested that particles of <5 μm are needed for homogeneous deposition and penetration of the distal air spaces (34), we contend that small particles, being driven by inspired airflow, may reach only those alveoli that are already open. As a partial support of this theory, a recent small human trial of nebulized surfactant with 2.5-μm particles did not show a significant difference in outcomes compared with continuous positive airway pressure alone (15). As a consequence we aimed for particles that were large but also not too big to be detrimentally influenced by gravity. Even though we did not measure surfactant deposition, the similarities in outcomes compared with bolus delivery, and difference from control, suggest a reasonable fraction of surfactant was delivered to the deep lung using our system. This justifies further studies during noninvasive support and spontaneous breathing.

Finally, in designing our device, we targeted a delivery time of approximately 20 min for preterm infants to guarantee the tolerance of the atomizing catheter, and to achieve clinical efficacy in a relatively short time period. As discussed previously, in this study the 60-min atomization period was due to the weight of the lambs, which were more than three times heavier than a preterm infant.

Limitations of the Study

This study has some limitations not already mentioned. Although we tried to limit differences in lung immaturity and standardized the gestational age, we observed variability in the severity of RDS among animals, specifically in the groups at baseline, that may have led to differences in the response to surfactant treatment. The Atomizer group was heavier at birth, which may have been beneficial, although this group also had less female lambs. Predicting birth weight during fetal instrumentation is difficult and it was our intention to randomize interventions. The control group had worse baseline gas exchange, lung mechanics, and regional ventilation than the other two groups prior to any surfactant therapy. This suggests that the differences seen at study end may not have been related to surfactant therapy but could have been due to respiratory transition. We contend that the rate of change in OI and C_{dyn} after surfactant therapy makes it unlikely that there was not some treatment response influencing the differences in outcome. As this study was a proof-of-principle study, and bolus surfactant is well established as a method of reducing lung injury in RDS, we did not undertake injury analysis. Future studies in spontaneously breathing recipients should consider deposition and injury end points.

Conclusion

In our preterm lamb model of moderate RDS, intratracheal atomized surfactant via a novel atomization system had a similar influence on regional lung mechanics and gas exchange as bolus administration. This point-of-concept study suggests that atomization of surfactant if feasible and likely results in lung deposition. Further studies in spontaneously breathing subjects receiving noninvasive ventilation using a supraglottic administration technique are warranted.

METHODS

The study was performed at the Murdoch Children's Research Institute, Melbourne, Australia and approved by the Institution's Animal Ethics Committee. Animals were cared for in accordance with the guidelines of the National Health and Medical Research Council of Australia.

Animal Preparation

Border-Leicester/Suffolk lambs (n = 20) were studied at 127 ± 1 d of gestation (term ~145 d). Lambs were delivered by caesarean section to ewes receiving isoflurane and nitrous-oxide inhalational anaesthesia, after premedication with xylazine 2 mg IM and ketamine 150 mg IM. Antenatal corticosteroids were not administered to increase the degree of RDS in this short-term study. Whilst on placental support, the carotid artery and external jugular vein were cannulated. The other carotid artery was encircled with a 4-mm transit-time flow probe (Transonic Systems, Ithaca, NY). Animals were tracheotomized and intubated using a 4.5-mm internal diameter cuffed ETT (Atomizer group) or a 4.0-mm tube (other groups), with approximately similar internal diameters after inserting the atomizer catheter. Fetal lung liquid was passively drained prior to delivery and the ETT clamped. The fetal thorax was then exteriorized and dried. Sixteen custom-built EIT electrodes were placed equidistant around the fetal chest using our previously described methodology (35,36). The EIT electrodes were connected to a Goe-MF II EIT system (CareFusion, Hoechberg, Germany), and a 10-s baseline recording of the unaerated lung made as a reference. If the lamb was allocated to receive atomized surfactant a custom-built catheter system was introduced into the ETT via a Ballard closed suction ETT adaptor (Kimberly-Clark, Neenah, WI). At delivery, the umbilical cord was cut, the lamb weighed and placed supine before starting mechanical ventilation. Analgesia and anaesthesia were maintained throughout with midazolam and ketamine infusions titrated to suppress spontaneous breathing. Hydration was achieved with a 0.18% NaCl and 4% glucose intravenous infusions.

Study Design

Prior to birth, animals were randomly allocated to one of the following groups:

Control group (n = 8).
Animals, who did not receive surfactant.

Bolus group (n = 6).
Animals that received 200 mg/kg of poractant alfa surfactant (Curosurf; Chiesi Farmaceutici, Parma, Italy) at 10 min of life as a single bolus administered over 10–15 s via a 6-FG (French Gauge) feeding catheter, premeasured to the ETT tip, and a closed system (NeoLink, Carefusion, San Diego, CA).

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Atomizer group (n = 7).
Group of animals, in which 200mg/kg of poractant alfa surfactant was atomized over a 60-min period from 10 min of life.

All lambs were ventilated for 180 min, using a standardized protocol detailed below, to allow observation of time-based changes in regional ventilation and mechanics for at least 120 min after completing surfactant administration.

Experimental Set-up

The experimental set-up is schematically reported in **Figure 5**. Peripheral oxygen saturation, heart rate, arterial blood pressure (HP48S monitor, Hewlett Packard, Andover, MA), and CBF were recorded continuously from birth. Pressure (P_{ao}) and flow (V'_{ao}) were measured at the airway opening using a Florian respiratory mechanics monitor (Acutronic Medical Systems, Hirzel, Switzerland). All signals were sampled at 1,000 Hz and acquired using a Powerlab/Lab Chart system (Version 7, AD Instruments, Sydney, Australia). Relative changes in regional thoracic volume were measured using the Thorascan software package sampling at 25 Hz (37,38). Arterial blood gas analysis was performed at 5, 10, and 15 min of life, and then 15-minutely until 90 min life and then 30-minutely until completion of the study.

Atomized surfactant was delivered using an *ad hoc* system (22). Briefly, the system was composed of an atomizing catheter connected to a modified infusion pump, delivering surfactant at a rate of 0.4 ml/min, triggered to the pressure signal recorded at the airway opening, such that surfactant was only delivered during inspiration. The characteristics of Curosurf after atomization with our catheters were defined before the study. Specifically, particles size was measured using the laser diffraction method (Spraytech, Malvern Instruments,Malvern, UK) and retention of physiochemical properties confirmed *in vivo* in preterm rabbits. The atomizing catheters are made of a central lumen, through which the surfactant flows and several outer lumens in which atomizing gas (compressed air) flows at a rate of 0.7 l/min. At the tip of the catheter, high-velocity air breaks the liquid jet producing particles with a median (10th–90th percentile) of 91 (27–197) μm. The atomization catheter was kept centred in the ETT by a custom-built fine wire support to avoid spraying surfactant toward the ETT walls whilst not interrupting ventilator gas flow.

Ventilation Strategy

Mechanical ventilation was applied using positive pressure ventilation in a targeted tidal volume mode (SLE5000 infant ventilator, SLE, South Croydon, UK). Initially, tidal volume (V_T) was set at 7.0 ml/kg, maximum positive inspiratory pressure at 40 cm H_2O, positive end-expiratory pressure at 8 cm H_2O, respiratory rate at 60 bpm, inspiratory time at 0.4 s, and fraction of inspired oxygen at 0.3. Thereafter, fraction of inspired oxygen was adjusted to maintain peripheral oxygen saturation between 88 and 94% and set V_T adjusted in increments of 1.0 ml/kg to maintain $PaCO_2$ between 45 and 60 mmHg. Maximum positive inspiratory pressure could be increased to a maximum of 50 cm H_2O if set V_T was not being met and CO_2 clearance or oxygenation were compromised. Positive end-expiratory pressure was weaned (minimum of 6 cm H_2O) if the lamb's respiratory status was improving following these measures.

At 180 min, the lambs were humanely killed with a lethal dose of pentabarbitone and then exposed to atmosphere for 2 min to deflate the lung to functional residual capacity. A pressure–volume curve was performed using a calibrated glass super syringe from 0 to 40 cm H_2O. This allowed calculation of the static mechanics of the lung and calibration of EIT (35,36,39–41).

Data Analysis

OI was determined at each arterial blood gas. A modified ventilation efficiency index was calculated to quantify CO_2 exchange relative to magnitude of respiratory support, using the formula: $1/(V_T$ (ml/kg) × $PaCO_2$ (mmHg)). C_{dyn} was computed from the P_{ao} and V'_{ao} data during five consecutive inflations using the least means squared algorithm (42). The mean CBF during each time period was determined from the CBF waveforms.

Regional changes in relative C_{dyn} were generated from the EIT data. For each lamb, the aerated EIT pixels at vital capacity were first determined to generate segmented lung image. Vital capacity was defined as the difference in impedance between 0 and 40 cmH_2O during the postmortem super-syringe pressure volume curve (26,43). Functional EIT images of the pixels within the segmented regions were created from the tidal minimum to maximum impedance difference of the time-course signal (24). Functional EIT images were reconstructed from 20 to 30 inflations during each relevant time point that were free from artefact and containing stable V_T and pressure amplitudes. Functional EIT data were then calibrated to the average global C_{dyn} value coinciding with the inflations (40,44). Histograms of the gravity-dependent distribution of C_{dyn} in the right and left hemithorax were then generated (25,26).

A sample size of six lambs per group was required to identify a difference in C_{dyn} of 0.1 ml/cmH_2O/kg, assuming a SD of 0.05 ml/cmH_2O/kg (8), 80% probability and an alpha error of 0.05. All data were tested for normality and analyzed with an appropriate two-way ANOVA (using group and time as factors) and Holm-Sidak post hoc tests. Statistical analysis was performed with Sigmaplot 11.0 (Systat

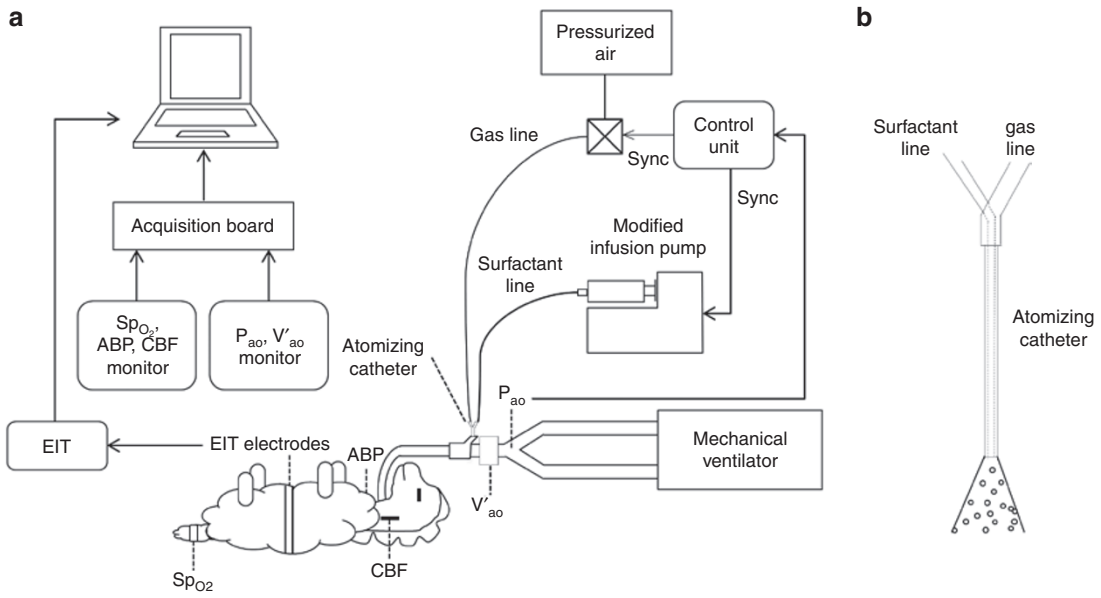

Figure 5. Set-up. Schematic representation of the overall experimental set-up (**a**) and the atomizing catheter (**b**).

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Software, San Jose, CA), with a P value < 0.05 being considered statistically significant.

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