RESPIRATORY MEDICINE (2001) **95,** 612–617
doi:10.1053/rmed.2001.1114, available online at http://www.idealibrary.com on IDEAL®

A comparison of surfactant delivery with conventional mechanical ventilation and partial liquid ventilation in meconium aspiration injury

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The objective of this study was to compare surfactant (SF) distribution and physiological effects after standard SF delivery during conventional mechanical ventilation (CMV) with that using partial liquid ventilation (PLV). A model of meconium aspiration syndrome (MAS) was developed using two groups of adult rats ($n=14$). After meconium instillation of $2{\cdot}5\,ml\,kg^{-1}$ (20% v/w), SF/CMV: ($n=7$) CMV and SF/PLV: ($n=7$) PLV, received ^{14}C-labeled surfactant ($4\,ml\,kg^{-1}$) delivered intratracheally in four aliquots over 20 min in both groups. Sequential measurements of arterial blood chemistry and lung mechanics were performed in all animals. At the conclusion of experiments, lungs were inflated ($30\,cmH_2O$), dried, sectioned and evaluated for radioactivity in disintegrations per minute (DPM). Surfactant distribution was improved ($P<0{\cdot}01$) with PLV as compared to CMV with 48·8% of the pieces *vs.* 30·9% of the pieces receiving within 25% of the mean amount of surfactant, respectively. Further, regional distribution was also significantly more uniform with PLV than CMV: left vs right ($P<0{\cdot}01$) lung and ventral *vs.* dorsal ($P<0{\cdot}01$) regions. Finally, arterial PO_2 and ventilation efficiency index were significantly ($P<0{\cdot}01$) greater post-treatment in SF/PLV than SF/CMV. These data demonstrate surfactant delivery with PLV, as compared to CMV alone, to be an improved method of delivering surfactant in MAS and suggest the possible utility of SF/PLV combination therapy for its treatment of other etiologies of neonatal respiratory distress.

Key words: liquid ventilation; surfactant; meconium aspiration syndrome; perfluorochemicals; rats.

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Introduction

While the incidence of meconium aspiration syndrome (MAS) has declined over the last two decades, it remains a significant cause of neonatal morbidity and mortality (1). The pathophysiology of MAS appears to be a complex process, both mechanical and chemical in nature (2,3). Acutely, aspirated meconium results in complete and partial airway obstruction leading to co-existent atelectatic and hyper-inflated lung regions. These complications result in ventilation/perfusion (V/Q) mismatch, pulmonary hypertension and the increased possibility of pneumothorax frequently seen in infants with MAS (4–6). Later sequelae include chemical pneumonitis and surfactant inactivation which many investigators have suggested as a significant contributor to MAS pathophysiology (7–17). Other studies demonstrating improved arterial oxygenation and lung mechanics following surfactant therapy further support this belief (18–20).

Partial liquid ventilation (PLV) with perfluorochemical (PFC) liquids has been shown to improve arterial oxygenation and gas exchange in a variety of animal injury models and humans with severe respiratory distress (21–26). Partial liquid ventilation employs conventional mechanical ventilation (CMV) to deliver gas tidal volumes into a lung filled up to functional residual capacity (FRC) with PFC. In addition, this technique has been found to improve arterial oxygenation and gas exchange in a MAS injury model in newborn lambs (23).

Liquid ventilation with PFC liquids has been shown to effectively deliver various biological agents to the alveolar surface of the lung (27–31). PFC liquids are an ideal vehicle for pulmonary administered drug delivery (PAD) because the low surface tension supports uniform distribution throughout the lungs. PFC liquids and surfactant have been found to be biocompatible with surfactant's surface tension reducing properties being unaltered after exposure to PFC (23,32–34). In this study, it was hypothesized that PLV will permit more homogeneous surfactant delivery

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Received 13 July 2000 and accepted in revised form 7 April 2001.

0954-6111/01/070612+06 $35·00/0

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than that achieved through CMV in a MAS injury model. Thus, uniform distribution should result in improved ventilation/perfusion matching as demonstrated by improved indices of oxygenation and gas exchange.

Materials and methods

Fourteen adult Sprague-Dawley rats (350–650 gm) were anaesthetized with intra-peritoneal injection of sodium pentobarbital (40 ml kg^{-1}) restrained and placed under a radiant heat lamp. The animals were managed following the principles of Guiding Principles in the Care and Use of Animals by the American Physiologic Society. All procedures in the protocol were approved by the Temple University Institutional Review Board. Supplemental doses of anesthesia were given as needed (6 mg $kg^{-1}h^{-1}$, i.v.). A subcutaneous injection of 1% lidocaine was given prior to insertion of a endotracheal tube by tracheostomy. Catheters were placed in the femoral artery and vein allowing for drug delivery and monitoring of arterial blood pressures and blood gases. Pancuronium bromide (0·1 mg kg^{-1}) was administered before initiating CMV (Infant Star; Infrasonics, San Diego, CA, U.S.A.). CMV was begun with peak inspiratory pressures (PIP) of 12–18 cmH_2O, positive end expiratory pressure (PEEP) of 2–4 cmH_2O, fractional inspired oxygen concentration (FiO_2) of 1·0 and a respiratory frequency between 35·45 breaths min^{-1}. Ventilation schemes were adjusted to maintain $PaCO_2$ between 35·45 mmHg. Arterial blood gas measurements were measured with a Radiometer ABL 330 Blood gas analyzer (Copenhagen, Denmark). The arterial catheters were connected to pressure tranducers for continued monitoring of blood pressure and heart rate via an Athena Neonatal monitor (Air Shields, Hatboro, PA, U.S.A.). Oxygen content and hemoglobin were measured using a Hemoximeter Radiometer OSM 3 (Copenhagen, Denmark).

Meconium for the study was obtained from the first stools of human infants. It was pooled and diluted with 0·9% NaCl to 25% by weight slurry which was then filtered to remove large particulate matter. The meconium solution was further diluted to yield a reproducible meconium-crit of 25%. Stock solution was divided into 5 ml aliquots and frozen until needed.

Surfactant (Survanta; Ross Laboratories, Columbus, OH, U.S.A.) was combined with ^{14}C radiolabeled dipalmitoyl phosphotidal choline (50 μ curie ml^{-1} to yield a solution with an activity of 0·24 μ curie ml^{-1}. Surfactant (4 ml kg^{-1}) was delivered intra-tracheally via bolus infusion over 20 min in four aliquots to all rats. Animals were positioned according to manufacturer's instructions and were removed from the ventilator during aliquot instillation for no longer than 15 sec. Surfactant was administered slowly in this model to reduce gas trapping and potential pneumothorax.

PARTIAL LIQUID VENTILATION

A total of 20 ml kg^{-1} of perflubron (LiquiVent®; Alliance Pharmaceutical Corp., San Diego, CA, U.S.A.) was instilled in the PLV group after surfactant administration. Via a side port on the endotracheal tube, 1/3 of the total dose was infused continuously over 10 min with a Gemini PC-2 infusion pump (IMED Corporation, San Diego, CA, U.S.A.). Animal position was varied to optimize liquid distribution. The remaining two-thirds of the PFC dose was then infused continuously in an identical manner over 20 min, with each surfactant bolus delivered halfway through the 5 minute infusion time allotted for each of the four lung regions.

EXPERIMENTAL PROTOCOL

Baseline values for arterial blood gases, ventilatory parameters, and arterial blood pressure measurement were documented. All rats received 2·5 ml kg^{-1} of meconium delivered intra-tracheally in four aliquots over 15–30 min. At 60 minutes from the time of injury, rats were then treated with exogenous surfactant either during CMV or PLV and managed for 2 h after treatment at a FiO_2 of 1·0. The PIP and PEEP were limited to 40 and 7 cmH_2O respectively; subsequent ventilation strategy was based on optimizing arterial blood gas profile within these limits. Arterial blood samples were taken every 15 min for the entire 3 h protocol.

At the conclusion of the experiment, animals were killed with an overdose of potassium chloride, the lungs were excised still attached to the endotracheal tube, inflated and dried using a PEEP of 30 cmH_2O. Equidistant horizontal sections were made to establish four apical to basilar regions, correspondingly lettered A-D in each lung. A vertical section was made down the midline of each lung to divide each into ventral and dorsal regions. These regions were further sectioned to yield 29 total lung pieces (10–70 mg each); 12 left, 16 right and one tracheal piece. As previously described (27,30), pieces of lung were digested in hyamine hydroxide (10 mg ml^{-1} (National Diagnostics, Atlanta, GA, U.S.A.). From the digestate, 1 ml aliquots were taken and added to 175 ml liquid scintillation cocktail (Amersham Corporation, Arlington Heights, IL, U.S.A.). Samples were analysed on a Wallac 1409 liquid scintillation counter (Wallaac Inc., Turku, Finland) with radioactivity measured in disintegrations per minute (DPM).

DISTRIBUTION ANALYSIS

Distribution of surfactant was expressed as normalized values for each piece of lung. Normalized values were calculated by dividing the (disintigrations per minute) DPM mg^{-1} for each piece by the DPM mg^{-1} of total lung tissue. These normalized values were then presented as histograms with interval widths of 10% about the mean value of 1·0. Comparison of regional distribution between surfactant and liquid/surfactant treated groups was determined in a similar manner.

STATISTICAL ANALYSIS

Results were analysed with multi-factorial two-way analysis of variance (ANOVA) for repeated measurements as a

function of time and group. Post hoc analysis of inter-group differences was determined using the Bonferroni/Dunn all means comparison test. Statistical significance was accepted at *P*-values less than 0·05.

Results

Measurements at baseline and following meconium injury are summarized in Table 1. All animals receiving meconium demonstrated a significant ($P<0{\cdot}01$) decrease in P_aO_2 and VEI, and an increase in P_aCO_2. As shown in Table 1, there were no significant differences between pre-injury and post-injury values as a function of group. Physiological changes over time with respect to SF/CMV and SF/PLV are shown in Figs 1 and 2. The P_aO_2 in the SF/PLV group was significantly ($P<0{\cdot}01$) higher than the SF/CMV group 30 min post-treatment with no significant difference noted throughout the remaining 90 min. No treatment difference existed between groups with regard to P_aCO_2, but ANOVA demonstrated a significant ($P<0{\cdot}01$) group difference for VEI over time (Fig. 2). Post-hoc analysis showed that VEI for the SF/PLV was greater at 30 min post-treatment as compared to the SF/CMV group at the same time.

Surfactant distribution throughout the entire lung and various lung regions is illustrated in Figs 3 and 4. With the SF/CMV group [Fig. 3(b)], surfactant delivery was relatively non-homogeneous with $30{\cdot}9\pm4{\cdot}9$ SE % of the pieces receiving within 25% of the mean amount of surfactant. In addition, the largest percentage of pieces, $12{\cdot}5\pm2{\cdot}4$ SE %, received greater than 1·94 times the mean. In contrast, SF/PLV (Fig. 3(a)) resulted in $48{\cdot}8\pm4{\cdot}8$ SE % of the pieces receiving within 25% of the mean amount of surfactant. Further, no pieces received less than 34% of the mean and only $2{\cdot}9\pm1{\cdot}5$ SE % received greater than 1·94 times the mean.

Regional distribution (Fig. 4) was also better with SF/PLV as compared to SF/CMV. Surfactant deposition was significantly ($P<0{\cdot}01$) more equal (50:50) in the left *vs*. the right lung and in ventral *vs*. dorsal lung regions with SF/PLV as compared to SF/CMV. While deposition was still preferentially basilar (regions C and D) with SF/PLV and SF/CMV, deposition in region A, the most apical, was closer to the mean with SF/PLV than with SF/CMV. No significant distribution differences in regions B–D were noted between SF/PLV and SF/CMV groups.

TABLE 1. Pre and post-injury gas exchange indices (mean ± SE) for the SF/CMV and SF/PLV study groups

	Pre-injury		Post-injury	
	SF/CMV	SF/PLV	SF/CMV	SF/PLV
PaO_2 mmHg	524±22	485±16	66±4*	75±7*
$PaCO_2$ mmHg	41±2	38±1	54±2*	56±6*
VEI	0·25±0·02	0·26±0·04	0·07±0·01*	0·09±0·02*

*$P<0{\cdot}01$ significantly different compared to pre-injury values.

Discussion

Meconium aspiration syndrome is believed to have multifactorial pathophysiology involving mechanical airway obstruction, chemical pneumonitis, and surfactant inactivation (4–10). Studies, both *in vivo* and *in vitro* have demonstrated surfactant's surface tension lowering properties to be reduced by meconium (14,16–18). It would stand to reason that surfactant replacement would improve pulmonary mechanics and gas exchange. Significant yet

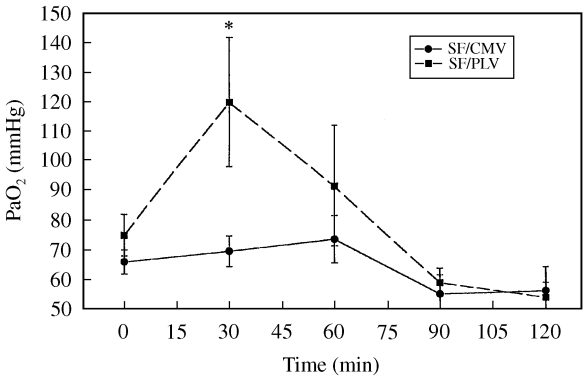

FIG. 1. Arterial oxygen partial pressure as a function of time. Treatment occurred immediately after time = 0. Significant difference at $t=30$ min. $P<0{\cdot}05$ when comparing SF/CMV (●) and SF/PLV ■).

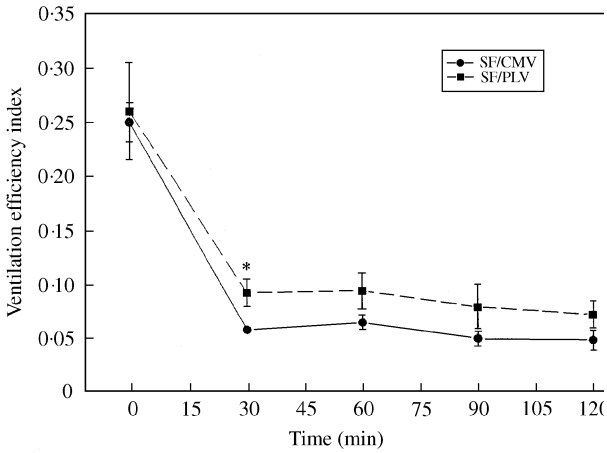

FIG. 2. Ventilation efficiency index (VEI) as a function of time. Treatment occurred immediately after time = 0. Significant difference at $t=30$ min. $P<0{\cdot}05$) when comparing SF/CMV (●) and SF/PLV (■).

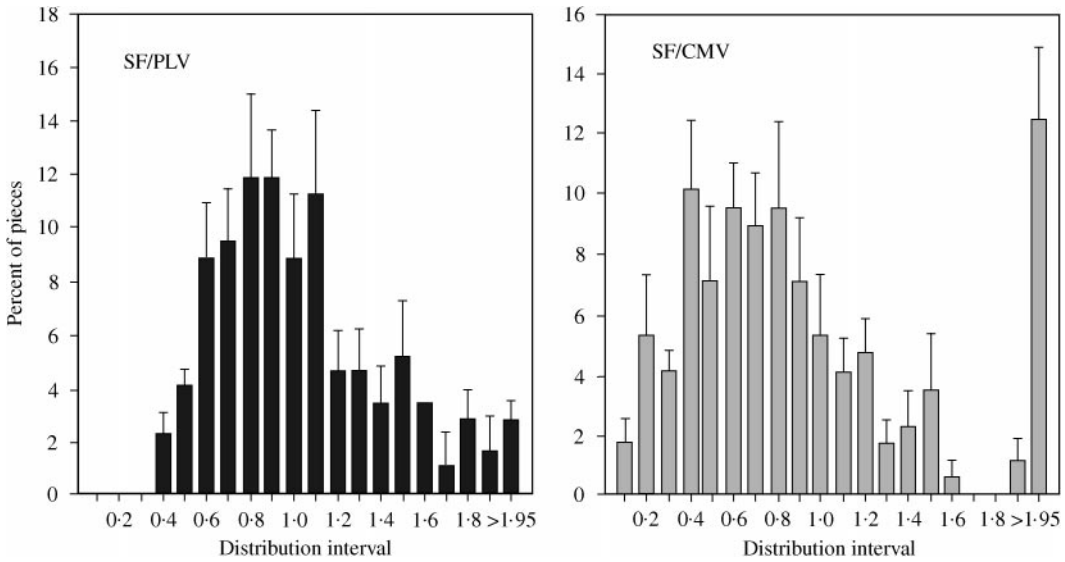

FIG. 3. Distribution of surfactant with (a) SF/CMV and (b) SF/PLV. All values are expressed as mean percentages of pieces of lung (mean ± SE) in 10% intervals.

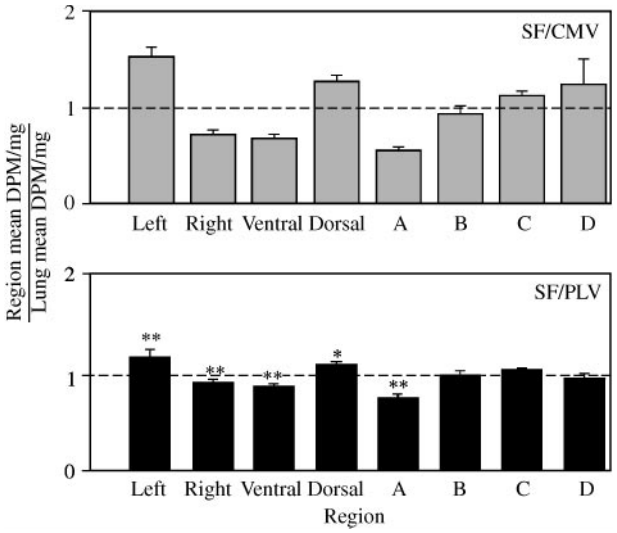

FIG. 4. Regional distribution of (a) SF/CMV and (b) SF/PLV. **Significance at $P<0{\cdot}01$ and *$P<0{\cdot}0{\cdot}05$ when comparing distribution between groups. Regions A–D represent apical to basilar lung segments, respectively.

transient improvement in oxygenation after surfactant treatment has been observed in various animal models of MAS and in a small uncontrolled study in human infants (35–38). Partial liquid ventilation has been found to be an efficacious treatment for a variety of lung injuries including respiratory distress and oleic acid injury (31–34). In a newborn lamb model with acute MAS, PLV resulted in a significant improvement of PaO_2 and $PaCO_2$ as compared to meconium controls (23).

It was our hypothesis that since each PLV and exogenous surfactant treatment was proven beneficial for MAS, that dual treatment might result in improvements additive in nature. Further, it was felt that this novel delivery technique utilizing PFC liquid as the vehicle would result in more uniform distribution of surfactant as compared to CMV controls.

It is currently believed that exogenous surfactant treatment will yield the greatest benefit when distribution is most homogeneous (19,22). Thus far, instillation in the fluid filled lung or rapid instillation of large volumes of surfactant suspension have afforded the most uniform distribution (35–38). These delivery methods have been problematic however as large bolus infusion frequently results in cyanosis, bradycardia, and hypotension secondary to transient airway obstruction and elicited vagal reflexes. Studies using saline as a vehicle/diluent for surfactant delivery in uninjured lungs have yielded conflicting distribution results (35). These studies, however, found no acute improvement in PO_2, or failed to evaluate resultant physiological affects altogether. Further, this same delivery technique resulted in inhomogeneous surfactant delivery in lung injury models. Efforts employing slow tracheal surfactant infusion in saline lavaged rabbit lungs eliminated the complication of hypotension but resulted in poor distribution (38).

In the presented delivery technique, improved surfactant distribution was achieved throughout the entire lung and various lung regions with PLV treatment as compared to treatment during CMV. Interestingly, the first of the four surfactant aliquots was delivered with animals positioned with the left lung dependent. This delivery paradigm appears to have resulted in preferential surfactant deposition in the left lung in animals treated with CMV. This effect was significantly reduced with PLV delivery. Likewise, CMV treatment resulted in greater dorsal (gravity dependent lung region) deposition than ventral with this effect also being significantly reduced through PLV treat-

ment. These results might be explained by the findings of Takashi *et al*. (37) who described the correlation between surfactant distribution achieved in first and second treatments. They found a remarkably close matching of distribution between the first and second surfactant treatment with preferential delivery to presumably better ventilated, aerated lung regions as a result of the initial treatment. This suggests that initial surfactant delivery can influence subsequent delivery by improving regional lung mechanics and ventilation. PLV delivery appears to have significantly reduced the preferential surfactant distribution observed in CMV treated animals. The ability of PFC liquid to stabilize alveoli and recruit atelectatic lung regions through reduction of interfacial surface tension combined with surfactant instillation during continuous PFC infusion are probable reasons for these observations.

While significant, albeit transient improvements were observed in oxygenation and ventilation efficiency index, it is unclear why such a significant difference in surfactant delivery between groups did not translate into physiological differences of similar magnitude. Surfactant inactivation is one explanation as several studies have documented the surfactant inactivation capabilities of meconium both *in vivo* and *in vitro*. Once inactivated, surfactant serves to increase colloid osmotic pressure within alveoli as opposed to decreasing surface tension. In alveoli with increased alveolar–capillary permeability this can result in significant pulmonary edema as was observed in nearly all animals in this study. This may have resulted in the layering of less dense edematous fluid atop PFC liquid. With the solubility of O_2 being 20 times greater in PFC compared to H_2O this could have presented a significant barrier to gas exchange and resulted in pronounced V/Q mismatch. Pulmonary edema, fluid was removed throughout experiments when observed in the trachea tube but not without the inadvertent removal of some PFC. Perhaps alternative positioning of the animals, such as in an upright position, would permit edematous fluid to rise and accumulate in the mainstem bronchi and trachea where it could be siphoned off with little PFC loss. Furthermore, due to small airway size, suctioning in the rat model was substantially more difficult than in infants or larger animal models. Finally, there was no PFC replacement throughout the study. Based on more recent PFC evaporative loss studies, loss of PFC liquid from the injured lungs over time has been shown to compromise gas exchange and lung function (39,40).

In conclusion, we have demonstrated a method for surfactant delivery utilizing PLV in an MAS injury model. This technique resulted in significantly better surfactant distribution than that achieved through the currently recommended method of administration during CMV. These findings warrant further evaluation of this delivery technique in MAS and other lung injury models. In addition, this technique may yield greater physiological improvement in a model of RDS from prematurity where surfactant is merely deficient and when replaced unlikely to be inactivated as can occur with MAS.

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