Single Versus Repetitive Doses of Natural Surfactant as Treatment of Respiratory Distress Syndrome in Premature Lambs

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ABSTRACT. After delivery by caesarean section at 117–120 and 125–130 days gestational age, 20 lambs were supported with infant ventilators at an FiO_2 of 1.0. Throughout the experimental period the respiratory settings were held constant. All lambs were treated by tracheal instillation of 50 mg natural sheep surfactant lipid/kg body weight at 1 h of age, after respiratory failure had been documented. All lambs responded with a decrease in PaCO_2 and increases in PaO_2 and pH, but these responses were of greater magnitude with advancing gestational age. Without waiting for respiratory failure to reappear, repetitive doses of natural surfactant were administered and the effects studied. Retreatment by instillation of 12.5 mg surfactant/kg at 2, 3, 4, 5, and 6 h of age was ineffective at 117–120 days gestational age, but sustained the initial response to the bolus of 50 mg surfactant/kg at 125–130 days gestational age. This indicates that retreatment with natural surfactant should be started before the reappearance of respiratory failure in order to be effective, and that the response to retreatment with small, repetitive doses of surfactant is dependent on gestational age. (*Pediatr Res* 19: 224–227, 1985)

Abbreviations

GA, gestational age
RDS, respiratory distress syndrome

The RDS of the premature neonate is considered to be primarily due to surfactant deficiency (2). Endotracheal instillation of a bolus of natural surfactant in studies on premature animals (1, 4–6, 10–17, 19, 22) and man (7, 8, 23) produces a prompt improvement of pulmonary gas exchange and lung compliance, decreases lung injury, and increases survival time. In premature lambs at 120–130 days GA, the effect of a single dose of natural surfactant lasts for about 8 h if administered at birth but only about 3 h if administered soon after the establishment of respiratory failure (14). Retreatment after the reoccurrence of respiratory failure was successful in only 40% of the animals (14). This observation suggests that a second instillation of surfactant has a favorable effect, but this is probably limited by the presence of respiratory failure. The purpose of this investigation is to determine whether endotracheal instillation of small repetitive doses of natural surfactant sustains the effect of a bolus of surfactant administered to premature lambs in respiratory failure.

MATERIALS AND METHODS

Animals. Twenty premature lambs of known gestational ages were delivered by caesarean section of yearling ewes of the Texel breed (weight 41.2 ± 1.2 kg). The ewes were premedicated intravenously with atropine and 20 mg xylazine hydrochloride (Rompun) and received local anesthesia. A hysterotomy was performed and the lambs were separated from the placenta and quickly weighed. Following intraperitoneal administration of 10 mg/kg of pentobarbital sodium a midtracheal tracheotomy was done and an uncuffed endotracheal tube (i.d. 4.5–5 mm) inserted and tied into the trachea. The lambs were then paralyzed with pancuronium bromide (0.2 mg/kg/dose iv). Both pentobarbital and pancuronium were repeated every 4 h. The lambs were ventilated from birth with constant flow, time-cycled, pressure-limited ventilators (Amsterdam Infant Ventilator, MK 2) delivering humidified and warmed 100% oxygen. No tubes were inserted to prevent pneumothorax. The ventilator settings were held constant throughout the experimental period at an FiO_2 of 1.0, a rate of 30–40 breaths/min, inspiratory time of 1 s, peak inspiratory pressure of 25–30 cm H_2O , and positive end expiratory pressure of 3 cm H_2O . The lambs were dried and placed on a heating blanket under an infant radiant heater (Air Shields) and supplemental heat lamps. Core temperature was monitored with a rectal temperature probe and, after a short period of initial fall in temperature, maintained at 38–39° C. Arterial blood gases and pH were taken every 15 min and measured with an AVL-940 blood gas instrument. A 5 French polyethylene catheter was placed in the distal aorta via the right femoral artery and was used for continuous recording of blood pressure. Airway pressure was recorded continuously from a tracheal catheter in the proximal end of the endotracheal tube. In order to maintain glucose levels, 10% glucose was infused at a rate of 100 ml/kg/24 h through a 3.5 French polyethylene catheter introduced into the inferior caval vein via the right femoral vein. After caesarean section maternal blood was drawn in heparinized syringes to transfuse the lambs for correction of hypotension soon after delivery or for blood replacement during the study period. No bicarbonate therapy was used to correct metabolic acidosis when it occurred in the lambs.

Natural surfactant. The surfactant was recovered by lung lavage of healthy adult sheep at a slaughterhouse. The procedure described by Jobe *et al.* (14) was followed with two modifications. Before the three-step centrifugation was performed, the lavage fluid was centrifuged at $200 \times g$ at 2° C for 20 min to remove tracheal debris and blood cells. Second, the last centrifugation step was repeated two times after resuspension of the resulting pellets in saline. Lipids were extracted according to the procedure of Bligh and Dyer (3). Phospholipid analysis was carried out by two-dimensional thin-layer chromatography followed by phosphorus determination of the isolated spots (24). Protein was measured using bovine serum albumin as standard (18).

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The necessary amount of surfactant was recovered in five batches (total 10.1 g), with a maximum of about 300 mg collected from each adult sheep. The phospholipid composition of each batch was identical; phosphatidylcholine comprised 76% of the total phospholipid (Table 1). The phosphatidylcholine to protein ratio was 22.62 ± 6.42 $\mu\text{mol}/\text{mg}$, indicating that very little protein was present.

Experimental protocol. One hour after birth all lambs were treated via tracheal instillation with an initial dose of 50 mg natural sheep lipid/kg body weight in a suspension of 18 mg/ml. At treatment all lambs were in respiratory failure as defined for elevated PaCO_2 levels and low pH values on at least two samples. The lambs were temporarily disconnected from the ventilator and the surfactant suspension at 37°C was instilled into the lungs over 20 s. The lambs were then changed in position and hand ventilated for 30 s with an FiO_2 of 0.21 and a peak inspiratory pressure of 25 cm H_2O to achieve as uniform a distribution of the suspension as possible. Thereafter the lambs were connected to the ventilator with no changes in the settings.

A total of 20 premature lambs were studied in three assigned groups. Group A consisted of six lambs at 117–120 days GA who received 50 mg surfactant lipid/kg body weight at 1 h of age, followed by hourly doses of 12.5 mg surfactant lipid/kg at 2, 3, 4, 5, and 6 h of age. Group B consisted of six lambs at 125–130 days GA who received 50 mg surfactant lipid/kg body weight at 1 h of age, but no repetitive doses. Group C consisted of eight animals at 125–130 days GA treated as those in group A. Each lamb at 125–130 days GA was assigned on a random basis to group B or C. The lambs were sacrificed by iv administration of 300 mg pentobarbital sodium 8 h after the initial dose of surfactant or after respiratory failure had reappeared ($\text{pH} < 7.00$ and $\text{PaCO}_2 > 75$ mm Hg).

All values are expressed as the mean $\pm$ SEM. Statistical comparisons between groups of lambs were made using Student's two-tailed *t* test.

Table 1. *Phospholipid composition of natural surfactant in five batches*

Phospholipids (% composition)	Natural surfactant (<i>n</i> = 5)
Phosphatidylcholine	76.02 ± 0.62
Phosphatidylglycerol	12.12 ± 0.20
Phosphatidylethanolamine	4.98 ± 0.23
Sphingomyelin	2.52 ± 0.18
Phosphatidylserine	0.94 ± 0.09
Phosphatidylinositol	0.96 ± 0.07
Lyso-phosphatidylcholine	0.90 ± 0.09
Unidentified	1.30 ± 0.54
μmol Phosphatidylcholine/mg protein	22.42 ± 6.42

RESULTS

Table 2 shows the gestational age; birth weight; age at first dose of surfactant; amounts of natural surfactant instilled; pre-treatment values of PaO_2 , PaCO_2 , and pH; mean arterial pressure; and peak inspiratory pressure. Soon after birth severe respiratory failure developed in all lambs despite high peak inspiratory pressures. The mean PaO_2 , PaCO_2 , and pH at the time of treatment were comparable. All lambs were normotensive and not tachycardic. The period from birth to initial treatment was similar for all lambs.

After the first dose of surfactant, the 117–120 days GA lambs of group A exhibited a prompt increase in mean PaO_2 from 73 ± 19 to 208 ± 38 mm Hg. The mean PaCO_2 decreased from 140 ± 30 to 48 ± 3 mm Hg $1\frac{1}{2}$ h after the first dose of surfactant and the pH values reflected this fall. After the initial response the lambs quickly deteriorated and were in respiratory failure at 3 h of age despite hourly doses of 12.5 mg surfactant lipid/kg (Fig. 1). PaO_2 values dropped before the PaCO_2 started to rise. The 125–130 days GA lambs of group B showed a rise in mean PaO_2 from 50 ± 10 to 240 ± 27 mm Hg after a single dose treatment. The mean PaCO_2 dropped from 98 ± 23 to 27 ± 3 mm Hg and the pH increased from 7.02 ± 0.06 to 7.37 ± 0.04 $1\frac{1}{2}$ h after treatment. The duration of effect in group B was about $4\frac{1}{2}$ h (Fig. 1). The 125–130 days GA lambs in group C had an initial response to the first dose of surfactant similar to that of group B: PaO_2 rose from 77 ± 13 to 294 ± 24 mm Hg, PaCO_2 dropped from 80 ± 10 to 33 ± 5 mm Hg, and pH rose from 7.10 ± 0.03 to 7.37 ± 0.05 $1\frac{1}{2}$ h after the first dose (Fig. 1). The administration of repetitive doses of 12.5 mg surfactant/kg to these lambs every hour through the 6th h after birth maintained a mean PaO_2 above 200 mm Hg at 9 h of age (Fig. 1).

The mean period from initial surfactant instillation to recurrence of hypoxia (defined by a PaO_2 below 75 mm Hg) was 2.0 ± 0.2 h in group A, 4.0 ± 0.5 h in group B, and a minimum of 6.9 ± 0.4 h in group C. These differences are statistically significant ($p < 0.001$). Four of the eight lambs in group C presented high PaO_2 levels and normocapnia until sacrifice 8 h after the instillation of surfactant. The mean PaO_2 values reached at peak response were higher in the lambs at 125–130 days GA than at 117–120 days GA ($0.05 < p < 0.10$). Pneumothorax occurred in 50% of the lambs in each group, usually after the reappearance of respiratory failure. In one lamb from group A and in two lambs from group C, sudden deterioration due to pneumothorax led to persistent hypoxia.

DISCUSSION

Tracheal instillation of a bolus of natural surfactant improves pulmonary gas exchange in premature animals and infants (1, 4–9, 11–17, 19, 22, 23), indicating that it can be delivered in an active form to the alveoli. Fifty mg surfactant lipid/kg body

Table 2. *Characteristics of three groups of lambs*

	Group A, repetitive dose treatment	Group B, single dose treatment	Group C, repetitive dose treatment	<i>p</i>
No. of lambs	6	6	8	$<0.001^*$
Gestational age (days)	117.8 ± 0.5	127.7 ± 0.8	127.5 ± 0.9	$<0.001^*$
Wt (kg)	2.3 ± 0.1	3.1 ± 0.2	3.0 ± 0.2	NS†
Age at treatment with surfactant (h)	1.03 ± 0.06	1.01 ± 0.04	1.14 ± 0.06	NS
Initial dose of surfactant (mg/kg)	48 ± 3	50 ± 1	53 ± 4	NS
pH before treatment	6.97 ± 0.07	7.02 ± 0.06	7.10 ± 0.03	NS
PaO_2 before treatment (mm Hg)	73 ± 19	50 ± 10	77 ± 13	NS
PaCO_2 before treatment (mm Hg)	140 ± 30	98 ± 23	80 ± 10	NS
Mean arterial pressure before treatment (mm Hg)	48 ± 3	56 ± 3	54 ± 3	NS
Peak inspiratory pressure before treatment (cm H_2O)	31 ± 2	29 ± 1	28 ± 1	NS

* A vs B and C.

† Not significant, $p > 0.05$.

Fig. 1. Sequential P_{aO_2} (in mm Hg), P_{aCO_2} (in mm Hg) and pH values. Group A (117–120 days GA) is represented by ●, group B (125–130 days GA) by △, and group C (125–130 days GA) by ★. All lambs were treated with 50 mg surfactant lipid/kg (NS) at zero time. Repetitive doses are shown at the top (NS doses). All values are expressed as mean $\pm$ SE.

weight improves clinical status as well as the pressure volume and surface balance characteristics of the lungs (10). However, only 19 mg surfactant lipid/kg seems to produce improvement in the clinical parameters (10). In this study we affirmed the positive effects of tracheal instillation of a bolus of 50 mg natural surfactant lipid/kg in lambs at gestational ages of 117–120 and 125–130 days with established respiratory failure soon after birth. Comparison of the responses in these two gestational age periods demonstrates that the response increases with advancing gestational age.

Tracheal instillation of a bolus of surfactant is effective for a limited period only (14) and does not change endogenous synthetic and secretory rates (20). As RDS in premature infants starts to improve after about 48 h of age due to *de novo* synthesis of surfactant, we tried to maintain the effects of the first dose of surfactant by repetitive administrations of smaller doses. Our results show that at 125–130 days gestational age the initial response to tracheal instillation of 50 mg surfactant/kg can be sustained by reinstallation of repetitive doses of 12.5 mg surfactant/kg at 1-h intervals. This positive effect of repetitive doses was not seen at 117–120 days gestational age and may thus be

dependent on gestational age. At 125–130 days gestational age, the quantities used are probably sufficient to cover the loss of surfactant in the lungs due to clearance from the airways and the presence of surfactant inhibitors which may influence the activity of exogenous surfactant (11, 14). However, the repetitive doses of 12.5 mg surfactant lipid/kg (one-fourth of the initial dose) were estimated on the basis of the reported 2- to 4-h duration of response to a bolus of 50 mg surfactant/kg in lambs at 120–130 days gestational age with established RDS (10, 13, 14). It is possible that smaller repetitive dose could be used to obtain similar effects.

In the study of surfactant instillation, the method of administration alone may have beneficial effects (9). Repetitive doses of saline with manual ventilation were not administered to control lambs in this study. However, by performing manual ventilation with peak inspiratory pressures and F_{IO_2} well below the ventilator settings, we probably avoided any positive effect of method of administration.

It remains speculative whether it is necessary to administer a large bolus of surfactant when initial treatment is followed by repetitive doses. It could be the case that the effects of a smaller initial dose can be sustained by repetitive doses. In neonates at 27–30 wk gestational age, RDS stabilizes for 8–15 h after the instillation of a bolus of 60 mg human surfactant/kg (8). Retreatment of these neonates with smaller doses of surfactant at 4- or 6-h intervals might prevent sudden changes in their clinical condition associated with complications such as patent ductus arteriosus (7), air leaks, and intraventricular hemorrhage (21).

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Correlation between Fetal and Maternal Serum Bile Acid Concentrations

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ABSTRACT. Serum concentrations of different bile acids (BA) were determined by radioimmunoassay in 56 human fetuses and mothers. Serum was obtained immediately after legal abortion, performed between the 14th and the 21st wk of gestation. Conjugated cholic (CCA) and chenodeoxycholic acid (CCDCA) concentrations were determined in 33 cases, conjugated lithocholic (CLCA) and deoxycholic acid (CDCA) in 20, and sulfolithocholyglycine (SLCG) in 15. In fetal blood, mean concentrations of CCA ($0.80 \pm 0.40 \mu\text{mol/liter}$), CCDCA ($4.50 \pm 2.70 \mu\text{mol/liter}$), and CLCA ($1.70 \pm 1.04 \mu\text{mol/liter}$) were significantly higher than those in the mother (CCA $0.34 \pm 0.17 \mu\text{mol/liter}$; CCDCA $0.79 \pm 0.34 \mu\text{mol/liter}$; CLCA: $0.70 \pm 0.30 \mu\text{mol/liter}$; $p < 0.001$); fetal serum levels of CDCA ($0.46 \pm 0.32 \mu\text{mol/liter}$) and SLCG ($0.15 \pm 0.09 \mu\text{mol/liter}$) were lower than in the mothers (CDCA $1.20 \pm 0.80 \mu\text{mol/liter}$, $p < 0.001$; SLCG $0.40 \pm 0.30 \mu\text{mol/liter}$, $p < 0.01$). There was no correlation between levels of BA and gestational age. Serum total protein and albumin concentrations were both reduced in 10 fetuses as compared with the mothers. These data support the concept of a state of physiologic cholestasis during development and suggest that placental transfer of primary BA occurs mostly in the fetal to maternal direction. This transfer could be facilitated by the reduced fetal plasma albumin concentration, since BA in free solution diffuse more easily through the placenta. There is evidence of lithocholic acid synthesis in the fetal liver, while deoxycholic acid appears to be mostly of ma-

ternal origin. Finally, sulfation of BA is poorly developed at this age of gestation. (*Pediatr Res* 19: 227-231, 1985)

Abbreviations

BA, bile acids
CCA, conjugated cholic acid
CCDCA, conjugated chenodeoxycholic acid
CDCA, conjugated deoxycholic acid
CLCA, conjugated lithocholic acid
SLCG, conjugated sulfolithocholic acid

We recently reported that the fasting serum concentrations of the two primary BA conjugates, CCA and CCDCA, are significantly elevated during the first few months of life (3). However, during the 1st yr of age, concentrations decrease to values similar to those of adults. This state of hypercholanemia, reflecting an immaturity of the enterohepatic circulation, has since been corroborated by other investigators using similar radioimmunoassays (4, 25, 27), and "physiologic cholestasis" is now recognized as a normal developmental condition. The factors responsible for physiologic cholestasis have not yet been completely defined in the human infant; data obtained in animal models have documented the immaturity of several of the processes involved in the enterohepatic circulation of BA (hepatic uptake, conjugation, excretion, and intestinal absorption) (5, 10, 23, 24, 26). However, the presence of marked species differences, as has been well demonstrated for other fetal hepatic excretory mechanisms, makes it difficult to extrapolate these data to the human infant. Data concerning metabolism and placental transfer of BA in the human fetus are extremely limited.

The aim of this study was to determine the relationship between fetal and maternal serum BA concentration and compo-

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