Cardiovascular Effects of Surfactant Suspensions Given by Tracheal Instillation to Premature Lambs

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Summary

After delivery by cesarean section at 133-136 days gestational age, 18 lambs were supported with infant ventilators. Peak inspiratory pressure was the only ventilator setting that was changed in an attempt to normalize PCO_2 . The lambs were retrospectively divided into two groups based on their respiratory status before treatment. Ten lambs with PCO_2 values of 38 ± 2 mmHg (mean $\pm$ S.E.) at 5 h of age were treated with a 15-ml suspension containing 50 mg natural sheep surfactant lipid/kg body weight. After surfactant instillation, pH fell, PCO_2 increased and compliance/kg decreased transiently. Surfactant treatment did not reduce the respiratory support that these lambs required. Eight lambs with PCO_2 values of 68 ± 3 mmHg at 3 ± 0.8 h of age responded to surfactant instillation with a decrease in PCO_2 and an increase in PO_2 and pH. In both groups of lambs heart rates, mean aortic pressures, and mean pulmonary artery pressures changed little after surfactant instillation. Cardiac outputs and regional blood flows measured before and after surfactant treatment were unchanged. Instillation of surfactant suspensions to premature lambs did not adversely affect cardiovascular status; however, some lambs did have a transient deterioration of lung function after surfactant instillation.

Abbreviations

RDS, respiratory distress syndrome
Fr., French

Suspensions of surfactant given via the trachea either at birth or after the onset of respiratory failure dramatically improve lung function in premature monkeys (5), rabbits (6), and lambs (1). This encouraging experience has resulted in two clinical trials of the administration of surfactant suspensions as therapy for infants with RDS (7, 17). A concern is the safety of delivering surfactant suspended in a large volume of aqueous solution to infants in respiratory failure (15). The fluid volume could further compromise lung function and/or secondarily affect cardiovascular function (13). Prematurely delivered lambs have a syndrome of respiratory failure that is physiologically similar to RDS and results in histologic changes comparable to those observed in infants with RDS (18). We have assessed cardiovascular function in prematurely delivered lambs before and after surfactant treatment by tracheal instillation.

MATERIALS AND METHODS

Animal preparation. Eighteen lambs were delivered by Cesarean section from date-mated Western mixed breed ewes carrying twins at 133-136 days gestational age. The ewes, each weighing approximately 40 kg, were sedated with intramuscular injections of 800 mg ketamine and 3 mg atropine sulfate. The ewes then received spinal-epidural anesthesia with 4 ml 2% lidocaine and 4 ml 0.5% marcaine. The head and neck of each lamb were exposed through

an uterine incision and a 5-mm endotracheal tube was secured by tracheotomy (12). After sampling fetal lung fluid, each lamb was delivered, a sample of cord venous blood for blood gas and pH analysis was taken, and the lamb was ventilated with 100% oxygen with an anesthesia bag at peak inspiratory pressures of 30 cmH₂O (12). The lambs were weighed and then ventilated with humidified and warmed 100% oxygen on Sechrist pressure limited infant ventilators with the following initial settings: rate, 20 breaths/min; inspiratory time, 1 sec; positive end expiratory pressure, 2 cmH₂O; peak inspiratory pressure, 28 cmH₂O; flow, 8 liter/min. The lambs were paralyzed with pancuronium bromide given through a 5 Fr. catheter placed in the distal aorta via an umbilical artery. This catheter was used for continuous blood pressure and heart rate recording and for frequent arterial blood gas and pH measurements. Only the peak inspiratory pressure of the ventilator was changed in an attempt to keep the PCO_2 between 30-40 mmHg; no other ventilator changes were made for the duration of the experiment. Each lamb received an infusion of 10% dextrose at 100 cc/kg·24 h, and body temperature was maintained with radiant warmers and heat lamps. The tidal volume of each lamb was measured with a Fleisch pneumotachygraph (12). Compliance/kg was calculated as ml tidal volume/kg body weight divided by the difference between peak inspiratory pressure and positive end expiratory pressure.

Shortly after birth, a 3.5 Fr. infant feeding tube was passed into the left ventricle via the right carotid artery and secured. A second 3.5 Fr. infant feeding tube or a 4 Fr. Swan Ganz balloon catheter was passed into the pulmonary artery. Catheter positions were verified by pressure wave form. The left ventricular and pulmonary artery catheters were continuously infused at 1 ml/h with saline containing 1 unit heparin/ml. Pressures were recorded continuously from the aortic and pulmonary artery catheters using a Beckman Dynograph Recorder, Model R611 and Beckman transducers. Mean pressures were measured electronically.

Surfactant treatment. All lambs were treated by tracheal instillation with 50 mg natural sheep surfactant lipid/kg suspended in 15 ml water. The surfactant was isolated from lung lavage from adult sheep as previously reported (12). This surfactant was very effective at improving lung function in premature lambs delivered at 120 days gestational age. Surface properties and composition have been reported (12). To instill the surfactant suspension, the lamb was disconnected from the ventilator and the 15-ml suspension was injected into the endotracheal tube over 5 sec as the lamb was rotated. The endotracheal tube then was reconnected to the ventilator within 10 sec with no changes in ventilator settings. The lamb was manually rotated for about 15 sec after surfactant instillation and placed with the sternum down on the bed for the duration of the experiment. The lambs were treated arbitrarily with surfactant at 5 h of age if PCO_2 values were within the normal range ($n = 10$). If PCO_2 values were greater than 60 mmHg, despite peak inspiratory pressures over 30 cmH₂O, the lambs were treated before 5 h of age ($n = 5$). Three additional lambs were also in respiratory failure, but were not treated until approximately 5 h of age.

Radiolabeled microsphere injections. Radiolabeled microsphere injections were used to detect changes in blood flow before and after surfactant treatment. Shortly before surfactant treatment, and about 20 min and 3 h after surfactant treatment, each lamb received an injection into the left ventricle of $15 \pm 2 \mu\text{m}$ (mean $\pm$ S.D.) diameter microspheres (New England Nuclear) labeled with [^{57}Co], [^{113}Sn] or [^{46}Sc] (9). Starting just before the injection, a reference sample was withdrawn at a rate of 6 ml/min for 2 min into a heparinized glass syringe from the aortic catheter with a Harvard pump. The blood volume of each reference sample immediately was divided into three scintillation vials and sequential rinses of the syringe were added to the vials. After sacrifice, the organs of each lamb were weighed. Each adrenal was placed in a separate vial for counting whereas all other organs and the carcass were carbonized before counting (9). The radioactivity in the reference sample and the sum of radioactivity recovered in all the organs and carcass were used to calculate cardiac output and regional blood flows (9). The left and right hemispheres of the brain and the left and right kidneys were carbonized and counted separately. No disparity of flow/g tissue between these paired organs was noted, despite the fact that the right carotid artery had been ligated to place the catheter in the left ventricle. Final data for brain include both cerebral hemispheres and the brain stem. The radioactivity of the isotopes was determined simultaneously and the counts were cross-channel corrected by a computer program using the appropriate pure isotope standards. All radiolabeled microspheres were assumed to have been trapped on the initial pass through the microcirculation (9). A single reference sample was drawn from the distal aorta because we and others have not detected right to left ductal shunts in this animal preparation (4).

All values are given as means $\pm$ S.E. Comparisons between groups of lambs were made using Student's two tailed *t* test. Significant differences in blood flow distributions were evaluated by an analysis of variance followed by the Student Newman-Keuls multiple comparison procedure.

RESULTS

The 18 lambs were divided retrospectively into two groups based on their respiratory status before treatment (Table 1). Group 1 contains those lambs with elevated PCO_2 values before surfactant treatment whereas the lambs in group 2 had normal PCO_2 values at treatment at 5 h of age. The blood gas and pH responses to surfactant therapy of the 2 groups are shown in Figure 1. The lambs in group 1 initially had more severe lung disease with elevated PCO_2 levels despite peak inspiratory pressures of $37.6 \pm 2.1 \text{ cmH}_2\text{O}$. All eight lambs responded to surfactant instillation with a prompt increase in mean PO_2 from 63 to over 200 mmHg. The surfactant therapy also resulted in a fall in PCO_2 to a mean value of 43 mmHg at 1 h after treatment, a concomitant rise in pH as a result of the decrease in PCO_2 , and an improvement in

compliance (Fig. 2). The peak inspiratory pressure was not changed after treatment with surfactant (Fig. 2). Surfactant therapy did not permit a decrease of ventilatory support.

In contrast, lambs in group 2 required less peak inspiratory pressure before surfactant instillation to maintain good oxygenation and PCO_2 values less than 40 mmHg. After surfactant instillation, the PCO_2 increased slightly and the pH and compliance fell. An increase in peak inspiratory pressure was required to normalize the blood gas values (Fig. 2).

The cardiovascular status of both groups of lambs was remarkably stable despite surfactant instillation (Fig. 3). Lambs responding to surfactant treatment (group 1) had consistently lower mean blood pressures than group 2 lambs. Mean heart rates were not different between the two groups. The pulmonary artery pressures in the more acidotic lambs with lower PO_2 values were not different than those measured in the group 2 lambs either before or 1 h after surfactant instillation. Pulmonary artery pressures appeared to fall transiently in group 1 lambs after instillation of surfactant. We observed very transient bradycardia and arrhythmias during and immediately after the surfactant instillation.

The cardiac outputs and distribution of blood flows in the two groups of lambs are given in Tables 2 and 3. Mean cardiac outputs in either group of lambs did not change after surfactant treatment. Blood flow to the heart, lungs, brain, and kidneys did not change after surfactant treatment. Of note was the large flow to the adrenal glands of both groups of lambs.

The flow to the lungs should be only from the bronchial circulation if the ductus arteriosus were fully constricted. Mean values of $29 \pm 9\%$ and $25 \pm 8\%$ of the left ventricular output of group 1 lambs went to the lungs before and after therapy, respectively. Four of these lambs had large left to right shunts with $50 \pm 5\%$ and $41 \pm 8\%$ of the cardiac output entering the lungs before and after surfactant instillation, respectively; the other four lambs had only $8.2 \pm 2.3\%$ of the cardiac output to the lungs. Mean values for % cardiac output directed to the lungs for group 2 lambs were $12.0 \pm 2.3\%$ and $9.6 \pm 2.2\%$ before and after treatment, respectively. These lambs had a constricted ductus with small or no shunts as pulmonary artery pressures were less than systemic pressures.

DISCUSSION

In several editorials, optimism has been expressed about the potential benefits of surfactant replacement therapy for RDS (2, 19); however, the safety of administration of a relatively large volume of surfactant suspension into the airways remains a concern (15). Infants with RDS have very labile blood gases, and severe hypoxemia can occur with such routine nursing procedures as airway suctioning (14). The ability of the lungs of an infant with RDS to clear a bolus of fluid may also be less effective than in an infant without lung disease (16). Although the beneficial effects of surfactant suspensions given to infants (7) or lambs (1) with very severe lung disease may overcome any deleterious effects of the fluid volume, the fluid volume might be more detrimental to infants or premature animals with less severe degrees of lung immaturity.

In previously reported protocols using surfactant suspensions in prematurely delivered lambs, animals at 120–130 days gestational age have been used (1, 10, 12). These lambs had a degree of pulmonary immaturity more severe than most infants with RDS. Ten of the lambs described in the present study (133–136 days gestational age) had a degree of respiratory failure at 5 h of age that was successfully treated to achieve normal PCO_2 values with infant ventilators at peak inspiratory pressures of $25 \pm 1 \text{ cmH}_2\text{O}$. We have documented that at this gestational age the pool size of surfactant disaturated phosphatidylcholine recovered by alveolar wash was proportionate to the peak inspiratory pressure that was needed to normalize the PCO_2 values (11). Lambs that require about $25 \text{ cmH}_2\text{O}$ peak inspiratory pressure would be expected to have surfactant pool sizes about 25–50% of that found in term newborn lambs (11); thus, these 10 lambs were assumed to have a mild form of a respiratory distress like syndrome.

Table 1. Characteristics of two groups of lambs

	Group 1	Group 2	P
Number of lambs	8	10	
Gestational age (days)	133.8 ± 0.2	134.8 ± 0.3	NS ²
Weight (kg)	2.4 ± 0.2	2.9 ± 0.2	NS
Sex M/F	1/5 ¹	5/5	
Age at treatment with surfactant (h)	3.0 ± 0.8	5.1 ± 0.1	<0.01
pH before treatment	7.15 ± 0.02	7.34 ± 0.03	<0.001
PO_2 before treatment (mmHg)	63 ± 6	237 ± 37	<0.001
PCO_2 before treatment (mmHg)	67 ± 3	38 ± 1	<0.001
Peak inspiratory pressure at treatment (cmH_2O)	37.6 ± 2.1	25 ± 1	<0.001

¹ The sex was not recorded in two lambs.

² NS, not significant, $P > 0.05$.

Fig. 1. Blood gas and pH responses of lambs to surfactant suspensions. Mean $\pm$ S.E. of pH, P_{O_2} and P_{CO_2} in mmHg for the two measurements before and for 3 h after surfactant instillation (SA) are given. The time of SA instillation is given as time 0 and indicated by the vertical dotted line. The actual elapsed time from birth for SA instillation was 3.0 ± 0.8 h for group 1 lambs (Δ - Δ) and was 5.1 ± 0.1 for group 2 lambs ($\circ$ - $\circ$).

Eight lambs had lung disease characterized by elevated P_{CO_2} values in spite of mean peak inspiratory pressures at 3 h of age of 37.6 ± 2.1 cmH₂O. Only lambs with elevated P_{CO_2} values responded to the instillation of natural sheep surfactant with an improvement in lung function; however, the effect of the surfactant therapy was of short duration, as P_{O_2} values fell and P_{CO_2} values again increased within 3 h of treatment. We demonstrated previously that respiratory failure recurred after surfactant treatment of lambs delivered at 120 days gestational age concurrently with the measurement of elevated surface tensions in surfactant recovered by alveolar wash (10). The high minimum surface tensions seemed to result from the presence of soluble proteins that inhibited the ability of surfactant to lower the surface tension at an airwater interface (10).

Surfactant given to 10 lambs with normal P_{CO_2} values did not result in any improvement in pulmonary function. In fact, we observed a small fall in compliance and an elevation of P_{CO_2} values that returned to pretreatment values only after a mean increase in peak inspiratory pressure of 2 cmH₂O. These lambs did not demonstrate the decrease in ventilatory support after surfactant administration that Fujiwara *et al.* (7) noted in infants with RDS; thus, there may be distinct differences in the responses of relatively mature lambs and infants to surfactant instillation. These observations in lambs suggest that one should be cautious

about treating infants with surfactant suspensions unless the infants have relatively severe RDS.

Surfactant instillation did not result in large changes in the cardiovascular status of either group of lambs. Cardiac outputs before and about 20 min after surfactant treatment were not different. Although a large fall in cardiac output could have occurred immediately after surfactant instillation, the small changes in heart rates and systemic pressures suggest that cardiac output was preserved. The cardiac outputs and organ flows are very similar to the values reported by Furzan *et al.* (8) for healthy newborn lambs.

Mean pulmonary artery pressures were less than systemic pressures in both groups of lambs. Four of eight of the lambs who improved with surfactant therapy had large left to right shunts of cardiac output across a patent ductus arteriosus whereas none of the lambs with less severe lung disease had similarly large ductal shunts. Despite a rapid increase in P_{O_2} and pH after surfactant instillation in the lambs with severe lung disease, no prolonged fall in mean pulmonary artery pressures occurred. After surfactant therapy initiated soon after birth in lambs delivered at 120 days gestational age, the left to right shunt across the ductus arteriosus increased (3). In the more mature lambs used in the present experiment, the mean shunt was similar before and after surfactant instillation. These results suggest that more mature animals may

Fig. 2. Compliance and peak inspiratory pressures (PIP) of lambs. Mean $\pm$ S.E. values are given for compliance/kg body weight and PIP in cmH_2O used to ventilate group 1 lambs (Δ - Δ) and group 2 lambs ($\bigcirc$ - $\bigcirc$). Surfactant instillation (SA) is indicated as time 0 by the vertical dotted line.

Fig. 3. Heart rates (HR), mean aortic pressures (Ao pres), and mean pulmonary artery pressures (PA pres) of lambs. Mean $\pm$ S.E. values of HR in beats/min, Ao press and PA press in mmHg are shown for lambs before and for 3 h after surfactant instillation (SA) at time 0 (vertical dotted line). Group 1 lambs are indicated by (Δ - Δ) and group 2 lambs are indicated by ($\bigcirc$ - $\bigcirc$).

Table 2. Cardiac output (C.O.) and blood flow in group 2 lambs

	15 ± 2 min before surfactant (n = 10)	21 ± 2 min after surfactant (n = 10)	3 h after surfactant (n = 8)
C.O./kg cc/min/g tissue	264.5 ± 14	236 ± 16	218 ± 29
Carcass	0.18 ± 0.02	0.15 ± 0.01	0.15 ± 0.02
Heart	1.62 ± 0.25	1.67 ± 0.36	1.53 ± 0.35
Lung	1.18 ± 0.17	0.96 ± 0.29	0.75 ± 0.30
Brain	0.50 ± 0.07	0.52 ± 0.07	0.41 ± 0.08
Kidney	1.38 ± 0.26	1.35 ± 0.16	0.71 ± 0.13
G.I.	0.69 ± 0.08	0.98 ± 0.05	0.93 ± 0.15
Liver	0.46 ± 0.07	0.39 ± 0.06	0.47 ± 0.08
Adrenals	4.47 ± 0.42	3.36 ± 0.28 ¹	3.54 ± 0.54
Spleen	4.57 ± 1.07	3.15 ± 0.72	1.78 ± 0.49

¹ P < 0.05 versus before surfactant.

Table 3. Cardiac output (C.O.) and blood flow in group 1 lambs

	8 ± 1 min before surfactant (n = 5)	26 ± 3 min after surfactant (n = 5)
C.O./kg cc/min/g tissue	229 ± 41	203 ± 16
Carcass	0.10 ± 0.03	0.09 ± 0.01
Heart	2.31 ± 0.89	2.36 ± 0.71
Lung	1.28 ± 0.72	0.75 ± 0.37
Brain	0.96 ± 0.27	0.68 ± 0.20
Kidney	0.81 ± 0.11	0.85 ± 0.05
G.I.	0.47 ± 0.11	0.52 ± 0.11
Liver	0.41 ± 0.05	0.42 ± 0.01
Adrenals	7.20 ± 2.22	11.59 ± 6.35
Spleen	2.78 ± 0.80	0.77 ± 0.29

be less sensitive to increased ductal shunting after surfactant treatments than more immature lambs. Surfactant instillations did not result in dramatic changes in the cardiovascular status of premature lambs at 133–136 days gestational age who were paralyzed and ventilated for lung immaturity.

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