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Pediatrics 1985;76;145-153

VOLUME 76 • AUGUST 1985 • NUMBER 2

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ABSTRACT. With a randomized clinical trial, the possibility was assessed that a tracheal instillation of pulmonary surfactant prior to the first breath might prevent the development of some of the signs of neonatal respiratory distress syndrome. Of the 72 infants in the trial, all born at a gestational age of less than 30 weeks, 39 received 3 or 4 mL of surfactant, prepared from the lipids extracted from calf lung lavage. The treatment resulted in a significantly improved gas exchange during the first 72 hours of life. On the average, the arterial/alveolar P_{O_2} ratio was 0.15 higher for the treated infants, and only about half as much extra oxygen had to be supplied. The respiratory support (peak inspiratory pressure × frequency) could be lowered significantly. Pulmonary interstitial emphysema occurred in 13 of the 33 control infants, but in only three of the 39 treated infants. Six of the control infants died in the neonatal period, but only one treated infant died. It is concluded that surfactant supplementation prior to the first breath is feasible and is of value as protection against the respiratory distress syndrome and the negative effects of hypoxia and ventilatory support. *Pediatrics* 1985;76:145–153; *surfactant phospholipids, bubble surfactometer, arterial/alveolar ratio, natural surfactant, bovine surfactant.*

In 1959, Avery and Mead[1] reported that infants dying of the respiratory distress syndrome had a deficiency of pulmonary surfactant, compared with those born at the same gestational age and also dying in the neonatal period but for reasons other than respiratory distress syndrome. Adams et al[2] confirmed this observation and were able to add that in the airways of infants dying of respiratory distress syndrome, there were less surface-active phospholipids. With the knowledge obtained from these pioneering studies, it was logical to conclude that most likely the main cause of respiratory distress syndrome is the surfactant deficiency and that it might be possible to prevent this condition by supplying, prior to the first breath, the missing surfactant right where it is initially needed: at the air-liquid interface of the upper airways. We have ample evidence, from animal experiments, that if concentrated natural surfactant, obtained from the lung lavage of adult animals, is instilled into the trachea of a preterm neonate immediately after birth, it will facilitate aeration,[3–6] be evenly distributed to small airways and alveoli,[7] stabilize the lung,[3–8] and improve gas exchange[8–11] and survival.[4,5] When results of these animal experiments first became available, it seemed obvious that the principle could be clinically applied. It was also clear, however, that it would not be possible to use a crude, unsterilized, natural surfactant, and any attempts to sterilize with heat or γ-irradiation resulted in drastic loss of surface activity (G. Enhorning, unpublished observations). We tried to produce

Received for publication Feb 20, 1985; accepted April 5, 1985.
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an artificial surfacant consisting of the main components of the natural product and mimicking its surface properties, but for several years we were not successful. At an early stage, though, we learned that if the lipids of natural surfactant are extracted with chloroform/methanol and then resuspended, a product with low protein content is obtained and, even after autoclaving, this product is as active as crude surfactant.[12] This was the preparation used in our clinical trial, the aim of which was to evaluate the possibility of preventing respiratory distress syndrome in the infant born so very much prior to term (less than 30 weeks of gestation) that there is almost certain to be a surfactant deficiency.

MATERIAL AND METHODS

Surfactant

The raw material for the surfactant, supplied by Canada Packers Ltd, Toronto, was the lung lavage fluid of newly slaughtered calves. Centrifugation yielded a white pellet from which the surface-active lipids were extracted,[12,13] precipitated with acetone, and given a concentration of 25 mg/mL when resuspended by vortexing in 100 mM NaCl and 0.5 mM $CaCl_2$. Phospholipids constituted 97% of the material, of which 80% was phosphatidylcholine; approximately two thirds of the molecules were disaturated. With the lipid extraction procedure, the proteins were reduced from more than 10% to less than 1% of the phospholipid weight. The surface properties of this surfactant, consisting of extracted and resuspended lipids, were evaluated with the pulsating bubble surfactometer.[14,15] The principle of this instrument is to measure the pressure difference, ΔP, across the surface of a bubble expanded and made to pulsate in the liquid to be evaluated. Knowing the bubble radius, R, surface tension, γ, can be calculated with the Laplace formula, $\Delta P = 2\gamma/R$. The extracted and resuspended lipids were found to have ideal surface properties even after autoclaving. Almost immediately after the bubble had been expanded to maximal size, ΔP reached a value of 1 cm H_2O, which corresponds to a surface tension of 27 mN/m. With the first pulsation, a surface tension of close to zero was reached at minimal bubble size, and a value of approximately 35 mN/m was observed at maximal size. These values indicated fast adsorption and excellent stabilizing ability.

Following extensive animal testing[16,17] and a successful clinical pilot study,[18] surfactant, consisting of extracted and resuspended lipids, was produced in a single batch, enough to suffice for the entire clinical trial. The surfactant was divided into separate 4-mL vials, one for each infant to be treated. The vials were sterilized by autoclaving and kept frozen until needed. When the seal of a vial had been broken and an infant had been treated, the remaining surfactant was checked intermittently for sterility and the surface properties were evaluated with the bubble surfactometer. Bacterial growth was never encountered and the surface properties did not vary significantly; they were as good at the end of the study as at the beginning.

Power and Sample Size

Our clinical trial was carried out at Women's College Hospital in Toronto, an institution to which women with high-risk pregnancies are referred from several obstetrical departments with a total of 55,000 deliveries per year. We limited our study to infants born at a gestational age of less than 30 weeks, when almost all will suffer from a surfactant deficiency. We could expect 120 to 180 deliveries per annum at this early gestation. Assuming that signed consent could be obtained in only about 50% of those cases, there would still be 60 to 90 infants in a 1-year study, and we felt this would give a sample size large enough to enable us to prove that the surfactant treatment resulted in a significantly improved gas exchange. We decided that blood gas changes during the first 72 hours of life would be our most important end points, because they are measured fairly exactly and objectively.

Based on our experiences from studies on preterm rhesus monkeys,[19,20] six treated with surfactant instillation into the trachea immediately after birth and six serving as control subjects, we surmised that the treatment would result in an improved oxygenation. For the clinical trial, we were planning to use a higher dose of surfactant and our sample size was going to be at least five times greater than in the study on rhesus monkeys. Would that be adequate? Using the nomogram of Altman and Gore,[21] we tried to find an answer to that question.

At Women's College Hospital, the arterial/alveolar (a/A) P_{O_2} ratio for infants born at a gestational age of less than 30 weeks is, on the average, 0.35. If the surfactant treatment were to raise the a/A ratio to 0.55, and the standard deviation is 0.2, the standardized difference becomes 1.0. With a power of 0.90, the sample size would be 45 and 60 for a significance level of 5% and 1%, respectively. Thus, with a sample size of 60 to 90 in a 1-year study, we should be able to demonstrate that the surfactant treatment resulted in significantly improved ventilation.

Randomization

Women at risk of delivering infants at a gestational age of less than 30 weeks, by menstrual dates and ultrasound, were considered for inclusion in the study. If they were in danger of imminent delivery or were under the influence of hypnotic medication, they were deemed unable to sign informed consent. Otherwise, they were approached by a member of the investigating team. The clinical trial was fully explained, so that a signed, informed consent could be obtained, from both the patient and her spouse if possible. Because the treatment was to be given immediately after delivery, the amount of surfactant to be administered had to be related to the gestational age rather than birth weight. Infants born at less than 27 weeks of gestation were to receive 3 mL of surfactant, ie, 75 mg of phospholipids, whereas those born at 27 to 29 weeks of gestation, and estimated to weigh about 1,000 g, were to be given 4 mL of surfactant (100 mg of phospholipids). A placebo was not used for the control subjects. With preterm labor, attempts are usually made at Women's College Hospital to accelerate the maturation process of the fetal lungs. The mother is given two intramuscular doses of betamethasone, 12 hours apart, and an attempt is made to arrest labor with isoxsuprine administered intravenously. Therefore, as seen from the flow diagram of Fig 1, the study was stratified not only for age but also as to the glucocorticoid treatment, whether complete (>36 hours), incomplete, or not given at all. There were thus six strata, and following allocation of a patient to one of them, randomization was carried out in subgroup blocks of ten. Numbered, sealed envelopes were used in consecutive order, each containing a card with instructions as to whether or not surfactant was to be administered. The five cards indicating that surfactant was to be given were randomly mixed with the other five in each subgroup block.

Surfactant Administration

Just prior to delivery, the envelope from the appropriate subgroup block was opened and, if surfactant was to be administered, a vial was warmed and the proper volume was drawn into a syringe. At birth, the infant's chest was gently compressed in an attempt to prevent mechanically an effective first breath. The neonate was immediately taken to the resuscitation table and, after careful suctioning of the oropharynx, was intubated. The actual instillation was carried out by a member of the perinatal team who would not be involved in the care of the infant for the next five to six days. The syringe containing surfactant, or air for the control

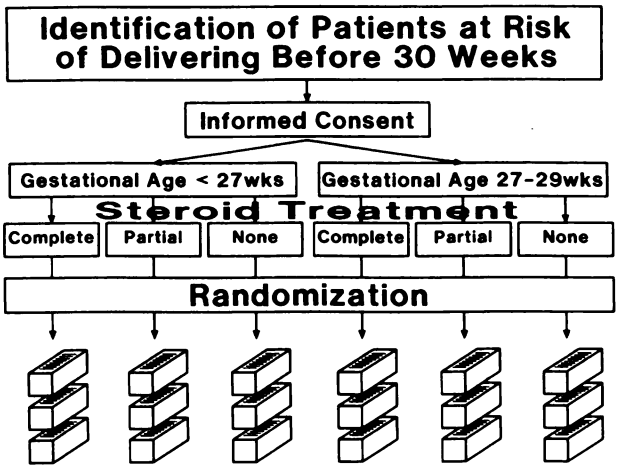

Fig 1. Study design with stratification according to gestational age and steroid treatment. Subgroup boxes at bottom of figure each contain ten consecutively numbered envelopes.

infants, was taped to conceal its content. A catheter, made from a No. 5 Argyle feeding tube, was attached. The length of the latter had been adjusted so that its tip protruded no more than 2 to 3 mm beyond the end of the endotracheal tube. While the neonatologist who had intubated the baby and all other members of the resuscitation team closed their eyes or looked away, the surfactant, or air, was instilled into the trachea, and following two or three manual inflations with a bag, the infant was handed back to the resuscitation team. Attempts were thus made to "blind" the study, but these attempts may not always have been completely successful. However, the ongoing care was identical for treated and untreated infants and conformed to the standards of the neonatal unit.

Evaluation of Treatment

The efficacy of the treatment was ascertained primarily on the basis of blood gas values. The first 72 hours after birth were considered particularly important and likely to be affected by the surfactant supplementation. If respiratory problems could be significantly alleviated during those first three days of life, it was conceivable that hyaline membrane disease would never develop. Specifically, blood gases were determined 1, 6, 12, 24, 36, 48, and 72 hours after birth. The blood samples were usually obtained through an indwelling catheter in the umbilical artery, but when it had not been possible to introduce a catheter, or it had been removed, a peripheral artery was punctured. Other important end points were the respiratory adjustments that were necessary in the attempts to maintain the blood gases at normal values.

It was generally possible to maintain a normal $Paco_2$ of 30 to 45 mm Hg by adjusting the respira-

tory settings. To compensate for low pulmonary compliance, the peak inspiratory pressure (PIP) might have to be raised and/or the frequency (F) of the ventilator set at a higher value. The product of these two factors, the ventilation index (VI), expressed the ventilation requirements: VI = PIP × F.

The oxygen fraction of the inspired air (FiO_2) was adjusted to maintain arterial oxygen tension (PaO_2) in the range of 50 to 70 mm Hg. The barrier effect counteracting transfer of oxygen from alveoli to blood was expressed in two ways: as the conventional A-a DO_2, ie, the difference between alveolar (A) and arterial (a) oxygen tension, and as a/A ratio, the latter being preferable because it is less influenced by changes in FiO_2.[22] To calculate A, a modified equation was used: $A = FiO_2 \times$ (barometric pressure minus water vapor pressure) $- PaCO_2/R$. Assumptions were made that barometric pressure was 760 mm Hg, water vapor pressure 47 mm Hg, and the respiratory exchange ratio, R, 0.8; therefore, $A = FiO_2 \times 713 - PaCO_2/0.8$.

The diagnosis of respiratory distress syndrome was made blindly and independently by two physicians, neither of whom knew which infants had received surfactant and which had not. One, a member of the neonatal team, based the diagnosis on results of blood gas analysis, ventilator settings, and chest radiograms from the first five days of life. The other physician, an experienced neonatal radiologist from another hospital, based the diagnosis on the chest radiogram findings only.

Surfactant-treated infants and control infants were also compared with respect to various respiratory distress syndrome complications and total time of neonatal intensive care. Pneumothorax was confirmed by transillumination, withdrawal of air, or chest radiographic findings; pulmonary interstitial emphysema was defined as air seen within the lung tissue, which in the opinion of the consultant radiologist was not within the airways. Patency of the ductus arteriosus was diagnosed by clinical signs of a murmur with full pulses and deterioration in pulmonary status consistent with a left-to-right shunt. In most instances, ECG and echocardiographic criteria were also confirmed by a consultant cardiologist as being consistent with a significant shunt at ductal level.

All the babies have been enrolled in a perinatal follow-up program and will be assessed with regard to development and hospitalizations, as well as infective, allergic, and respiratory problems. However, the results of the follow-up are not yet available.

Statistical analysis was carried out using χ^2 tests for incidence data and, for numerical, one-tailed unpaired Student's t tests. Means ± SD are given unless otherwise noted. No values were excluded from analysis. When an infant died within the study period, the values were included until the moment of death, and no adjustments were made to allow for the fact that the sick infant was no longer in the group.

RESULTS

There were 72 infants enrolled in the trial; fully informed parental consent was obtained prior to the delivery for 39 in the treatment group and 33 in the control group. Among the treated were two neonates with congenital abnormalities: one had esophageal atresia and one had myotubular myopathy. In the control group, two infants had possible pulmonary hypoplasia. However, none of the 72 infants was excluded from the study. The majority of infants were born to mothers who had received betamethasone, and in the younger age group all had been exposed to the steroid (Fig 2). The randomization process functioned well; in each stratum there were approximately equal numbers of treated infants and control infants. There were no essential differences between the two groups except for sex; there were more female infants in the group receiving surfactant and more male infants among the control subjects (Table 1).

Of the 39 infants treated with surfactant, 31 received the instillation prior to the first breath. In six cases, the infants had already taken a breath prior to the instillation; in two cases, the infant's condition was deemed by the resuscitator to warrant immediate active bagging with 60% oxygen.

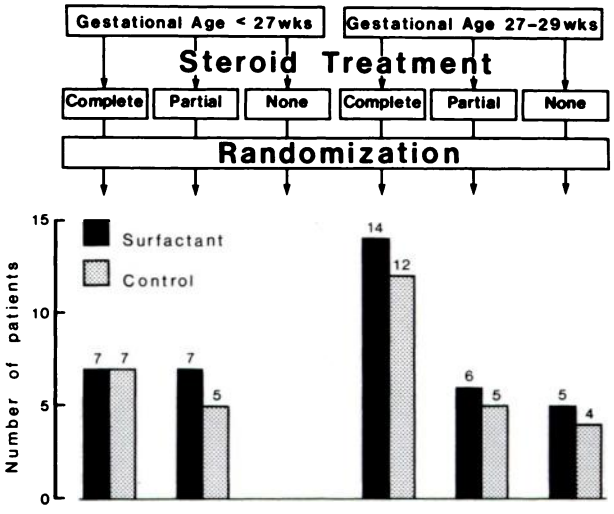

Fig 2. Steroid treatment according to gestational age. All infants in the younger age group were exposed to steroids. In each stratum, randomization resulted in approximately equal numbers of treated and control infants.

TABLE 1. Clinical Data for Surfactant-Treated and Control Groups

	Surfactant-Treated Group (N = 39)	Control Group (N = 33)	*P* Value
Gestation (wk)	27.4 ±1.4	27.2 ± 1.4	NS
Weight (g)	976 ± 252	974 ± 207	NS
Steroid >36 h	21	19	NS
Steroid <36 h	13	10	NS
No steroid	5	4	NS
Cesarean section	23	20	NS
Vaginal delivery	16	13	NS
1-min Apgar score	4.7 ± 1.5	4.3 ± 1.6	NS
5-min Apgar score	7.9 ± 0.8	7.3 ± 1.2	.019
Cord vein pH	7.35 ± 0.08	7.34 ± 0.11	NS
Cord base deficit	1.8 ± 3.3	2.9 ± 3.7	NS
Small-for-dates	5	5	NS
Sex (M/F)	17/22	21/12	NS

This resulted in an improvement and the surfactant was then administered.

For maintenance of normal $PaCO_2$, and as compensation for low compliance, the ventilation index (peak inspiratory pressure × frequency) had to be high immediately after birth for treated neonates as well as control infants. However, from the very beginning, ventilation index was higher for the control infants, and it could be lowered faster for the treated infants (Fig 3).

The A-a gradient was significantly lower in the surfactant-treated group at one hour after birth, and the difference remained highly significant throughout the study period of 72 hours (Fig 4).

The improved oxygenation was also reflected in the increased a/A ratio calculated for the treated group. The normal adult level is approximately 0.8. The values for the treated group did not reach this level but were, on the average, 0.15 higher than for the control infants (Fig 5).

In an effort to avoid hypoxia, FiO_2 had to be raised for both groups, but significantly more so for the control infants (Fig 6). The total need for extra oxygen during the first three days was termed "oxygen hours" and was defined as the sum of the 72 hourly increments above the 21% of room air. The control infants required twice as many oxygen hours (Table 2). However, the total number of days of this therapy was not significantly lower for the treated infants, and the number of days they required intermittent mandatory ventilation was not less. Bilateral pneumothorax and interstitial emphysema were observed significantly more often in the control infants than in the treated infants. These and other complications are listed in Table 3. Intraventricular hemorrhage in mild form, which might develop as a complication of respiratory distress syndrome, was seen more often among the control infants, but the more severe forms, grades

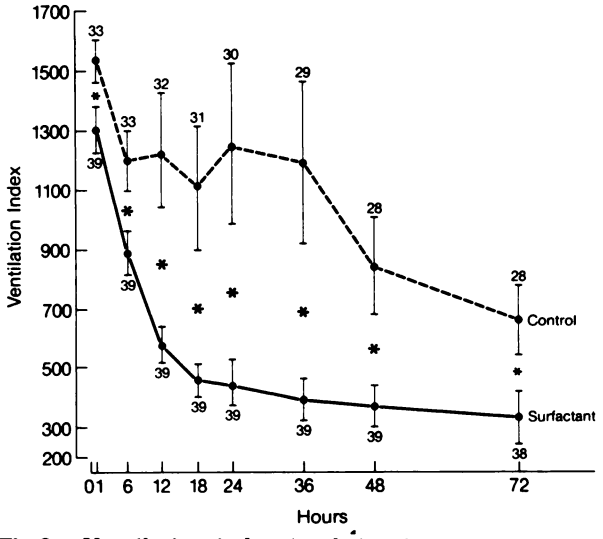

Fig 3. Ventilation index (peak inspiratory pressure × frequency) could be kept lower for treated infants. Above and below values, which are means ± SE, are indicated n values, number of infants alive and contributing to value of specific hour. Asterisks between values indicate statistical significance. Large asterisk indicates $P < .005$; small asterisk indicates $P < .05$.

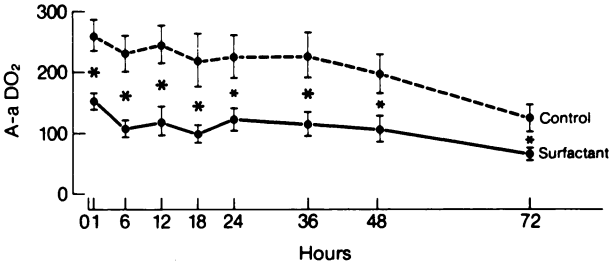

Fig 4. Difference between alveolar and arterial Po_2 (A-a Do_2) for surfactant-treated group and control group (mean ± SE). n values and asterisks are defined in Fig 3 legend.

III and IV, as defined by Papile et al,[23] were observed with equal frequency in the two groups. We defined bronchopulmonary dysplasia as the need

for oxygen therapy for more than 30 days. With this definition, 22 of the 38 survivors in the surfactant-treated group were affected by bronchopulmonary dysplasia and the same number among the 27 survivors in the control group ($P < .05$). Patent ductus arteriosus is a complication that has been associated with surfactant treatment.[24] In our experience, when the surfactant was given prior to the first breath, a patent ductus was not diagnosed more often among the treated infants.

There were two deaths in the treated group and seven in the control group. If only the neonatal deaths are counted, mortality was significantly higher among the control infants. Clinical data for the nine infants who died are presented in Table 4. Of the two treated infants who died, one weighed 580 g at birth and the other 700 g. Both were born at a gestational age of 25 weeks. The smaller baby died at the age of 4 days with minimal lung disease, but with bilateral grade IV intraventricular hemorrhage. The larger baby died at age 14 months of chronic lung disease due to tracheal stenosis and reflux aspiration; this infant initially had a diagnosis of mild respiratory distress syndrome. In contrast, of the seven control infants who died, four had severe respiratory distress syndrome with complications, two had pulmonary hypoplasia, and one was a victim of sudden infant death syndrome at age 6 months.

The two physicians who, independently and without knowledge of whether or not surfactant had been given, assessed the respiratory problems of the 72 neonates, were in almost complete agreement as to the total number of infants with respi-

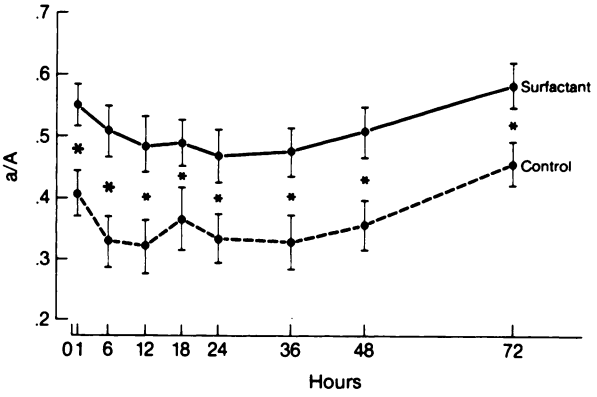

Fig 5. Arterial/alveolar (a/A) P_{O_2} ratio for surfactant-treated group and control group (mean ± SE). n values and asterisks are defined in Fig 3 legend.

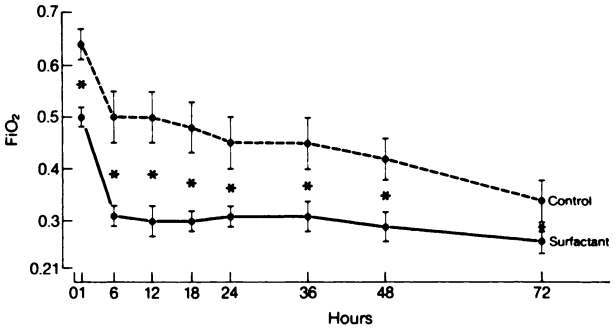

Fig 6. Oxygen fraction of inspired air (F_{iO_2}) for surfactant-treated group and control group (mean ± SE). n values and asterisks are defined in Fig 3 legend.

TABLE 2. Oxygen Therapy and Ventilatory Requirements

	Surfactant-Treated Group	Control Group	*P* Value
Oxygen hours*	660 ± 972	1,390 ± 1,288	<.005
Duration of O_2 therapy (d)	52.3 ± 67.5	64.5 ± 57.7	NS
Duration of intermittent mandatory ventilation (d)	27.2 ± 25.7	37.8 ± 27.5	NS

* Sum of the 72 hourly increments above the 21% oxygen of room air.

TABLE 3. Complications in Surfactant-Treated and Control Groups

	Surfactant-Treated Group	Control Group	*P* Value
Pneumothorax (all)	6	11	NS
Unilateral	4	4	NS
Bilateral	2	7	<.05
Pulmonary interstitial emphysema	3	13	<.005
Intraventricular hemorrhage (total)	11	20	<.01
(Grades III and IV)	4	3	NS
Bronchopulmonary dysplasia (O_2 > 30 d)	22/38	22/7	NS
Patent ductus arteriosus	18	19	NS
Mean stay in neonatal intensive care unit (d)	53.6 ± 43.6	70.1 ± 49.5	NS
Deaths (total)	2	7	NS
(Neonatal)	1	6	<.05

TABLE 4. Deaths in Surfactant-Treated and Control Groups*

	Birth Weight (g)	Gestation (wk)	Cause of Death
Surfactant-treated group (n = 39)			
1	580	25	Grade IV IVH—minimal lung disease
2	700	25	Died at 14 mo—chronic lung disease due to tracheal stenosis, reflux aspiration (initial disease—mild RDS)
Control group (n = 33)			
1	640	25	Severe RDS and pneumonia
2	600	26	Severe RDS and low output state
3	950	27.5	Pulmonary hypoplasia
4	920	27	SIDS at 6 mo (mild RDS initially)
5	1,000	27	Severe RDS—air embolism
6	730	27	Severe RDS, probable sepsis
7	880	27.5	Pulmonary hypoplasia, probable sepsis

* Abbreviations used are: IVH, intraventricular hemorrhage; RDS, respiratory distress syndrome; SIDS, sudden infant death syndrome.

TABLE 5. Primary Respiratory Diagnosis

	Clinical			Radiologic		
	Surfactant-Treated Group	Control Group	*P* Value	Surfactant-Treated Group	Control Group	*P* Value
Normal	6	2		15	5	
'Wet lung'	18	7		10	6	
Respiratory distress syndrome (RDS)						
Mild	4	2		6	3	
Mild-moderate	2	3		0	1	
Moderate	6	5		1	4	
Moderate-severe	2	7		1	3	
Severe	0	4		6	9	
Hypoplasia	1	2		0	2	
Pneumonia	0	1		0	0	
Total RDS	14	21	.0204	14	20	.0384
Moderate to severe	8	16	.0138	8	16	.0138
Normal or 'wet'	24	9	.0042	25	11	.0102

ratory distress syndrome. When the diagnosis was based on the chest radiogram findings only, the respiratory problem was considered to be more severe than when clinical data were also taken into account (Table 5). When the two physicians had stated their opinion in writing, their diagnosis was correlated with our notes as to whether or not surfactant had been administered. It turned out that those who had received the surfactant had less respiratory distress syndrome, both in incidence and severity.

DISCUSSION

As could be expected, the number of treated infants was approximately equal to the number of control infants within each stratum created. We did not stratify for sex, for two reasons. If we had, there would have been 12 strata rather than six and that would have increased the risk of administrative errors. Furthermore, we would not have known the sex until after the baby was born, so for each delivery we would have had to prepare for the two possibilities of the randomization. In half the cases, the surfactant, in readiness in a syringe, would have been wasted.

Because there are reports[25,26] that female neonates are less prone to respiratory distress syndrome than male neonates, we were hoping for an even sex distribution or a preponderance of boys in the treated group, but this was not to be. However, as seen in Table 6, there was no indication that female infants fared better than male infants with regard to respiratory problems.

The surfactant used for this 1-year trial was dispensed in 90 doses of 4 mL each. We felt it important that the surfactant originate from a single batch, prepared prior to the start of the trial. Only then would it be possible to ensure that the

TABLE 6. Sex Distribution of Respiratory Diagnosis

	Male Neonates (N = 38)	Female Neonates (N = 34)	*P* Value
Normal or 'wet' Respiratory distress syndrome (RDS)	18	15	NS
Mild	3	3	NS
Mild-moderate	4	1	NS
Moderate	6	5	NS
Moderate-severe	3	6	NS
Severe	1	3	NS
Total RDS	17	18	NS
Other	3	1	NS

surfactant had a consistently high quality. For an optimal effect, we wanted to make certain that the surfactant was deposited into the trachea prior to the first breath. The moment just after birth offers a golden opportunity to administer the surfactant. The supension, in high concentration, would then be at the air-liquid interface, exerting its surface-tension-lowering effect. Resistance to initial aeration would be reduced, and distribution would be even. The surfactant instilled into the trachea, prior to the first breath, can be expected to leave a monolayer film outlining cylindrical airways and alveoli, thus offering stability to the terminal units. Furthermore, it has been demonstrated that surfactant supplementation to the preterm neonate will protect the airway epithelium from injury, which otherwise follows assisted ventilation.[27]

At Women's College Hospital, an active and aggressive program of treatment for infants born at a gestational age of less than 30 weeks has been developed and it has been in use for several years. The neonates are immediately intubated and given assisted ventilation. Intratracheal instillation of surfactant, prior to the first breath, was thus possible with minimal interference of routine; yet, it was not always accomplished without problems. Eight of the 39 infants assigned to treatment did not receive the surfactant prior to the first breath. Under less ideal circumstances, the success rate would have been even lower. To implement the principle of surfactant supplementation on a large scale, it probably would be necessary to relax the rule that the surfactant be instilled into the trachea prior to the first breath. The eight infants who received the surfactant after the first breath did not differ from the others in the treatment group in gestational age, weight, or any of the parameters measured during the first 72 hours. It is conceivable, though, that they were in better condition at birth as most of them were able to overcome our attempts to inhibit an inspiration.

The control infants were never given a placebo. We wanted to test the value of giving surfactant as opposed to nothing at all. Administering saline solution to the control infants in a volume equal to the 3- or 4-mL bolus of surfactant likely would have done no harm but, because absorption of airway fluid is a problem for the preterm neonate, there was a risk that saline instillation might bias the trial in favor of the surfactant treatment.

The numerical differences observed were often of high statistical significance when evaluated with the Student's *t* test. It was clear, however, that in some instances the values were not scattered in a normal distribution. Hence, the Wilcoxon-Mann-Whitney test was also used for evaluating the differences observed. In no instance was a significance seen with the *t* test lost when evaluation was made with nonparametric statistics. Thus, we felt confident that we had indeed observed an improved gas exchange following the surfactant treatment. For maintenance of normal $PaCO_2$, respiratory support could be less, as reflected in the lower ventilation index, and near normal levels of oxygenation were also easier to achieve. It is of interest to note that the improvement was apparent from the very beginning, and there was no sign of deterioration indicating the need for a repeat surfactant treatment. This finding is in agreement with the observation of Fujiwara et al[24] that one surfactant instillation was all that was required, but it is at variance with experiences from experiments on preterm lambs[9] and with a pilot study on infants severely affected by respiratory distress syndrome.[18] We felt that it was essential that the surfactant be administered at a very early stage, even before the neonate had any need for a surface-tension-lowering agent, ie, prior to the first breath. Only then would it be possible to completely avoid structural damage due to surfactant deficiency.

Even though there was clear evidence that the treatment dramatically improved the situation for the severely surfactant-deficient infants, these infants were not totally exempt from the need for respiratory support. This is not surprising considering that these infants were born at a time when the respiratory system is not fully developed anatomically. It is conceivable, however, that the surfactant administered was not optimal in quality or quantity. Therefore, there is reason in future work to compare the bovine surfactant we used for this trial with other preparations. A totally synthetic surfactant with no protein certainly has its appeal,[28,29] and eventually a product will likely be developed that has the ideal surface properties also when administered as a suspension.

We have probably defined bronchopulmonary

dysplasia incorrectly. The need for prolonged oxygen supplementation may be related to factors other than chronic lung disease. Possibly, it would be better to relate the need for extra oxygen to gestational age rather than time of extrauterine life. For instance, if an infant who has reached a gestational age of 36 weeks still requires supplemental oxygen, this may more accurately indicate true bronchopulmonary dysplasia. We found it encouraging that following treatment there were trends toward reduced incidence of severe complications likely to have effects on long-term outcome, and we will be following the future development of these infants.

We did not anticipate a measurable reduction in mortality. However, there clearly was a difference, when taking into account that the two deaths in the group of 39 treated infants were unrelated to severe respiratory distress syndrome, whereas four of the seven deaths in the control group were due to severe hyaline membrane disease and its complications.

The trend toward a reduced length of stay in a neonatal intensive care unit suggests that there may be immediate, not to mention long-term, humanitarian, but also economic benefits to the prevention of respiratory distress syndrome with surfactant treatment. We consider our trial to have demonstrated that surfactant prior to the first breath will improve pulmonary compliance and gas exchange, thereby preventing some of the negative effects of assisted ventilation and hypoxia. We have shown that instilling a surfactant with the required surface properties directly into the trachea prior to the first breath is feasible, and this treatment is a valuable addition to current management of the very preterm infant (less than 30 weeks of gestation).

ACKNOWLEDGMENTS

This project was supported by grants from the Medical Research Council of Canada and from Women's College Hospital Research Fund.

We thank Dr R. Donovan for encouragement, Dr A. C. Bryan for advice, Dr D. Van Binnendyk for statistical assistance, Dr B. J. Reilly for evaluating chest radiograms, and Dr M. Skidmore for clinical assessments.

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Pediatrics 1985;76;145-153