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Surfactant Function in Neonates with Respiratory Distress Syndrome

Key Words
Surfactant phospholipids
Pulsating bubble surfactometer
Protein leakage
Inhibition
Sequential small-volume lavage
Premature infants
Very low birth weight
Respiratory distress syndrome

Abstract
The function of pulmonary surfactant of a group of 14 preterm neonates (birth weight 907 ± 60 g) who suffered from severe respiratory distress syndrome (RDS) and who had received exogenous bovine lipid extracted surfactant on the first day of life was compared to that in a second group of 8 neonates (birth weight 940 ± 110 g) with mild RDS who had not received surfactant treatment. Mechanical respiratory support from day 2 on was the same in both groups. The minimal surface tension (γ_{min}) improved steadily, falling from about 30 mN/m initially to less than 20 mN/m before extubation. A consistent but loose correlation was found between γ_{min} and mechanical respiratory support necessary, as quantitated by the oxygenation index. Total protein was about 0.8 ± 0.2 mg/mg of phospholipids and did not change during the first week of life. There were no correlations between total protein and γ_{min} or the oxygenation index. The data suggest that inhibition of surfactant function by proteins leaked into the airspaces does not play a major role during recovery from RDS. Instead, endogenous remodelling of surfactant might be of greater relevance.

Introduction

Avery and Mead [1] were the first to propose that lung extracts from very small premature infants had a higher than expected surface tension, indicating that their surfactant is deficient. It has also been reported that in the sequential airway samples of infants with respiratory distress syndrome (RDS) who had not received exogenous surfactant treatment, the minimim surface tension (γ_{min}) fell steadily from about 28 mN/m to values less than 10 mN/m at the time of extubation [2]. In a more recent investigation, no differences in γ_{min} between postnatal day 1 and days 2–4 were observed [3]. While surfactant substitution is now an established and essential element in the care of the preterm neonate with RDS, relatively little is known about surfactant function in human neonates during the course of RDS, especially regarding the effect of exogenous surfactant administration. Such biophysical data would be a useful basis for understanding how this mode of therapy could be optimised. Several reports on the changes of biochemical parameters in the airway secretions, like the phospholipid composition [3–

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5], the surfactant proteins present [3–6] and pharmakokinetics of surfactant [7–9] suggest that substantial rearrangements in the pulmonary surfactant system take place following exogenous surfactant replacement therapy. In addition, surfactant dysfunction due to the leakage of plasma proteins into the airspaces [10] may severely affect surfactant function [11, 12]. Whether exogenous surfactant substitution reduces protein leakage, as estimated by reduced protein contents in lavages, or whether it corrects the function of a primarily impaired surfactant, is not yet clear. The aim of this study, therefore, was to investigate the surfactant function of sequential small-volume lavages in neonates with RDS who had received a bovine natural surfactant in comparison to neonates who had not received an exogenous surfactant.

Materials and Methods

The study was approved by the authorised Human Research Committee of our university and informed consent from the parents or guardians was obtained. Tracheobronchial small-volume lavages of 22 infants less than 30 weeks of gestation who were ventilated from postnatal day 1 to day 7 were collected. The diagnosis of RDS was established clinically and by typical chest radiograph appearance, in the absence of other causes for respiratory distress like infection, sepsis, cardiac defects or congenital malformations. Surfactant was administered on the first day of life as a bolus through the endotracheal tube when the inspiratory oxygen fraction (FiO_2) was greater than 0.4 or the peak inspiratory pressure was higher than 20 cm H_2O (196 Pa) for infants less than 27 weeks of gestation or 22 cm H_2O (216 Pa) for infants older than 27 weeks, respectively. After the procedure, the neonates were immediately reconnected to the ventilator at pretreatment settings. On average, a dose of 83 ± 6 mg/kg body weight of the lipid extracted bovine surfactants Alveofact (Thomae, Biberach, Germany) or Survanta (Abbott, Wiesbaden, Germany) were given. Fourteen neonates had to be treated with surfactant, 1 infant received three doses, 1 two doses, the others a single dose. In infants receiving no surfactant (n = 8), no placebo was used. The mode of ventilation during the study period was standardised, with inspiratory time in the range of 0.2–0.4 s, initial respiratory rates of 60–80/min, and positive endexspiratory pressure at 3 cm H_2O (29 Pa). Ventilatory pressure support was adjusted, so that the arterial oxygen partial pressure was in the range of 50–70 mm Hg (6.6–9.3 kPa), oxygen saturation was more than 90% and the carbon dioxide partial pressure in blood was between 40 and 50 mm Hg (5.3 and 6.6 kPa). Postductal arterial blood gases were drawn every 3–6 h. The oxygenation index (MAP × FiO_2/PaO_2) was calculated as described previously [9].

The small-volume lavages of the nasotracheally intubated neonates were routinely obtained by lavage with 1 ml/kg body weight of 0.45% saline injected through the endotracheal tube and recovered through a suction catheter into a Leuken trap. This material was gently vortexed for 1 min and centrifuged at 150 *g* at 4°C for 10 min. The supernatant was recovered and stored at –80°C. From an aliquot, the lipids were extracted and total amounts of phospholipids determined [9]. The sample was concentrated in a centrifuge under reduced pressure. Surface activity of the samples was measured at a final phospholipid concentration of 1 mmol/l. One aliquot was directly used (native), a second was substituted with $CaCl_2$ (3 m*M*) and third aliquot was substituted with 5 m*M* ethylenediaminetetraacetic acid (EDTA). Surface tension was recorded in a pulsating bubble surfactometer (Electronetics, Amherst, Mass., USA) during static conditions after 12 s (adsorption), and γ_{min} was read after 3 min of pulsations, while the radius of the bubble oscillated from 0.55 to 0.40 mm (corresponding to 50% surface compression) at a rate of 20 oscillations/min [13].

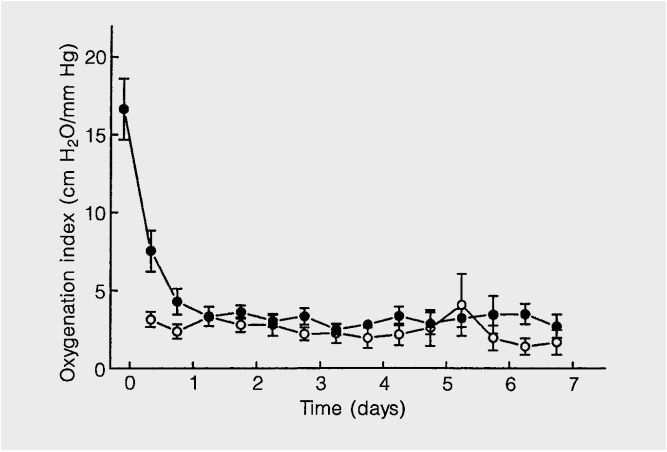
Total protein was determined with Coomassie Brilliant Blue G-250 (Bio Rad, Munich, Germany) according to Bradford [14].

Statistical evaluation was performed on Graphpad software packages (San Diego, Calif., USA). Linear correlation analysis was performed, and coefficients of correlation were tested according to Sachs [15]. The two groups were compared by the unpaired t test. The level of significance was set at $p < 0.05$. First, infants receiving either of the two surfactant preparations Alveofact (n = 9) or Survanta (n = 5) were separately analysed, and no differences for gestational age, birth weight, changes in γ_{min} and protein content were observed. Therefore, in further analyses, these infants were treated as a single group having received surfactant. The data were expressed as means ± SEM from n different subjects investigated.

Results

Two groups of neonates, with and without surfactant treatment, did not differ in gestational age (26.2 ± 0.4 vs. 27.3 ± 0.6 weeks), birth weight (907 ± 60 vs. 940 ± 110 g), prenatal corticosteroids (38 vs. 42%), Apgar scores, the rate of cesarean section (75% vs. 80%) and socio-economical background. In accordance with the study design, at about 3.9 h of life, the average time of surfactant treatment, FiO_2 and respiratory support were higher in infants that required surfactant therapy (fig. 1). In the case of exogenous surfactant therapy, the respiratory support necessary dropped rapidly (fig. 1). In agreement with previous studies [16, 17], no differences between the two bovine surfactant preparations used were noted, and therefore, the data were reported for the group of surfactant-treated neonates as a whole. During the following 6 days, no differences in respiratory support necessary were observed between this group of surfactant-treated neonates and the neonates not treated with exogenous surfactant (fig. 1). The time of extubation was the same in both groups. At 28 days of life, about half of the neonates in each group still needed oxygen or mechanical ventilation, and they had x-ray changes which were characteristic of bronchopulmonary dysplasia.

The phospholipid mass recovered during the first week of life was consistently lower in neonates with RDS and surfactant treatment (table 1). To assess the surfactant activity of the material recovered and to compare differ-



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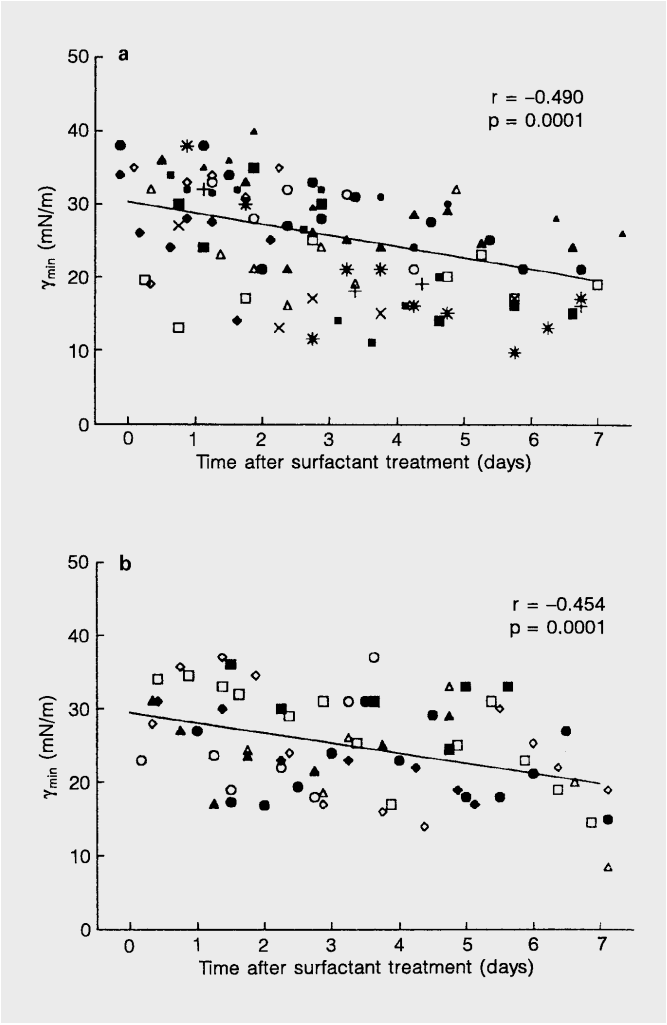

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Fig. 1. Respiratory support necessary during the first week of life was quantiated by the oxygenation index in premature neonates with the RDS. On average, surfactant was given 3.9 h after birth. Values are means ± SEM. ○ = Data of 8 neonates with time after birth that did not receive exogenous surfactant; ● = data from 14 neonates with time after surfactant therapy.

Fig. 2. Time course of γ_{min} of surfactant isolated from the small-volume lavages of neonates with RDS without (**a**) and with treatment with a bovine surfactant (**b**). Individual values of each neonate (represented by a particular symbol) and the linear regression line for all values are given.

Table 1. Phospholipid mass recovered on average in small-volume lavages during the first week of life in premature infants with RDS with or without surfactant therapy

	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7
No surfactant	855 ± 435	1,016 ± 382	752 ± 179	599 ± 65**	453 ± 76*	465 ± 153	800 ± 331*
Surfactant	708 ± 166	582 ± 142	481 ± 103	316 ± 50	244 ± 53	295 ± 30	256 ± 62

Data are expressed as nmol phospholipid/sample recovered and are means ± SEM of 8 infants receiving no surfactant treatment and of 14 neonates receiving surfactant. * $p < 0.05$, ** $p < 0.01$ by the unpaired t test.

ent small-volume lavages, measurements were made at a fixed phospholipid concentration. The minimum surface tension was initially about 30 mN/m and fell on average linearly with time. However, as there was a substantial variation, the individual values are given (fig. 2). No differences were noted between neonates treated with exogenous surfactant and those without treatment. In both groups, the addition of calcium significantly improved surface tension (table 2), whereas a deterioration was noted with calcium chelation by EDTA (table 2).

The adsorption to an air-liquid was estimated from surface tension data of the non-pulsating bubble after 12 s. No changes with time or differences between the two groups were found (with surfactant treatment $\gamma_{ads} = 47 \pm 0.7$ vs. 49 ± 0.5 mN/m in neonates without surfactant treatment). The total content of protein was the same in both groups, i.e. about 0.8 ± 0.2 mg/mg phospholipid, and did not change with time (data not shown).

The respiratory support which was necessary for appropriate oxygenation and ventilation was quantitated by the oxygenation index. Consistent correlations were found with γ_{min} (fig. 3). Although the correlations were

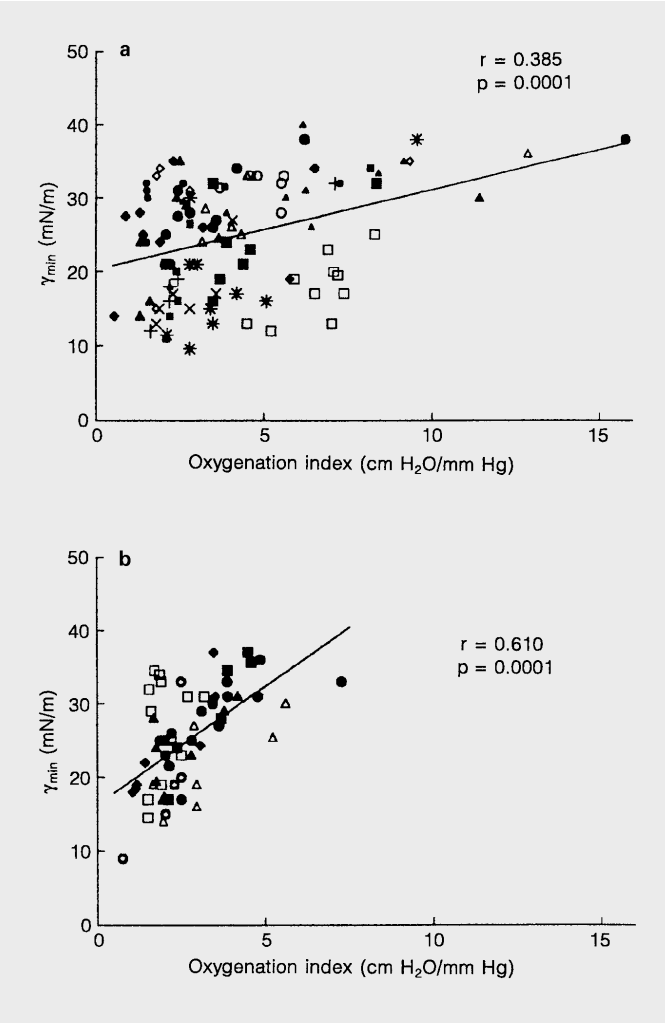

Fig. 3. Correlation between oxygenation index and γ_{min} of the small-volume lavages of neonates with RDS. These infants were treated without (**a**) or with a bovine surfactant (**b**). Individual values of each neonate (represented by a particular symbol), the linear regression line and the results of correlation analysis are given.

Table 2. γ_{min} (mN/m) in the presence and absence of calcium or EDTA

Days after birth	Surfactant			No surfactant		
	native	Ca^{2+} 3 m*M*	EDTA 5 m*M*	native	Ca^{2+} 3 m*M*	EDTA 5 m*M*
1	29.3±1.7	22.6±1.9	31.9±2.1	30.1±1.4	25.6±2.6	32.7±1.6
2	28.7±1.5	24.7±2.1	32.9±2.0	26.5±2.1	23.6±2.4	29.5±2.0
3	25.5±1.6	22.3±1.7	31.3±1.9	23.1±1.4	19.6±1.8	25.2±1.9
4	21.8±2.0	18.2±1.5	22.5±2.5	25.9±1.9	23.5±2.3	29.9±1.8
5	22.1±1.6	18.5±2.1	25.3±3.0	24.1±1.9	19.0±2.2	27.1±2.1
6	19.1±1.8	18.2±2.7	21.3±5.9	24.6±2.0	17.7±1.2	25.2±2.4
7	19.1±2.1	16.8±2.5	25.0±4.2	20.5±2.0	18.4±2.9	23.2±2.4

Data are means ± SEM from 14 surfactant-treated neonates and 8 neonates not treated with surfactant.

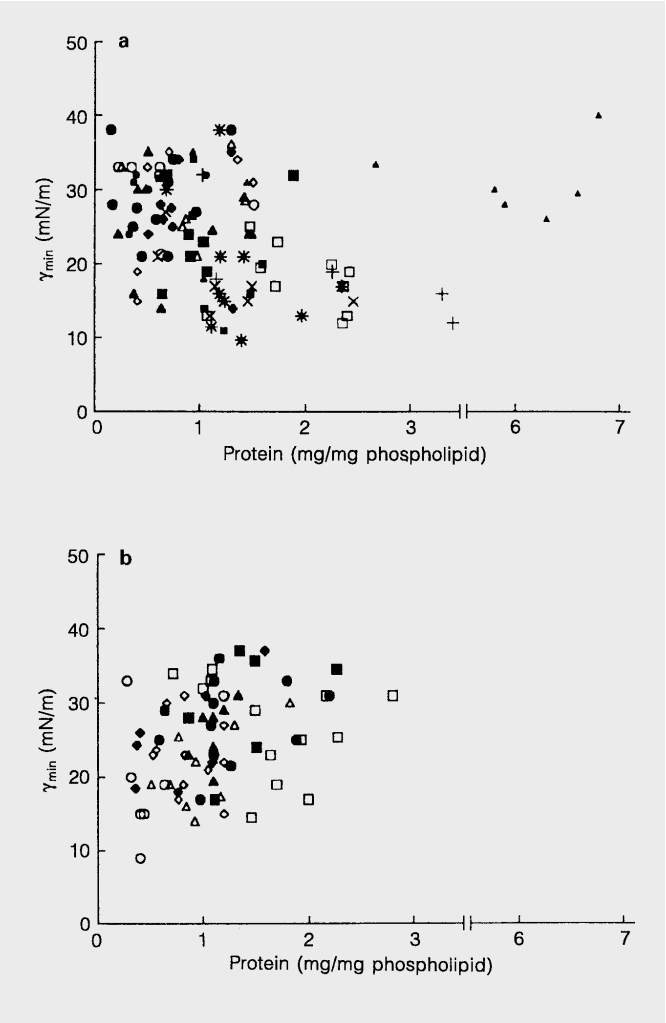

Fig. 4. Correlation between protein content and γ_{min} of small-volume lavages from premature neonates with RDS. These infants were treated without (**a**) or with a bovine surfactant (**b**). Individual values of each neonate represented by a particular symbol are given. No correlation was observed.

not very tight, they indicated that the better the surface activity, e.g. the lower γ_{min}, the less mechanical respiratory support was needed. In contrast, no correlation was found either between the protein content and γ_{min} (fig. 4) or between the protein content and the oxygenation index (data not shown). All these findings held consistently for both groups of neonates, also when the FiO_2 or the ventilation index (peak inspiratory pressure × respiratory rate) were used instead of the oxygenation index (data not shown).

Discussion

We studied the functional properties of the lung surfactant system in very low birth weight premature infants with RDS. One group of neonates received exogenous surfactant therapy for severe RDS. The mechanical respiratory support which was then necessary as quantitated by the oxygenation index did not differ from that of the group without surfactant treatment. So, all neonates levelled off on day 2 with a mild form of RDS. It is not clear whether a deficiency in surfactant mass was still present in both groups or not. The somewhat lower phospholipid total mass recovered in the small-volume lavages made this more likely for surfactant-treated infants (table 1). This issue could only be resolved by measuring total lung surfactant content, which is not feasable in humans, or clinically by the effect of a repeated administration of surfactant for very mild RDS beyond day 1 of life. Speer et al. [18] have demonstrated beneficial effects of repeated dosages of exogenous surfactant for infants with severe RDS, whereas this has not yet been demonstrated in very mild RDS.

The primary goal of this study was to assess the quality of surfactant, but not its mass. This was achieved by normalising measurement conditions to a fixed phospholipid concentration of 1 mmol/l. This value was chosen since a concentration dependency up to 0.5 mmol/l, but not above, has been observed [unpubl. data; see also ref. 2]. On average, γ_{min} was relatively high on the first day of life and linearly decreased to lower but not normal (e.g. 10 mN/m) values. Such a steady improvement was seen in most infants. The substantial variation over time may be connected with sampling from variable sites in the lungs [19], inhomogeneous lung injury or problems with the assessment of surface tension in the pulsating bubble surfactometer. The latter is unlikely, as daily quality control measurements of aliquotted surfactants showed coefficients of variation less than 5%. Additionally, repeated measurements of the same samples and measurements in the presence or absence of EDTA and calcium were close in range.

In both groups of neonates with and without exogenous surfactant treatment, a steady improvement of surfactant functional properties was observed. These changes correlated with the reduced need for mechanical respiratory support. While such a correlation appears evident, the factors that determine these changes in biophysical activity are much less clear.

Based on the studies of Jefferies et al. [10], which had shown an impaired alveolar-capillary barrier in neonates, we had anticipated substantial leakage of plasma proteins

into the air space. Evidence supporting some role of surfactant inhibition may be derived from the enhancing effects of the additional calcium (table 2), which may indicate inhibition by serum [20]. On the first and second days of life, the effect of in vitro addition of calcium was about twice as great as later, when chelation of Ca^{2+} ions with EDTA was more pronounced. Some studies indicated that in animals, a ratio between proteins and phospholipid above 1 results in the impairment of surfactant function [11, 21]; if our neonates had higher ratios, we did not fractionate the small-volume lavages into a surfactant-rich fraction (which may be more relevant for function) and a supernatant fraction. Our previous data suggest that only a minority (about 10%) of the total protein would localise in the surface-active fraction [22]. Other arguments against the inhibition of surfactant as being the major mechanism of surfactant dysfunction in these infants are the consistent lack of correlation between protein content and the oxygenation index or γ_{min}. No changes over time were observed, and there was no difference between surfactant-treated infants and those who had not received treatment, especially during the first day of life. This data is consistent with data in pre-term ventilated lambs [23]. The fact that about 50–60% of the protein recovered was albumin and that this fraction was very constant (data not shown) makes it likely that plasma is the source of the protein. Taken together, our measurements of total protein do not explain the observed changes in γ_{min}. But one has to keep in mind that bidirectional fluxes of proteins and possibly specific inhibitory proteins which may only represent less than 10% of the protein mass [2] might play some role. As the assessment of the biophysical activity of surfactant was the primary goal, other potential inhibitors such as amino acids [4] or changes in neutral lipids and cholesterol [24] remained undetermined. In the same way, due to lack of material, protecting factors like the surfactant proteins A, B and C [5] could also not be measured directly in this study. In comparable neonates, we had previously found no evidence for changes of dipalmitoyl-phosphatidylcholine content, the principal phospholipid of surfactant, during the first week of life. However, substantial changes in other phospholipids [9] indicated that lung metabolic processes may continuously modify endogenous and exogenous surfactant composition and properties [25].

To conclude briefly, exogenous surfactant administration almost instantaneously ameliorated severe RDS. During the first week of life that followed with mild RDS, the functional properties of surfactant obtained with small-volume lavages steadily improved, and no differences to comparable premature infants who did not receive surfactant were observed. These data suggest that not the removal of leaked proteins, which might act as inhibitors of surfactant function, plays the predominant role during the recovery of RDS, but that the endogenous metabolism possibly improved surfactant functions.

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