

Surfactant abnormalities in infants with severe viral bronchiolitis

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Abstract

To determine whether abnormalities of pulmonary surfactant occur in infants with acute viral bronchiolitis, surfactant indices were measured in lung lavage fluid from 12 infants with severe bronchiolitis and eight infants without lung disease. Compared with controls, the bronchiolitis group showed deficiency of surfactant protein A (1.02 v 14.4 µg/ml) and disaturated phosphatidylcholine (35 v 1060 µg/ml) which resolved as the disease improved. Surfactant functional activity was also impaired (minimum surface tension 22 v 17 mN/m). These findings indicate that surfactant abnormalities occur in bronchiolitis, and may represent one of the pathophysiological mechanisms causing airway obstruction.

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Acute viral bronchiolitis (AVB) is a common condition that leads to the hospitalisation of approximately 1% of infants in the first year of life. Treatment for the condition has changed little in the past 30 years, and remains essentially supportive.^{1,2} Efforts to improve respiratory status using a variety of different agents have met with little success. Inhalation of bronchodilators has been associated with short lived improvements in lung function at best, and both methylxanthines and corticosteroids have not proved to be effective.² This lack of efficacy is hardly surprising in view of the marked derangement of airway architecture seen in postmortem histopathological specimens, with bronchiolar wall inflammation, mucosal oedema, and plugging of the airway lumen with mucus and debris.^{3,4} What is less clear is whether these anatomical changes completely explain the striking clinical picture of small airway obstruction in infants with AVB. Indeed, many of the histopathological features of AVB persist for days or even weeks after the clinical signs have resolved,³ suggesting that during the phase of respiratory distress and hypoxaemia, a functional disorder may be superimposed on the anatomical factors that lead to small airway obstruction.

The possibility that a disorder of pulmonary surfactant may be important in the pathogenesis of bronchiolar obstruction in AVB has yet to be fully explored. Apart from its well documented role in maintaining alveolar patency,

there is evidence that surfactant also influences the patency of the small airways in the normal lung. A layer of surfactant phospholipid covers the surface of the small airways,⁵ and bronchiolar Clara cells synthesise and secrete surfactant proteins A and B.^{6,7} Theoretical modelling of fluid lined narrow tubes suggests that this lining of surfactant, by virtue of its property of lowering surface tension, may prevent small airway closure as luminal radius decreases, for example during normal expiration.⁸ The presence of functional surfactant would thereby become even more important in the context of AVB, where the radius of the small airway lumen is already compromised.

On this basis, we hypothesised that surfactant deficiency or dysfunction may be potentiating airway obstruction in AVB. Such abnormalities might, in part, explain the generally poor response of the airway obstruction to conventional bronchodilator treatment, and also the preponderance of ex-premature infants among those with severe disease. The aim of this study was to determine, by measuring surfactant indices in lung lavage fluid, whether there was evidence of surfactant deficiency or dysfunction in AVB. We measured surfactant protein A (SP-A), disaturated phosphatidylcholine (DSPC), and surfactant functional activity in 12 patients with severe AVB, and eight infants without lung disease.

Subjects and methods

Infants were enrolled in the study if they had a clinical and radiological syndrome consistent with AVB³ and required endotracheal intubation because of respiratory failure or apnoea. Infants with normal lungs, anaesthetised and intubated for elective surgery, served as the control group. Lung lavage fluid was collected by tracheal aspirate, conducted as follows. A standard suction catheter was premeasured so that, upon suctioning, the catheter tip would be positioned in the lower trachea, 1-2 cm beyond the tip of the endotracheal tube. With the infant lying supine, and head in the midline, the ventilator was disconnected, and 0.5 ml 0.9% saline was instilled down the endotracheal tube. Ventilation was then re-established for five breaths, the circuit disconnected, and the suction catheter passed into the trachea. Suction was applied using 200 mm Hg negative pressure, and the lavage fluid was collected in a mucus trap. This procedure was repeated for a total of four aliquots of saline, taking approximately two minutes to complete. The catheter was then rinsed with 1 ml of

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Table 1 Surfactant indices in controls and bronchiolitis (first sample) median (interquartile range)

	Controls	Bronchiolitis	<i>p</i> Value*
Tracheal aspirate return volume (ml)	1.9 (1.7 - 2.0)	2.0 (1.7 - 2.1)	0.50
ELF recovery (µl/ml)	106 (47 - 187)	179 (162 - 237)	0.08
[SP-A] _{ELF} (µg/ml)	14.4 (5.6 - 58.7)	1.02 (0.35 - 4.67)	0.008
[DSPC] _{ELF} (µg/ml)	1060 (690 - 4020)	350 (140 - 540)	0.01
MST - adsorption (mN/m)	34 (26 - 37)	44 (42.5 - 45)†	0.003
MST - compression (mN/m)	17 (13 - 20)	22 (20 - 25)†	0.01

MST = minimum surface tension.

* Mann-Whitney test.

† n = 8.

saline, the specimen centrifuged for 10 minutes at 150g and 4°C to remove cells, mucus and debris, and the supernatant stored at -20 °C for later analysis.

In the AVB group, a first specimen of tracheal aspirate was collected within 24 hours of ventilation being started, or soon after admission to our hospital if already ventilated elsewhere. Where practicable, a further sample was taken before extubation in the recovery phase of the disease. As a marker of severity of lung disease, the alveolar-arterial oxygen difference (AaDo₂) was calculated at the time of each sample, using the formula $AaDo_2 = pAo_2 - paO_2$, where pAo_2 is derived from the alveolar gas equation.

In the tracheal aspirate fluid, the DSPC concentration was measured using alumina column chromatography after oxidation of unsaturated phospholipids with osmium tetroxide,⁹ and SP-A concentration was determined by enzyme immunoassay, employing two different monoclonal antibodies to SP-A.¹⁰ Urea concentration in plasma and lavage fluid was measured, and, as a marker of dilution, the volume of epithelial lining fluid extracted in the tracheal aspirate sample was estimated.¹¹ The concentrations of SP-A and DSPC ([SP-A]_{ELF}, [DSPC]_{ELF}) in the epithelial lining fluid were then calculated.

For measurement of surfactant functional activity, tracheal aspirate fluid was centrifuged at 25 000g and 4°C for one hour, producing a lipid pellet which was resuspended in buffer (140 mM sodium chloride, 10 mM HEPES, 0.5 mM EDTA, 2.5 mM calcium chloride at pH 6.9) and adjusted to a DSPC concentration of 1mM (750 µg/ml DSPC). Standardising the DSPC concentration in this way meant that the functional assay reflected activity of other components of the surfactant system, rather than mere variations in the amount of DSPC in the lipid pellet. Surfactant activity was then assessed by creating an air bubble within the pellet suspension in a pulsating bubble surfactometer (Electronetics Corporation, Amherst, USA).¹² Adsorption of surfactant onto the bubble surface was evaluated by noting the lowest surface tension achieved over a 10 minute period with the bubble radius held constant. The capacity of surfactant to reduce surface tension on compression was determined by pulsating the bubble and recording the lowest surface tension achieved at minimum bubble radius.

Non-parametric tests of statistical significance (Mann-Whitney test for unpaired data, Wilcoxon rank sum test for sample pairs) were

used in data analysis. The study was approved by the human ethics committee at our institution, and informed consent was obtained from the parents of infants involved.

Results

The 12 infants with AVB were of median age 2.0 months (range 0.4–6.4), weight 3.1 kg (2.3– 5.4), and gestation at birth 34 weeks (24–41). For the eight controls, median age was 1.5 months (0.03–18), weight 4.2 kg (2.5–9.6), and gestation 39 weeks (34–40). All infants with AVB had hyperinflation with or without atelectasis on chest radiograph; 11 had respiratory syncytial virus. They were ventilated for a median of 99 hours (16–519), and all survived.

In the AVB group, the median time at which the first tracheal aspirate sample was taken was 18.5 hours after intubation (range 4.0–121). The median volume of tracheal aspirate fluid returned (including the catheter rinse) was 2.0 ml (interquartile range 1.7–2.1) and 1.9 ml (1.7–2.0) in controls. Epithelial lining fluid recovery per ml lavage fluid was 179 µl (162–237) in AVB, and 106 µl (47–187) in controls (*p* = 0.08, Mann-Whitney test).

Surfactant indices in lavage fluid from controls and infants with AVB are shown in table 1. Compared with controls, infants with AVB had marked deficiency of both SP-A and DSPC at the height of their disease. These differences were still apparent if the concentrations of each surfactant index in tracheal aspirate fluid were compared without reference to epithelial lining fluid volume (DSPC (µg/ml): controls 134 *v* AVB 67.3, *p* = 0.046; SP-A (ng/ml): 2750 *v* 169, *p* = 0.004). In addition to the observed surfactant deficiency, there was also evidence of surfactant dysfunction in the AVB group. Surfactant derived from the first tracheal aspirate in AVB demonstrated inferior adsorption capacity, and impaired ability to lower surface tension on compression (see table 1). These are the biophysical measures which best indicate the quality of surfactant material.

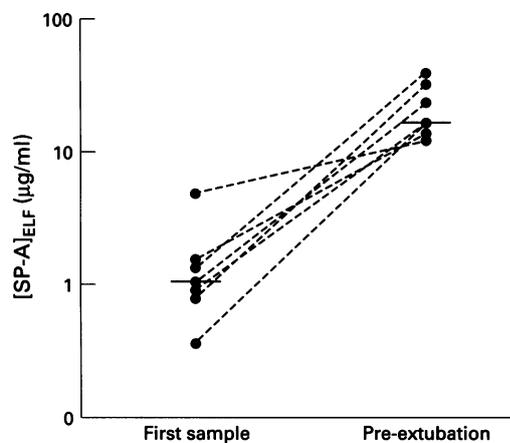
Figs 1 and 2 show [SP-A]_{ELF} and [DSPC]_{ELF} in first and pre-extubation samples from the seven infants with AVB in whom repeated samples were taken. Median [SP-A]_{ELF} increased during the course of the illness (1.03 to 16.07 µg/ml, *p* = 0.02), as did [DSPC]_{ELF} (140 to 920 µg/ml, *p* = 0.035). Tracheal aspirate return volume and epithelial lining fluid recovery were similar in the two samples (volume 2.0 *v* 1.8 ml, *p* = 0.09; epithelial lining fluid recovery 170 *v* 168 µl/ml, *p* = 0.80). The changes in surfactant indices occurred in parallel with an improvement in lung function indicated by change in median AaDo₂ from 349 at the time of the first sample to 134 at pre-extubation sampling (*p* = 0.02).

Discussion

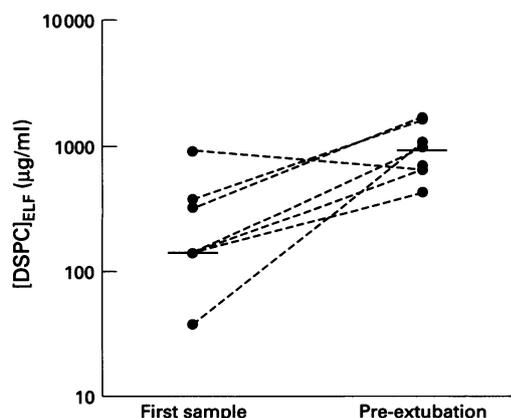
The low concentrations of SP-A and DSPC we observed in infants with severe AVB would seem to indicate that a significant abnormality of pulmonary surfactant is present in this condition. In neonatal lung diseases involving sur-

factant deficiency, concentrations of SP-A and DSPC in lavage fluid closely parallel the severity and clinical course of the illness. This relationship applies not only to respiratory distress syndrome,¹³ but also to meconium aspiration syndrome, and neonatal pneumonia.¹⁴ There are no data directly examining whether SP-A and DSPC are indicative of surfactant status in infants with small airway disease, as distinct from parenchymal lung disease. However, the increase in concentration of SP-A and DSPC seen in the AVB group during recovery occurred in clear association with improvement in lung function, and would not be accounted for by alterations in the degree of ventilatory support or variation in the quality of the sample. It is therefore likely that the low SP-A and DSPC concentrations in lavage fluid reflect real surfactant deficiency in the lungs of infants with AVB.

Inferior functional activity of surfactant was also seen in infants with AVB compared with controls, despite standardisation of the DSPC concentration in the samples. This finding is most likely explained by protein inhibition of surfactant function. Exudation of large molecular weight plasma proteins into the air-spaces occurs in virtually all forms of severe lung disease, and is a potent cause of surfactant dysfunction.¹⁵ SP-A counteracts these inhibitory effects,¹⁶ and the SP-A deficiency we observed in AVB will amplify any protein mediated inhibition occurring in our samples.



[SP-A]_{ELF} in bronchiolitis: first and pre-extubation samples; median indicated by horizontal bar.



[DSPC]_{ELF} in bronchiolitis: first and pre-extubation samples; median indicated by horizontal bar.

Decreased activity of surfactant proteins B and C, not measured in this study, may also contribute to surfactant dysfunction.

There is evidence that the surfactant abnormalities we have found could contribute to small airway obstruction in AVB. Theoretical analysis of the factors that might lead to small airway closure has predicted that airway collapse will occur more readily and more rapidly where there is higher surface tension due to a deficiency of surfactant.⁸ These findings have been confirmed by physiological study of the influence of surfactant on the patency of fluid lined glass capillaries,¹⁷ and of small airways in rats.¹⁸ In both cases, depletion of surfactant promoted closure of the tubular lumen. In the rat, impairment of surfactant function by addition of plasma proteins also led to airway obstruction. In our study, both surfactant depletion, and surfactant inhibition were present in infants with AVB, and we contend that these abnormalities may directly contribute to obstruction of the small airways.

Surfactant abnormalities may also interact with other pathophysiological mechanisms that exist in AVB. Airway mucus production is markedly increased in this condition.³ Surfactant depletion has been shown to increase the viscosity of mucus, and to impair mucociliary clearance.¹⁹ Surfactant abnormalities in AVB may therefore worsen mucus plugging, both in small and large airways. Similarly, the degree of small airway oedema, which is critical to the development of airway obstruction,⁸ will be increased where surface tension is high due to deficiency or dysfunction of surfactant.

Given that surfactant abnormalities may contribute to impairment of respiratory function in AVB, an investigation of the potential benefit of exogenous surfactant treatment would seem warranted. The logical first step is to gain a complete understanding of the contribution of surfactant deficiency to small airway obstruction. The means of delivering surfactant to the small airways in sufficient concentration to overcome dilutional and inhibitory effects must be developed. Clear evidence of the efficacy of surfactant treatment in an animal model of AVB would then be needed before any human trial could reasonably be conducted. Development of surfactant treatment in AVB would by necessity focus initially on infants requiring ventilatory support, but the prospect of aerosolisation of surfactant could allow for treatment in non-ventilated infants who have significant disease.

In conclusion, we propose that deficiency and dysfunction of pulmonary surfactant occur in AVB. Further investigation of the role of surfactant in development of airway obstruction in bronchiolitis, and the potential role of exogenous surfactant treatment for the disease, is indicated.

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