

Abstracts

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according to first authors.

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Synthetic Pulmonary Surfactant: Effects of Increased Amount of Unsaturated Phospholipids

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Background: In synthetic pulmonary surfactants, a commonly used phospholipid mixture is dipalmitoylphosphatidylcholine/phosphatidylglycerol (DPPC/PG) (7:3) (w/w). Due to the high melting temperature of DPPC, viscosity problems can arise when larger amounts at high concentrations (up to 80 mg/ml) are produced. On the basis of earlier results obtained with extracted liver phospholipids [Some et al, Biol Neonate 2003;84:37–38] we evaluated physical and physiological properties of surfactant containing more unsaturated phospholipids (about 50%) and less of acidic phospholipids (about 15%), thus resembling natural surfactant. **Methods:** The synthetic pulmonary surfactant protein C (SP-C) analogue, SP-C33, was mixed with different phospholipids in organic solvents, evaporated to dryness under N₂ and resuspended in saline by repeated aspiration in a 1-ml syringe, without viscosity problems. The surface activity of the different surfactant preparations at a concentration of 10 mg/ml was measured in duplicate during cyclic area compression in a captive bubble surfactometer. Values for minimum and maximum surface tension were recorded in first and fifth compression cycle. Physiological properties were studied in immature newborn rabbits (gestational age of 27 days). Animals (n = 6 in each group) were tracheotomized at birth, treated with surfactant (80 mg/ml, 2.5 ml/kg) and ventilated for 30 min with a standardised sequence of insufflation pressures (P), with or without a positive end-expiratory pressure (PEEP) of 3–4 cm H₂O. Lung-thorax compliance was calculated from recordings of tidal volume and P. Lung gas volumes (LGV) were measured after the experiment. The different preparations used

were: (1) 2% SP-C33 in DPPC/palmitoyloleoylphosphatidylglycerol (POPG) (68:31) (by weight); (2) 2% SP-C33 in DPPC/palmitoyl-lineoylphosphatidylcholin (PLPC)/POPG (55:30:15) (by weight); (3) Curosurf as a positive control.

Results:

Lipid Mixture	Surface tension (mN/m)			
	min	max	Area compression, %	
<i>Cycle 1</i>				
DPPC/POPG (68:31)	1	37	42	
DPPC/PLPC/POPG (55:30:15)	17	51	48	
Curosurf	1	30	36	
<i>Cycle 5</i>				
DPPC/POPG (68:31)	1	33	30	
DPPC/PLPC/POPG (55:30:15)	2	43	29	
Curosurf	2	33	31	
	Compliance 30 min ml/cm H ₂ O · kg		LGV ml/kg	
	+PEEP	-PEEP	+PEEP	-PEEP
DPPC/POPG (68:31)	0.99	0.92	17	5
DPPC/PLPC/POPG (55:30:15)	0.93	0.55	20	1
Curosurf	0.91	0.82	23	16
Non-treated controls	0.03	0.05	0	0

Conclusions: Synthetic surfactant with about 55% disaturated phospholipids has a higher minimum surface tension during the first compression cycle than the other two preparations used, despite larger compression. This preparation also has a higher maximum surface tension. In the fifth cycle film refinement has occurred and all three surfactant preparations have minimum surface tension close to 0 mN/m at ~30% area compression. Animals treated with synthetic surfactant have relatively high lung thorax compliance also in the absence of PEEP, but require a low PEEP for establishment of adequate LGV.

Methods for in vivo Studies of Surfactant Metabolism in Newborn Infants Using a Stable Isotope Technique

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Background: Stable isotope technique using labeled precursors of pulmonary surfactant has yielded new insights into surfactant metabolism in newborn infants. Several methodological approaches have been used: different tracers, surfactant extraction techniques and mass spectrometry instrumentation for measuring tracer incorporation. **Aims:** To determine if the indices of surfactant metabolism differ depending on 1) the phospholipid pool extracted (total phosphatidylcholine (PC), total phospholipids (PL), or disaturated phospholipids (DSPL)), or 2) instrumentation (gas chromatography-mass spectrometry (GC-MS) or gas chromatography/combustion interface isotope-ratio mass spectrometry (GC-IRMS)). **Methods:** To compare phospholipid pools, 7 premature infants with chronic lung disease (CLD) underwent 24-hour intravenous infusions of [$1-^{13}\text{C}$]acetate. PL and DSPL were extracted from serial tracheal aspirate samples. The amount and fatty acid composition were measured with quantitative gas chromatography (quant-GC) and ^{13}C incorporation over time was measured with GC-MS. To compare instrumentation, 20 premature infants with respiratory distress syndrome underwent 24-hour infusions of [$\text{U}-^{13}\text{C}_6$]glucose. PC was extracted from samples and the ^{13}C enrichment was measured with both GC-MS and GC-IRMS. Indices of surfactant metabolism included time of first appearance of label (T_{app} , h); fractional synthetic rate of surfactant from tracer (FSR, %/day) and fractional catabolic rate of surfactant turnover (FCR, %/day). **Results:** In comparing surfactant phospholipid pools with quant-GC, PL was significantly greater than DSPL (178 ± 27 vs. 86 ± 20 nmol, $p < 0.001$); the relative proportion of palmitate in the samples prepared as DSPL, was significantly greater than PL (76 ± 4 vs. $57 \pm 2\%$, $p < 0.001$). However, the indices of surfactant metabolism were similar. In comparing instrumentation, T_{app} and FSR were similar but FCR was slightly slower with GC-IRMS than GC-MS (24 ± 5 vs. $28 \pm 9\%$, $p < 0.01$). With GC-MS, with which the species of labeled palmitate can be distinguished, the ratio of single-labeled to double-labeled palmitate increased over time (from 0.4 ± 0.1 at peak enrichment to 1.3 ± 0.7 at the end of the study, $p < 0.001$), suggesting tracer recycling. GC-IRMS, which gives the total ^{13}C enrichment in palmitate, thus including the recycled label, resulted in a slower apparent turnover. **Conclusions:** With stable isotope technique in newborn infants the indices of surfactant turnover are similar regardless of phospholipid pool measured, suggesting that these pools are turning over at the same rate. The GC-MS instrumentation has a higher specificity compared to GC-IRMS and it is important to avoid masking tracer recycling when [$\text{U}-^{13}\text{C}_6$] glucose is used as tracer. Further studies comparing different tracers are needed.

Selective Surfactant Prophylaxis in Preterm Infants ≤ 31 Weeks' Gestation Using the Stable Microbubble Test in Gastric Aspirates

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Background: There is evidence that surfactant prophylaxis yields better clinical results than rescue therapy for respiratory distress syndrome (RDS) in very immature infants (less than 30–32 weeks' gestation) when the diagnosis is already established. The stable microbubble test (SMT) in gastric aspirate is a quick test that has shown potential for the selection of babies for prophylactic surfactant therapy. **Objective:** To evaluate selective surfactant prophylaxis of RDS based on the SMT. **Methods:** Preterm infants from 23 to 31 weeks' gestation had their gastric secretions collected immediately after birth. The SMT was readily performed by the house staff on duty, who had been trained in the technique. Newborns with < 25 microbubbles (MB; diameter $< 15 \mu\text{m}$)/ mm^2 (low count) received surfactant (Curosurf[®] 200 mg/kg) upon their admission to the neonatal intensive care unit (prophylactic approach). If the count was ≥ 25 MB/ mm^2 (high count) surfactant was given as soon as possible after the clinical and radiological diagnosis of RDS had been established (treatment approach). Additional doses were given to all infants if the ratio $\text{PaO}_2/\text{FiO}_2$ was < 200 mm Hg. **Results:** Ninety-eight infants were included. The mean gestational age and standard deviation was 28.4 ± 2.1 weeks. Fifty-four (55%) had a low MB count and received prophylactic surfactant. Of the remaining 44 infants with a high MB count only three met the criteria for rescue therapy (negative predictive value of 93% and confidence interval of 81.3–98.6%). The median and interquartile range regarding the time for surfactant administration after birth in the low MB count group was 20 (17–27) min. Surfactant was used in 23 of 28 (82%) infants with less than 28 weeks' gestation and in 34 of 70 (49%) infants between 28 and 31 weeks. The mortality of preterm infants ≤ 31 weeks during the study period was lower when compared with the corresponding mortality in the previous two years, but the difference was not statistically significant. **Conclusions:** We have shown that the SMT can be used in a clinical setting to determine surfactant administration still on a prophylactic basis (less than 30 min after birth). Comparatively to indiscriminate prophylaxis, this approach reduces the number of patients receiving unnecessary intervention and probably brings costs down too. The advantage is clearer for patients between 28 and 31 weeks.

Effects of Dexamethasone and Retinoic Acid on *Hox* Gene Expression in the Murine Type II Pneumocyte Cell Line MLE-12 and Fetal Lung Fibroblasts

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Background: Lung epithelial development and maturation are dependent upon soluble factors and interactions with subepithelial fibroblasts resulting in changes in the expression of transcription factors. The most important soluble factors involved are glucocorticoids and retinoids which have both synergistic and antagonistic effects on different aspects of pulmonary development. The *Hox* genes are a family of transcription factors involved with lung pattern formation and may be involved with epithelial cell differentiation. There are 39 *Hox* genes in mammalian genomes arranged into 13 paralogue groups (1 to 13) in 4 clusters (A to D) such that the low numbered genes (e.g. *Hoxa1*) are at the 3' end of each cluster and higher numbered (e.g. *Hoxa13*) are at the 5' end. **Objectives:** To examine the effects of dexamethasone (dex), a glucocorticoid, and all-trans retinoic acid (ATRA), a retinoid, on *Hox* gene expression in the transformed murine type II pneumocyte cell line MLE-12 and to examine the effects of dex on *Hox* gene expression in murine fetal lung fibroblasts (FLFs). **Methods:** Fibroblasts were obtained from day 16 fetal mouse lungs and cultured in medium with or without 10^{-7} M dex for 24 h. MLE-12 cells were cultured in medium containing 10^{-7} M dex, 10^{-5} M ATRA or a control solution for 1, 4 or 24 h. Total RNA was extracted and converted to cDNA by reverse transcription. Surfactant protein (SP) B, SP-C and *Hox* gene mRNAs were measured by real-time PCR using the TaqMan[®] system and were standardised to 18S ribosomal RNA. At least 3 experiments were performed for each condition. **Results:** ATRA caused a wave of increased expression from 3' to 5' along the A *Hox* cluster in the MLE-12 cells. *Hoxa3* expression peaked after 4 h exposure to ATRA with expression 4-fold that of control. *Hoxa4* and *a5* expression was highest 24 h after ATRA exposure; *Hoxa4* with a 4-fold increased expression compared to control and *Hoxa5* a 6-fold increased expression. Dex had no effect on SP-B in MLE-12 cells but caused a reduction in SP-C to 22% of control at 24 h and a reduction in *Hoxa3*, *Hoxa4* and *Hoxa5* to below 50% of control at 1 h after exposure with further decreases to about 15% at 24 h. ATRA or dex had no effect on SP-B, *Hoxb5* or *Hoxb6* expression. Dex exposure for 24 h did not affect *Hox* expression in FLFs. **Conclusions:** Dexamethasone does not increase SP-B expression in the MLE-12 cell line and causes a decrease in SP-C. This suggests that either the MLE-12 cells respond differently to hormonal factors compared to normal type II pneumocytes or that indirect factors, possibly from FLFs, are required to mediate the stimulation of SP. There is no evidence that *Hox* genes are involved with the regulation of SP expression although they may be involved with other aspects of lung development affected by ATRA.

Influence of Modified Natural and Synthetic Surfactant Preparations on Bacterial Killing by Polymorphonuclear Leucocytes

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Background: In addition to its biophysical functions, surfactant plays an important role in pulmonary host defense. **Objective:** In this investigation we studied the influence of various commercially available surfactants on the phagocytosis of bacteria that are common pathogens in the neonatal period. **Methods:** Non-encapsulated, high density (HD) Group B streptococci (GBS), *E. coli* and *S. aureus* were cultured with isolated human polymorphonuclear leucocytes (PMN) and non-specific serum in the presence or absence of different modified natural (Curosurf[®], Alveofact[®], Survanta[®]) or totally synthetic, protein-free surfactant preparations (Exosurf[®], Pumactant[®]). Prior to and after 30 and 60 min of incubation with PMN at different surfactant concentrations (1, 10 or 20 mg/ml), the number of viable bacteria was determined by colony counting. **Results:** Killing of *S. aureus* by PMN was not influenced by any of the surfactants with the exception of Exosurf at 20 mg/ml (see table). Alveofact[®] and Curosurf[®] had no significant negative effect on phagocytosis. At 20 mg/ml, Curosurf[®] reduced the number of viable *E. coli*. Survanta[®] at 10 and 20 mg/ml and Exosurf[®] at all concentrations impaired the killing of non-encapsulated GBS and *E. coli*. Pumactant[®] at 1–20 mg/ml interfered with the phagocytosis of *E. coli*. In further experiments we demonstrated that Curosurf[®] did not interfere with the phagocytosis of an encapsulated GBS-strain opsonised by a specific antiserum either.

Surfactant	mg/ml	Bacteria		
		GBS HD	<i>S. aureus</i>	<i>E. coli</i>
<i>Modified natural</i>				
Curosurf [®]	1	ns	ns	ns
	10	ns	ns	ns
	20	ns	ns	↑
Survanta [®]	1	ns	ns	ns
	10	↓	ns	↓
	20	↓	ns	↓
Alveofact [®]	1	ns	ns	ns
	10	ns	ns	ns
	20	ns	ns	ns
<i>Synthetic</i>				
Pumactant [®]	1	ns	ns	↓
	10	ns	ns	↓
	20	ns	ns	↓
Exosurf [®]	1	↓	ns	↓
	10	↓	ns	↓
	20	↓	↓	↓

Decrease (↓) or stimulation (↑) of phagocytosis in comparison with saline controls (ns, not significant).

Conclusion: We found that killing by PMN was influenced by the bacterial species and the composition and concentration of the different surfactant preparations. The strongest impairment in phagocytic function of PMN was observed with the protein-free synthetic surfactant Exosurf[®], a phospholipid preparation that contains the alcohols hexadecanol and tyloxapol as spreading agents.

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Intravenous Endotoxin Results in Umbilico-Placental Vasoconstriction and Induces Pulmonary Inflammation in Preterm Fetal Sheep

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Background: Chorioamnionitis and fetal systemic inflammatory response syndrome are associated with neonatal morbidity and mortality. However, chorioamnionitis is associated with reduced risk for respiratory distress syndrome but increased risk for fetal inflammatory response syndrome. In an experimental model of chorioamnionitis intraamniotic *E. coli* endotoxin rapidly induced fetal pulmonary and mild systemic inflammation with consecutive lung maturation, resulting in higher surfactant protein B contents and thinner alveolar walls. **Objective:** To clarify the role of systemic inflammation in the association of chorioamnionitis and lung maturation. **Methods:** Thirteen fetal sheep were chronically instrumented at 107 d gestational age (term is 147 d). A transonic flow probe was placed around the common umbilical artery. Three days after surgery fetuses of the control group (n = 6) received saline injection, while fetuses of the study group (n = 7) received 100 ng endotoxin (*E. coli*, 0127:B8) intravenously. Umbilical blood flow (Q_{umb}), oxygen saturation, pH, fetal heart rate (FHR) and mean arterial blood pressure (MAP) were monitored for 72 h. Paraffin embedded lung tissue was stained for nuclear factor (NF)- κ B for proinflammatory gene transcription, cyclooxygenase-2 for inflammatory changes, surfactant protein B for lung maturation and May-Grünwald to detect inflammatory cells. Immunohistochemistry was analyzed in a four-step semi-quantitative scale. **Results:** Q_{umb} began to fall 1 h after endotoxin, was minimal (-40%, $p < 0.05$) at 5 h and returned to control value at 12 h after endotoxin. Oxygen saturation fell to $32 \pm 4\%$ at 6 h after endotoxin vs $53 \pm 3\%$ in control animals ($p < 0.05$), while pH did not change. FHR increased to 221 ± 9 /min at 5 h after endotoxin vs 181 ± 6 /min in control animals ($p < 0.05$). MAP increased 1 h after endotoxin injection from 39 ± 2 to 46 ± 3 mm Hg ($p < 0.05$) and returned to control value at 5 h after endotoxin injection. Pulmonary inflammatory changes occurred in both alveoli and interstitium. Alveoli were infiltrated with neutrophils, monocytes and lymphocytes after intravenous endotoxin injection (inflammatory index 3.8 ± 0.9 vs 0.3 ± 0.1 , $p < 0.05$). The alveolar walls were not different between groups. NF- κ B was detected in the nucleus of endothelial cells (3.5 ± 0.8 vs 1.1 ± 0.5 , $p < 0.05$). Cyclooxygenase-2 stained the

cytoplasm of endothelial cells (3.6 ± 0.7 vs 1.5 ± 0.4 , $p < 0.05$). Surfactant protein B was detected in alveolar type II cells in control and study group with no difference in intensity (2.6 ± 0.6 vs 2.1 ± 0.5 , $p > 0.05$). **Conclusions:** Intravenous injection of endotoxin caused fetal hypoxemia without acidosis by a vasoconstriction of umbilical blood flow. Inflammatory activation was induced in the endothelial cells of the fetal lungs. Inflammatory changes were not limited to the blood vessels and the interstitium but involved the alveoli as well without inducing lung maturation.

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Effect of SP-A and Curosurf[®] on T-Cell Proliferation and Smad-Signaling in Human CD4⁺ T-Lymphocytes

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Background: Surfactant apoproteins SP-A and SP-D have been identified as modulators of various immune functions of macrophages and monocytes. In addition, phospholipids present in the natural porcine surfactant preparation Curosurf[®] have been shown to downregulate generation of proinflammatory cytokines [Bauer et al, *Pediatr Res* 1998;44:32-36]. Also transforming growth factor beta (TGF- β) is a crucial immunosuppressive regulatory cytokine. Most of the TGF- β effect is exerted via the Smad signaling pathway. The aim of the present study was to examine the possible effects of SP-A and Curosurf[®] on T-cell proliferation and Smad signaling in human CD4⁺ T-lymphocytes. **Methods:** The effect of SP-A (1 to 50 μ g/ml) isolated from bronchoalveolar lavage fluid of patients with alveolar proteinosis and Curosurf[®] (1 to 80 μ g/ml) on anti-CD3 mab (1 μ g/ml) induced CD4⁺ T-lymphocytes proliferation was measured by a ³[H]-thymidine proliferation assay after 72 h. IL-2 mRNA expression was analysed by RT-PCR. The effect of surfactant constituents on Smad signaling was evaluated on the promoter level with a Smad2/3 specific luciferase construct pGL3ti(CAGA)₁₂, transfected in human T-cells by nucleofection. In addition, the phosphorylation state of Smad2 was studied by Western blot and the mRNA expression of Smad7 – a negative regulator protein of Smad signaling – by RT-PCR. **Results:** SP-A had no effect on T-cell proliferation. However, for Curosurf[®] a concentration dependent suppression of human CD4⁺ T-lymphocytes proliferation (inhibition of 90% (standard deviation $\pm 5\%$)) was observed. Furthermore, Curosurf[®] inhibited IL-2 mRNA production (62% (standard deviation $\pm 9\%$)). SP-A and Curosurf[®] had no effect on the activation of the Smad2/3-specific luciferase construct, the phosphorylation of Smad2 and the expression of Smad7 mRNA. **Conclusion:** The inhibitory effect of Curosurf[®] on proliferation of human CD4⁺ T-lymphocytes is most likely associated with a decreased IL-2 production of lymphocytes. Neither SP-A nor Curosurf[®] affected Smad signaling in human CD4⁺ T-lymphocytes. The anti-inflammatory effect of Curosurf[®] may have potential implication for the treatment of different cytokine-mediated lung diseases in humans.

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Does Systemic Inflammation Occur in Meconium Aspiration Syndrome?

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Background and Objective: Meconium, the intestinal content of the fetus, may cause lung injury (meconium aspiration syndrome, MAS). Treatment is symptomatic by ventilatory support or in the worst cases extracorporeal membrane oxygenation (ECMO). MAS is the most frequent cause for the use of ECMO in the neonatal period. The pathophysiology is complex including a substantial inflammatory reaction in the lungs, but according to our results this inflammation may be systemic as well. We recently showed for the first time that meconium is a potent activator of complement [Castellheim et al., *Pediatr Res* 2004;55:310–318], leading us to hypothesize that complement activation is an essential part of the pathophysiology of MAS. **Methods:** MAS was induced by instillation of meconium into the lungs of newborn pigs according to a well established method of experimental MAS. To mimic the asphyxia in clinical MAS, hypoxia was induced by supplying 8% oxygen in nitrogen until base excess reached -20 mmol/l. Anaesthesia was induced by halothane and maintained intravenously by fentanyl and midazolam. Control animals received saline under otherwise identical conditions. The observation period was 7 h. Hemo- and lung dynamics were recorded. Systemic complement activation, revealed by the terminal sC5b-9 complex (TCC), and cytokines were measured in plasma samples by enzyme immunoassays. Granulocyte expression of CD18 and CD11b, as well as oxidative burst, were measured by flow cytometry. **Results:** Plasma TCC increased rapidly in the MAS animals, but not in the controls ($p < 0.0005$). The TCC concentration correlated closely with oxygenation and ventilation indices ($r = 0.67$ and 0.84 , $p = 0.004$ and < 0.0005 , respectively), and inversely with compliance ($r = -0.69$, $p = 0.01$), all three reflecting severe deterioration in pulmonary function. Granulocyte oxidative burst declined significantly in the MAS animals compared with the controls ($p = 0.02$) and correlated inversely with TCC ($p = 0.02$), probably reflecting a paralysis of granulocytes as part of a systemic inflammatory response. IL-6 ($p = 0.001$) and IL-8 ($p = 0.003$) increased in MAS animals versus controls. **Conclusion:** We have for the first time demonstrated that complement is rapidly and systemically activated in experimental MAS. We suggest that this activation may induce secondary inflammatory reactions such as cytokine production and oxidative burst, which contribute to the pathogenesis of MAS. Anti-complement therapy may be rational for treatment of this disease.

Bronchoalveolar Lavage with Porcine-Derived Surfactant in Acute Respiratory Distress Syndrome: Hemodynamic and Gas Exchange Assessment

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Introduction: The aims were to assess the ability of surfactant bronchoalveolar lavage (BAL) to remove material and inactivating substances from the lung, prevent chemical pneumonia and acute respiratory distress syndrome (ARDS), and to evaluate the efficacy of natural surfactant supplementation during and after BAL in recruiting and re-expanding the lung in ARDS triggered by aspiration. **Patients and Methods:** This was an open label, prospective, uncontrolled pilot study. 14 patients aged 2 months to 37 years were recruited for the study. All of them had ARDS caused by aspiration of gastric content from different origin and required artificial ventilation with tidal volumes > 12 ml/kg, positive-end expiratory pressure > 8 cm H₂O and mean PaO₂/FiO₂ < 300 mm Hg. The dose regimen consisted of five aliquots of 5 ml of surfactant diluted with normal saline (10 mg/ml) in infants, five aliquots of 10 ml (5 mg/ml) in children under 8 years, and 5 doses of 20 ml (2.5 mg/ml) in adolescents and adults. Manual ventilation with FiO₂ of 1.0 was applied during the manoeuvre and previous ventilator settings were resumed at the end of BAL. In all patients an additional bolus dose of 250 mg/kg of surfactant was administered selectively in the same area where BAL was performed, 1 h after BAL, to replace removed surfactant and compensate suspected deficiency. Heart rate, non-invasive mean arterial blood pressure, end-tidal CO₂ and peripheral oxygen saturation were recorded continuously throughout BAL. Pulmonary function indicators including ventilation parameters and arterial blood gases were recorded at baseline (just before treatment), at completion of the procedure and after 15 min, 1, 3, 6, 12, and 24 h. Chest x-ray assessment was made at baseline, 3 h after treatment, the day after, and subsequently only if needed. **Results:** All children survived and no significant variations in heart rate or mean arterial pressure were observed during the treatment. An increase in end-tidal CO₂ and in airway resistance and a reduction in oxygen saturation were observed only during BAL. Elimination of CO₂ and PaO₂ returned to baseline on completion of the procedure, and at the latest within 1 h. An important improvement in PaO₂/FiO₂ ratio was noted starting from 1 h after BAL and surfactant supplementation. Better elimination of CO₂ was achieved from the 3rd hour. This allowed the patients to be ventilated with reduced tidal volume (mean 12 ml/kg) and oxygen concentration (< 0.4). Reductions in resistance and improved lung compliance were observed later than the improvement in gas exchange. **Discussion and Conclusions:** This therapeutic approach was an attempt to both cleanse the lungs, replace surfactant, recruit damaged lung areas and stabilize terminal bronchioles and alveoli as well as to improve gas exchange. In particular, the method may mitigate some of the pathophysiological events involved in aspiration syndrome (obstruction of small airways, migration and diffusion of inhaled material into the entire lung and development of chemical pneumonia and ARDS).

Prenatal Inflammation and Betamethasone Do Not Affect Markers of Oxidative Stress in Fetal Lung Fluid from Preterm Lambs

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Background: Antenatal corticosteroids and chorioamnionitis induce prenatal lung antioxidant defenses; however inflammation may increase oxidative stress by the neutrophil respiratory burst. We determined the balance between these opposing effects by measuring markers of oxidative stress in lung fluid from preterm lambs prior to atmospheric oxygen exposure. **Objective:** To study effects of chorioamnionitis and antenatal corticosteroid therapy on protein carbonyl levels (C=Os) in fetal lung fluid (FLF) taken from preterm lambs at birth. **Method:** 38 pregnant ewes were randomly assigned to 4 treatment groups at 15 days prior to birth. (1) Chorioamnionitis induced by intra-amniotic injection of endotoxin (Chorio); (2) intra-muscular injection of 0.5 mg/kg betamethasone (Beta); (3) both of these (Both) and (4) intra-amniotic saline (Control). Lambs were born by caesarean section at 125 d (term = 150 d) and tracheotomised. FLF was aspirated and differential cell counts obtained before snap freezing. C=Os in FLF were measured by ELISA and expressed in ng/mg protein and cell counts as $n \times 10^3/\text{ml}$. Kruskal-Wallis test was used to test significance of differences. **Results:** Cell counts ($n \times 10^3/\text{ml}$) and C=O levels (ng/mg protein) are shown in the 4 groups (see table). *p* values demonstrate that there are significant differences among groups.

	Control	Chorio	Beta	Both	<i>p</i>
N	8	13	7	10	
Neutrophils	0 (0-0)	13 (5-32)	0 (0-0)	106(82-232)	<0.0001
Monocytes	1 (1-1)	120(61-133)	37 (2-143)	28 (18-72)	0.0001
Lymphocytes	0 (0-1)	19 (4-51)	9 (6-22)	189 (169-238)	0.0005
C=O	10.0(4.6-16.5)	8.5(4.5-16.3)	7.9 (6.0-11.3)	11.1(6.0-13.8)	0.99

Results shown as median (interquartile range).

C=O levels were not correlated with any of the FLF cell counts.

Conclusions: Inflammation and antenatal steroids had no overall effect on markers of oxidative stress in fetal lung fluid prior to atmospheric oxygen exposure. This may be because oxidative stress from the neutrophil respiratory burst is counteracted by upregulated lung antioxidant levels.

The Molecular Specificity of Surfactant Phosphatidylcholine in Acute Respiratory Distress Syndrome

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Background: Considerable evidence suggests that impairment of surfactant function can be a major contributor to disease severity in patients with acute respiratory distress syndrome (ARDS), and that this may be mediated by influx of inhibitory components into the lungs or alteration to surfactant composition. The altered composition of bronchoalveolar lavage fluid (BALF) phospholipid in ARDS may be due to dysfunctional synthesis and secretion from the type II alveolar epithelial cell, degradation by phospholipase(s), infiltration of plasma lipoprotein phospholipid or contamination by cell membrane components. Knowledge of the molecular species compositions of phosphatidylcholine (PC), from BALF and its purified surfactant fraction can provide an insight into these possibilities and so may inform the formulation of therapeutic surfactants designed for treating specific diseases. **Objectives:** To compare the PC molecular species compositions of purified surfactant and BALF from patients with ARDS. **Methods:** BALF was obtained by bronchoscopy from 13 ventilated adult patients who met the clinical criteria for ARDS. After centrifugation at 400 *g* for 10 min, an aliquot (1 ml) of the supernatant was separated by centrifugation over a NaBr gradient into surfactant and pellet (membrane fragments) fractions. PC molecular species compositions and concentrations of purified surfactant and pellet were analysed by electrospray ionisation mass spectrometry. **Results:** The PC molecular species composition of the membrane pellet was grossly abnormal compared with previous analyses from healthy controls and was very similar to that of the cell-free BALF supernatant. Compositions of surfactant components were decreased (PC16:0/16:0, $22 \pm 7.9\%$; PC16:0/14:0, $5.4 \pm 2.6\%$) with corresponding increased amounts of longer chain PC species (PC16:0/18:1, PC18:0/18:2, PC18:0/18:1). By contrast, PC16:0/16:0 and PC16:0/14:0 concentrations were significantly ($p < 0.05$) greater in the purified surfactant (34 ± 6.2 and $10 \pm 4.2\%$). The ratio of surfactant:pellet concentrations in BALF was highly variable from 0.03 to 0.82 (median 0.22). **Conclusions:** The abnormal PC composition of BALF from ARDS patients is due to contamination with cell membrane material, but isolation of a fraction enriched in surfactant PC species shows that no covalent complexes were formed between surfactant and membrane. The low fractional content of surfactant PC suggests that concentrations of functional surfactant in ARDS may be even lower than suggested by analysis of total BALF phospholipid.

Intron 4 Variation of Surfactant Protein B Gene Associates with Bronchopulmonary Dysplasia, but Not with RDS

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Background: Bronchopulmonary dysplasia (BPD) is influenced by a number of antenatal and postnatal risk factors and is often preceded by RDS. Surfactant protein (SP-A1, -A2, -B, -C and -D) gene variations may play a role both in BPD and RDS. **Methods:** 365 preterm Finnish infants (86 with BPD, 188 controls with RDS and 91 controls without RDS) in a high-risk population with gestation age ≤ 32 weeks were genotyped for all five SP genes. A multiparameter analysis was performed using Agrawal's algorithm-based data mining and conventional statistical allelic association study methods. Transcription Element Search Software (TESS) was used to determine potential regulatory sequences in the SP-B intron 4 variable region. **Results:** SP-B Ile131Thr alleles and SP-A1 6A2 allele interactively associated with RDS but not with BPD. SP-B intron 4 deletion variant allele frequency was increased in BPD vs. controls (OR 2.0, 95% CI 1.2–3.4, $p = 0.0078$). The presence of deletion variant was a risk factor for BPD even if essential external confounding factors were included in the analyses. No other SP polymorphisms were associated with BPD, and the SP-B intron 4 variation did not associate with RDS. Allele-specific differences were found in several transcription binding sites between SP-B intron 4 deletion variant and intron 4 invariant alleles. These binding sites may be involved in regulation of SP-B transcription. **Conclusions:** Taking into account genetic and non-genetic parameters, all the analytical tools used in the study were clearly suggestive of SP-B intron 4 deletion variant as a risk factor for BPD, but not for RDS. We propose that altered genetic susceptibility to a variety of pulmonary diseases due to SP-B gene variations occur via separate molecular mechanisms and may relate to a dual role of SP-B both as a surfactant protein and as an anti-inflammatory mediator.

Surface Activity and Structure of Films Formed from Extremely Small Surfactant Volumes: Surfactant Alteration in an Air-Ventilated Newborn Rat Model

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Background: Only about one microgram of surfactant might be recovered from tracheal aspirates from newborn babies or from lung lavage of small animals. Previous studies on surface activity have shown that the surfactant concentration (total phospholipids) should be at least 1mg/ml for films adsorbed from a surfactant suspension.

Objectives: To evaluate how lung compliance and surfactant properties are altered by high tidal volume (TV) ventilation in the neonate. **Methods:** The captive bubble system has been modified to allow the investigation of the surface activity of extremely small surfactant volumes. To increase the density of the subphase in the captive bubble chamber to above that of the surfactant suspension, 10% sucrose is added to the 0.9% salt solution. A very small volume, 0.05–0.1 μ l of surfactant, but of a concentration of at least 10 mg/ml, is squeezed into the chamber with a microsyringe and an attached Teflon tubing. The surfactant floats up to the agarose ceiling by buoyancy. A 2-mm air bubble is placed onto the ceiling and the surfactant is spread at the air-fluid interface by expanding the bubble rapidly to 8 mm in diameter. The bubble shape is continuously recorded during bubble cycling. Newborn rats, 4–8 days old, were mechanically ventilated by using 40 or 10 ml/kg TV strategies. **Results:** In all animals, dynamic compliance progressively increased following initiation of mechanical ventilation and was significantly higher than control values after 60 min ($p < 0.01$). Lung lavage total surfactant and the large aggregate fraction were significantly ($p < 0.01$) increased by 60 min of ventilation with either TV, compared to the control animals. However, ventilation with 40 ml/kg TV led to a decrease in the surfactant activity in that the minimum surface tension upon dynamic cycling was significantly ($p < 0.01$) higher. By 180 min the lung total surfactant content and dynamic compliance decreased to values comparable to control animals. **Conclusion:** In the newborn rat, mechanical ventilation with higher than physiological TV promotes an initial but transient improvement in lung compliance, and the surfactant activity tends to decrease as the time of ventilation increases. The modified captive bubble method is suitable to measure the surface tension properties of surfactant volumes less than one microlitre, provided that the concentration is at least 10 mg/ml of total phospholipids.

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Phenotypic Variability in a Kindred with Chronic Pulmonary Diseases Associated with the I73T SP-C Gene Mutation

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Background: Surfactant proteins play important roles in lung function and disease; mutations in the gene encoding surfactant protein C (SP-C) have been found to be associated with interstitial lung disease (ILD). We studied a large Italian kindred where the I73T mutation in the SP-C gene is associated with chronic pulmonary diseases. **Case Report:** A female newborn infant (31 weeks of gestational age, birthweight 1,600 g) showed respiratory distress syndrome in the neonatal period and was treated with mechanical ventilation for 2 weeks. At the age of 2 months she was discharged home with oxygen, but subsequently had frequent respiratory tract infections,

then developed dyspnea, diffuse emphysema and cor pulmonale. At the age of 5 years she is still oxygen dependent. The proband is heterozygous for a T to C change in exon 3 resulting in substitution of threonine for isoleucine at codon 73 (I73T) and this has already been described in association with ILD. **Family Members:** Twenty-four subjects belonging to this pedigree have been assessed. Five members in the mother's family have respiratory diseases with great diversity in clinical features: her mother was affected by restrictive pneumopathy and emphysema, her grand-mother by asthma and recurrent pneumonia, 2 uncles underwent lung transplantation in adult age and an aunt was diagnosed clinically as having pulmonary fibrosis. For the remaining subjects a clinical examination is planned. **Methods:** Molecular analysis of the SP-C gene was performed by automatic direct sequencing of amplified genomic DNA. Confirmation of the I73T mutation was performed by restriction analysis, since the T to C substitution inserts a Bsp 12861 site. **Results:** All the family members affected by pulmonary diseases and one with no clinical symptoms showed the presence of the mutation I73T. Among the other family members the mutation was found in six subjects for whom no clinical data were available so far. **Conclusions:** Our results confirm that heterozygosity for the mutation I73T may cause chronic inflammation of the lung or progressive pulmonary fibrosis. In addition, the chance to study a large pedigree allowed us to perform a genotype-phenotype correlation indicating a marked phenotypic variability. The diversity in symptoms, age at onset, clinical course and duration of lung disease in the relatives sharing this mutation indicates an incomplete penetrance of the mutation. This might be due to the influence of other genetic factors thus indicating that the phenotype may be complicated by additional components.

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Molecular Testing of Surfactant Protein B Deficiency in Italy

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Background: Hereditary surfactant protein B (SP-B) deficiency is an autosomal recessive disease in which affected infants are unable to produce normally functional surfactant, resulting in neonatal respiratory failure and death within the first year. SP-B deficiency is caused by mutations in the gene encoding SP-B. **Case Report:** We performed molecular analysis of DNA for SP-B gene mutations in 7 newborn infants; 6 had died of intractable respiratory disease and one was the asymptomatic sister of one of them. All the symptomatic infants were born at term (38–40 weeks), their birthweight was between 2,680 and 3,220 g, onset of respiratory distress was in the first 2 days and they were unresponsive to exogenous surfactant administration. **Methods:** We looked for mutation within the SP-B gene in these Italian cases using denaturing high-performance liquid chromatography and a direct sequencing protocol developed in our laboratory. This technique allows detection of nucleotide substitutions and small deletions/insertions on PCR fragments with a high sensitivity (99%). Immunohistochemical staining for surfactant pro-

teins was performed in formalin-fixed lung tissue of affected infants. **Results:** In 2 infants from the same family DNA analysis revealed the 121ins2 mutation on one SP-B allele and a novel mutation, 122 delC, on the other allele. Heterozygosity for 122delC was found in their asymptomatic sister. Immunostaining of lung tissue obtained at biopsy in the 2 affected infants demonstrated absent staining for SP-B, and robust extracellular staining for proSP-C, findings characteristic for SP-B deficiency. **Conclusions:** Hereditary SP-B deficiency is a rare, newly diagnosed and probably under-recognised disease, which should be suspected in term newborn infants with unexplained and intractable respiratory failure. The availability of molecular testing allows rapid confirmation of the clinical diagnosis.

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Lysophosphatidylcholine Amplifies Meconium-Induced Inactivation of Curosurf® in vitro

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Background: Pathophysiology of meconium aspiration syndrome includes surfactant inactivation. Meconium contains substances that directly inactivate surfactant. In addition, phospholipase A₂ and enzymatic products like lysophosphatidylcholine (LPC) may contribute to the inhibitory effects [Schrama et al., Acta Paediatr 2001;90:412–416]. Some new synthetic surfactant preparations are more resistant to surfactant inactivation than the modified natural surfactants. This could be related to different contents of LPC. **Objective:** To determine (A) time dependent and (B) possible synergistic inhibitory effects of meconium and LPC on modified natural surfactant. **Methods:** A: Curosurf® (2.5 mg/ml) was incubated with and without human meconium (1.2 mg/ml) at 37.5°C. Aliquots of Curosurf®-meconium mixtures and aliquots of Curosurf® incubated without meconium and subsequently mixed with meconium were studied after 0, 3, 24, 48 and 168 h. The biophysical activity was measured at 37°C in a pulsating bubble surfactometer (PBS) during 50% cyclic area compression at a frequency of 20/min. B: Curosurf® was extracted with chloroform-methanol and aliquots were resuspended in saline at a concentration of 5 mg/ml phospholipids after addition of 0, 1, 5 and 10% LPC. The surfactant preparations were incubated for 30 min with meconium at final concentrations 0, 0.1, 1 and 10 mg/ml. Thereafter the biophysical activity was measured in the PBS. Results are expressed as surface tension at minimum bubble size (γ_{\min}) after 1 min of pulsation ($n = 3-5$ repeated measurements). Statistical analysis was made by one way ANOVA with Dunnett's multiple comparison test. **Results:** A: γ_{\min} (mean \pm SD) after 1 min was 1.6 ± 0.6 mN/m without incubation (control). After incubating a mixture of surfactant and meconium for 7 days γ_{\min} increased to 30 ± 12.5 mN/m ($p < 0.01$ vs. control) indicating surfactant inactivation. Curosurf® incubated for 7 days and thereafter mixed with meconium had a γ_{\min} of 3.5 ± 2.1 mN/m (ns vs. control). B: γ_{\min} of Curosurf® at 2.5 mg/ml in the absence of meconium and LPC was 3 ± 2 mN/m (control). After addition of 10% LPC it increased to 7.5 ± 5.7 mN/m ($p < 0.05$ vs. control). Curosurf® 2.5 mg/ml with meconium 10 mg/ml had a γ_{\min} of 15 ± 10 mN/m ($p < 0.001$ vs. control).

Samples of Curosurf® containing both 10 mg/ml of meconium and 10% LPC had a γ_{\min} of 30 ± 4.5 mN/m ($p < 0.001$ vs. addition of only meconium or LPC). **Conclusions:** Inhibitory effects of meconium on Curosurf® are time dependent suggesting enzymatic breakdown and are amplified by LPC either present in the original surfactant or resulting from decomposition during incubation.

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Addition of Polymyxin B to Surfactant/Meconium Mixtures Reduces the Growth of *E. Coli*

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Background: Meconium aspiration syndrome and bacterial pneumonia may occur simultaneously. Previously we have demonstrated that surfactant treatment may be useful in both clinical conditions and that the antibiotic peptide polymyxin B (PxB) improves the resistance of Curosurf® against inhibition of biophysical function by human meconium in vitro. **Objective:** To study the effects of PxB, surfactant and meconium on the growth of Gram-positive group B streptococci (GBS) and Gram-negative *E. coli*, two major bacterial pathogens in the neonatal period. **Methods:** A bacterial suspension with $\sim 10^7$ colony forming units in saline and 20 mg/ml meconium was incubated at 37°C under gentle agitation with and without 10 mg/ml Curosurf® and/or 1% PxB (100 units/mg; Sigma-Aldrich Chemistry, Steinheim, Germany) for 5 h. After 0, 1, 3 and 5 h samples were serially diluted and 100 μ l aliquots were transferred to petri dishes, mixed with warm agar and incubated at 37°C for 24 h. The

colonies were counted and the results expressed as mean \pm SD CFU/ml from 3 repeated measurements. **Results:** Meconium increases the growth of *E. coli* in the nutrient-poor environment saline. The addition of Curosurf® alone did not mitigate bacterial growth. PxB and PxB-surfactant mixtures significantly reduced the proliferation of *E. coli*. Relatively little effect was seen on Gram-positive GBS (data not shown). **Conclusions:** Surfactant/polymyxin mixtures reduce the growth of *E. coli* in meconium suspensions in vitro. These results warrant further animal studies and seem to indicate that the biophysical and antibacterial properties of polymyxin/surfactant may be combined as a possibly useful adjunct in the treatment of neonatal pneumonia and/or meconium aspiration syndrome.

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Surface Spreading of Native and Clinical Surfactants under Demanding Conditions

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Background: Efficient pulmonary surfactants must spread rapidly at air-water interfaces to form surface-active films able to achieve and sustain very low surface tensions (high surface pressures) under compression, during successive compression-expansion cycles. **Methods:** We have optimised a standard assay to analyze the kinetic behaviour of different pulmonary surfactants to spread rapidly at the air-liquid interface of a surface balance and to form surface-active films, in the absence of surface layer compression. **Results:** Whole native surfactant purified from pig lungs spreads very rapidly, reaching equilibrium pressures of around 45 mN/m in less than 25 s when deposited on top of the surface at concentrations in the order of ca. 2 μ g phospholipid/cm². Spreading kinetics was sensitive to surfactant concentration and temperature. Spreading at low concentration and/or low temperature (15°C) had biphasic kinetics, interpreted as originating from two distinct consecutive steps with different rate limits. Material obtained by organic extraction of native surfactant (containing the lipid fraction plus hydrophobic proteins SP-B and SP-C) and reconstituted in buffer, showed also rapid spreading kinetics, just slightly inferior to those of native surfactant, as did samples of the clinical surfactant Curosurf. Significant differences were observed in the spreading properties of native surfactant compared with its organic extract or Curosurf when applied onto subphases containing diluted concentrations of blood serum. Presence of serum at 1–10 μ l per ml of subphase completely inhibited spreading of suspensions prepared from surfactant organic extracts or Curosurf, while hardly affecting spreading of native preparations. On the other hand, serum had no effects on spreading of organic solutions (chloroform/methanol) containing lipids and proteins from either surfactant or Curosurf, suggesting that serum components mainly prevent interfacial transference of surface active species from bilayer-like structures formed in aqueous media. Inhibition by serum of spreading of organic extract suspensions or Curosurf could be nearly prevented by high subphase concentrations of several carbohydrate-based polymers like polyethylene glycol (10 kDa, 5% w/v) or hyaluronic acid

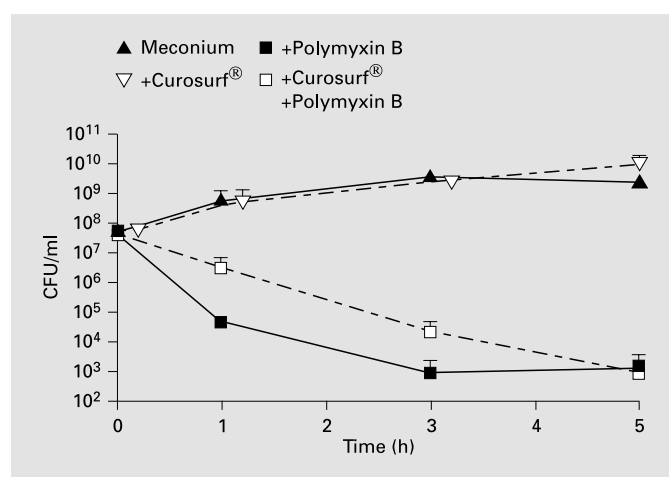


Fig. 1. Influence of meconium, Curosurf® and polymyxin B on the growth of *E. coli*.

(1,024 kDa, 0.125% w/v). Spreading on subphase that contained both serum and polymer produced spreading kinetics for organic-extracted native surfactant and Curosurf that were similar to those obtained in the absence of serum. **Conclusion:** These findings suggest that subphase polymers that are physiologically present in extracellular alveolar liquid subphase may be important additives to consider in treating lung diseases with exogenous surfactant.

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Surfactant Administration by Laryngeal Mask Airway in Preterm Infants with Respiratory Distress Syndrome

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Background: Surfactant therapy given to infants with respiratory distress syndrome (RDS) receiving continuous positive airway pressure (CPAP) reduces the need for subsequent mechanical ventilation. Currently, however, patients need to be intubated for a few minutes in order to deliver surfactant. This is an invasive procedure with attendant risks. We considered that use of a supraglottic airway device, the laryngeal mask airway (LMA), could offer a less invasive means of surfactant delivery. **Objective:** To evaluate the feasibility and practicality of administering surfactant using the LMA in preterm infants with RDS. **Methods:** A prospective observational study. Infants less than 72 h of age with gestational age (GA) <35 weeks and birth weight (BW) >800 g, treated with nasal CPAP (5 cm H₂O) for RDS were eligible for the study if the arterial to alveolar oxygen tension ratio (a/APO₂) was <0.20 over a period of >60 min. The LMA was positioned and the cuff was inflated with 2–3 ml of air. When an effective positive pressure ventilation, defined as adequate chest movements and oxygenation, was established, a bolus of surfactant (Curosurf® 100 mg/kg) was instilled into the lumen of the LMA. The infant then underwent manual ventilation for 2 min, after which the LMA was removed and the infant resumed nasal CPAP. Arterial blood gas measurements and a/APO₂ were recorded at baseline and 3 h after surfactant therapy. Clinical data including respiratory rate, heart rate, arterial blood pressure and respiratory support (CPAP level, FiO₂) were continuously monitored. The FiO₂ was changed to maintain preductal transcutaneous oxygen saturation (tcSaO₂) in the range 92–95%. **Results:** Eight patients (GA 31 ± 2 weeks; BW 1413 ± 519 g) were enrolled into the study at 32 ± 2 h of postnatal age. Six of them received 1 dose of surfactant; 2 doses were administered to the other 2 patients. Mean a/APO₂ increased significantly (from 0.13 ± 0.04 to 0.34 ± 0.13; p < 0.01) 3 h after treatment; no differences were found for other measurements (respiratory rate, heart rate, arterial blood pressure, tcSaO₂). No infant received sedative drugs for the procedure. No complications were reported during and after the treatment. Two infants needed subsequent mechanical ventilation due to progressive respiratory failure. **Conclusions:** The LMA can be used as a conduit to obtain rapid and non-invasive access to the trachea of the preterm infant with RDS to administer surfactant. This would reduce the risk of respiratory and extra-respiratory complications associated with tracheal intubation. We consider our results justify a comparative trial.

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Open Lung Ventilation Reduces Bacterial Translocation and Mortality in Experimental Streptococcal Pneumonia

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Background: We recently showed that exogenous surfactant reduces bacterial translocation and subsequent mortality during group B streptococcal (GBS) pneumonia in surfactant-deficient newborn piglets. A surfactant-mediated reduction in atelectasis might be one of the mechanisms responsible for these beneficial effects. **Objective:** To determine the effects of atelectasis on GBS translocation and subsequent mortality in a piglet model of GBS pneumonia. **Methods:** Thirteen healthy and twenty-six surfactant-deficient (whole lung lavage) newborn piglets (age 73 ± 16 h) received an intratracheal injection of a GBS solution (10 ml/kg, ~10⁸ CFU/ml) and were thereafter ventilated for 5 h. The healthy animals in group 1 (*Heal-CON*) and the lavaged animals in group 2 (*Lav-CON*) were conventionally ventilated using a positive end-expiratory pressure (PEEP) of 4 cm H₂O and a tidal volume of approximately 7 ml/kg. Lavaged animals in group 3 (*Lav-OLC*) were ventilated with the open lung concept aiming to recruit and thereafter stabilize previously collapsed alveoli using high levels of PEEP. Optimal recruitment was defined as a PaO₂ >450 mm Hg using an inspired oxygen fraction of 1.0. Samples for blood gas analysis and cultures were drawn every hour. Survival time after GBS instillation was recorded. At the end of the ventilation period the number of colony forming units (CFU)/lung was determined. **Results:** PaO₂ levels were significantly higher in the *Heal-CON* and *Lav-OLC* groups compared to the *Lav-CON* group (p < 0.001). PaO₂ levels were >450 mm Hg in the *Lav-OLC* group, indicating optimal alveolar recruitment. All animals in the *Heal-CON* and the *Lav-OLC* group survived the 5-hour ventilation period. In the *Lav-CON* group 11/13 animals died with a mean survival time of 211 ± 49 min (p < 0.001). Blood cultures were GBS positive in 0/13, 6/13 and 12/13 animals in the *Heal-CON*, *Lav-OLC* and *Lav-CON* group, respectively. Blood cultures became positive earlier in the *Lav-CON* group (97 ± 18 min, p < 0.001) compared to the *Lav-OLC* group (245 ± 23 min). The number of CFUs isolated per ml blood was also higher in the *Lav-CON* group compared to the *Lav-OLC* group (265 ± 165 vs 33 ± 53, p < 0.005). The number of CFU/lung (log₁₀CFU ± SD) at the end of the ventilation period was significantly lower in the *Heal-CON* (9.1 ± 0.3) and *Lav-OLC* (9.9 ± 0.3) compared to the *Lav-CON* group (11.2 ± 0.5, p < 0.001). **Conclusion:** Open lung ventilation attenuates bacterial growth and translocation and improves survival during GBS pneumonia in the surfactant-deficient lung. Treatment with exogenous surfactant previously showed comparable findings, suggesting that a reduction in atelectasis is one of the mechanisms responsible for these beneficial effects.

The Treatment Response after Early and Delayed Surfactant Administration Is Different during Conventional and Open Lung Ventilation

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Background: Randomized controlled trials have shown that early surfactant treatment is superior to delayed (>2 h) treatment in neonatal respiratory distress syndrome. However, most patients in these trials were treated with conventional ventilation (CV). CV often results in secondary lung injury which can reduce the surfactant treatment response (STR). Attenuating this secondary lung injury by open lung ventilation (OLV) might therefore improve the STR, especially after delayed administration. **Objective:** To explore the effect of CV and OLV on the STR after early and delayed surfactant administration in surfactant-deficient newborn piglets. **Methods:** In 25 newborn piglets (2.5 ± 0.4 kg) surfactant deficiency was induced by whole lung lavage. Twenty animals were randomized to receiving either early (2 h) or delayed (4 h) surfactant treatment (25 mg/kg). Prior to surfactant treatment, animals were ventilated using either CV (CV-2, CV-4) or OLV (OLV-2, OLV-4). Five control animals received surfactant immediately after lung lavage. Following surfac-

tant treatment all animals were ventilated for 2 h using CV. During CV the positive end-expiratory pressure (PEEP) was set at 5 cm H₂O and the tidal volume was maintained at approximately 7 ml/kg. During OLV, collapsed alveoli were actively recruited and thereafter stabilized with sufficient PEEP. FiO₂ was set at 1.0. Blood gas analysis and ventilatory pressures/volumes were recorded hourly before and every 30 min after surfactant treatment. **Results:** In both OLV groups mean PaO₂ levels increased immediately after lung recruitment and stabilized at levels above 500 mm Hg for the remainder of the pre-surfactant ventilation period. In contrast, mean PaO₂ levels in the CV-2 and CV-4 groups were significantly lower (< 100 mm Hg, $p < 0.001$) compared with both OLV groups. Following surfactant treatment, the control animals showed a gradual improvement in oxygenation, with mean PaO₂ levels of 415 ± 40 mm Hg after 2 h of ventilation. In both OLV groups PaO₂ levels remained high after surfactant treatment and switching to CV, with no difference between early (442 ± 40 mm Hg) and delayed administration (489 ± 58 mm Hg). This in contrast to the CV-4 group, where PaO₂ levels were significantly lower (146 ± 117 mm Hg, $p < 0.01$) 2 h after surfactant treatment compared to the CV-2 group (360 ± 133 mm Hg). Dynamic compliance showed a similar pattern with deteriorating lung mechanics after delayed treatment in the CV-4 group (0.38 ± 0.10 ml/cmH₂O), but not in the OLV-4 group (0.63 ± 0.07 ml/cm H₂O). **Conclusion:** In contrast to conventional ventilation, application of open lung ventilation prior to surfactant administration preserves the surfactant treatment response, even after delayed (4 h) treatment.

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