

Abstracts

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Abstracts in alphabetical order according to
first authors.

1

Recombinant Human Keratinocyte Growth Factor Compared to Betamethasone: Increased Surfactant Pools without Catabolic Side Effects during Alveolarization

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Background: Glucocorticoids are used to induce maturation of the surfactant system, which is at the expense of regular growth and alveolarization of the mostly injured, preterm lungs. Recombinant human keratinocyte growth factor (rhKGF) promotes differentiation and inhibits apoptosis of type II cells via epithelial KGF receptors. **Objective:** Prior to use in injured preterm lungs, the effects of rhKGF on surfactant pools must be addressed in healthy lungs. **Methods:** Rats at d1–19 were treated for 48 h with rhKGF (2 × 5 mg/kg), betamethasone (BM, 2 × 1 mg/kg) or rhKGF + BM. Body weight was determined prior to and after treatments. Lung lavage fluid (LLF) and lung tissue were analyzed for phospholipid pools, phosphatidylcholine (PC) molecular species and precursor concentrations using mass spectrometry. **Results:** Contrary to BM rhKGF did not impair weight gain. RhKGF increased surfactant pools in LLF up to the end of alveolar formation to a similar extent as BM, while later on (d19–21) only the combination was effective. However, in contrast to BM, rhKGF-induced increases in secreted surfactant were not at the expense of lung tissue pools. Similarly, rhKGF increased pool sizes of the PC-precursors choline, phosphocholine and CDP-choline by 41, 20 and 207%, while BM had no effect. The physiologic pattern of individual PC species in LLF (41 ± 1% dipalmitoyl-PC, DPPC) or lung tissue was not altered by rhKGF. BM, however, increased DPPC at the expense of other

saturated, monounsaturated and polyunsaturated components. **Conclusions:** RhKGF increases surfactant pools in LLF of immature lungs as BM does, but without catabolic side effects. In mature alveolarized lungs neither drug has an effect on its own, but the combination increases surfactant pools. RhKGF does not interfere with lipid homeostasis and might be useful in neonatal intensive care.

2

The Efficacy of Surfactant Lung Lavage by Curosurf Enriched with Dextran in Experimental Meconium Aspiration Syndrome

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Background: Removal of meconium from the airspaces is an important part of the therapeutic approach in meconium aspiration syndrome (MAS). **Objective:** To prove the hypothesis that addition of dextran to Curosurf[®] increases meconium removal during lung lavage and improves lung function in experimental MAS. **Methods:** A suspension of human meconium (30 mg/ml; 4 ml/kg) was instilled into the tracheal cannula of anaesthetized, paralyzed and conventionally ventilated (CV) adult rabbits to induce respiratory failure. Animals were then allocated to one of three groups (in each, n = 6): lavage with saline (Sal), surfactant without (Surf) and with dextran (Surf-dex). Lung lavage (10 ml/kg in 3 portions) was performed with diluted surfactant (Curosurf, 100 mg/kg) without or with dextran (30 mg/ml), or saline by asymmetric high-frequency jet ventilation (f = 300/min; Ti = 70%) and animals were ventilated with 100% O₂ for one additional hour with CV (f = 40/min; Ti = 50%). Blood gases, ventilatory settings and lung-thorax com-

pliance (CLT) were measured prior to and after meconium instillation, and 10, 30 and 60 minutes after lavage. The amount of removed meconium in bronchoalveolar lavage fluid was quantified. **Results:** Improved washout of meconium solid particles was found in both surfactant-lavaged groups in comparison to saline-lavaged animals (Sal: $4.8 \pm 1.0\%$) and more meconium was obtained in Curosurf with dextran than in only Curosurf lavaged animals (17.5 ± 3.5 vs. $12.4 \pm 3.9\%$, $p < 0.05$; both groups vs. Sal, $p < 0.01$). Values for $\text{PaO}_2/\text{FiO}_2$ were significantly higher in Surf-lavaged animals than in controls (at 60 min: 24.5 ± 4.2 vs. 9.1 ± 2.2 kPa, $p < 0.01$). Additional increase in oxygenation was seen in Surf-dex group (at 60 min: 34.2 ± 8.1 kPa, p vs. Surf group < 0.01). CLT was higher in Surf-dex group in comparison to Sal and Surf groups (at 60 min: 9.6 ± 0.9 vs. 7.6 ± 1.2 , $p < 0.01$ and 8.0 ± 0.7 ml/cm $\text{H}_2\text{O}/\text{kg}$, $p < 0.05$). **Conclusions:** Our data indicate that enrichment of Curosurf with dextran increases its efficiency in meconium removal and improves lung function in surfactant-lavaged rabbits with meconium aspiration.

3 Surfactant Disaturated Phosphatidylcholine Kinetics in Human Adults with Acute Respiratory Distress Syndrome Using Stable Isotopes

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Background: In patients with acute respiratory distress syndrome (ARDS) only a part of the lungs is aerated and surfactant composition and function are altered. The extent of lung damage and of changes in surfactant metabolism still remain unclear. This is because we lack suitable and safe methods to study surfactant metabolism in human ARDS. **Objective:** To evaluate surfactant disaturated phosphatidylcholine (DSPC) turnover in patients with ARDS using stable isotopes. **Patients:** 12 patients with ARDS and 7 subjects with normal lungs (controls). **Methods:** After the tracheal administration of a tracer dose of ^{13}C -dipalmitoylphosphatidylcholine, we measured, over time, the ^{13}C enrichment of the palmitate residues in DSPC from tracheal aspirates by mass spectrometry. Data were fitted to a model with two compartments (the airways and lung tissue) and kinetic indices were derived based on the existing evidence that alveolar macrophages degrade between 5 and 50% of DSPC in normal lungs, the rest being lost from lung tissue. In ARDS, due to the release of hydrolytic enzymes, it was assumed that between 5 and 100% of DSPC could be degraded in the airways. Some of the kinetic indices were uniquely determined, while for others only lower and upper bounds were resolvable. **Results:** The DSPC alveolar pool in ARDS was markedly and significantly smaller than in controls (mean \pm SD: 0.2 ± 0.05 vs. 1.7 ± 0.5 mg/kg, $p < 0.05$). Flux from tissue to alveoli was $0.03 \pm$

0.009 mg/h/kg in ARDS and 0.28 ± 0.09 mg/h/kg in controls respectively ($p < 0.05$) whereas recycling was not different. De novo synthesis ranged between 0.02 to 0.10 mg/h/kg in ARDS and from 0.23 to 0.47 mg/h/kg in controls ($p < 0.05$). **Conclusions:** In ARDS the alveolar DSPC pool is markedly decreased and DSPC turnover is altered. This model may be useful in assessing the alteration of surfactant DSPC in ARDS in relation to the time course and severity of the disease. The method is also suitable to evaluate the effect of different treatment modalities on surfactant metabolism.

4 Curosurf and an SP-C33 Based Synthetic Surfactant Are Both Effective in Experimental Acute Respiratory Distress Syndrome Induced by Surfactant Depletion and Injurious Ventilation

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Background: Although clinical trials have given conflicting results, exogenous surfactant might be of value in acute respiratory distress syndrome (ARDS). **Objective:** We tested Curosurf[®] and a synthetic surfactant based on SP-C33, an analogue to native SP-C [Johansson J et al., J Appl Physiol 2003;95:2055–2063] in experimental ARDS. **Methods:** In two separate series, the lungs of pigs were subjected to repeated washings with warm saline (30 ml/kg \times 8), and then to 45 min of injurious mechanical ventilation. The ensuing ARDS-like injury was treated with a 30 ml/kg lavage of dilute (2 mg/ml) Curosurf or synthetic surfactant, and then with undiluted (80 mg/ml) surfactant: 100 mg/kg in the prone and 100 mg/kg in the supine position. Another 100 + 100 mg/kg was given 30 min later, for a total of 460 mg/kg. Outcome was evaluated 2.5 h afterwards, with blood gases and compliance of the respiratory system (C_{rs}). Protein concentration in a diagnostic lung lavage was obtained in the synthetic surfactant series. Mean alveolar expansion in three lobes of the right lung was estimated in histologic sections with the observer (BR) unaware of group assignment. **Results:** Findings are shown in the table as median (range). **Conclusion:** Both surfactants were effective in this ARDS model.

	n	C_{rs} , ml \cdot kg ⁻¹ \cdot cm H_2O^{-1}	$\text{PaO}_2/\text{FiO}_2$ mm Hg	Protein g \cdot l ⁻¹	Alveolar expansion, %
Curosurf	8	2.4 (1.8–3.7)	518 (305–579)	–	87 (77–100)
Control	8	1.4 (0.8–2.0)	195 (91–534)	–	72 (27–97)
		$p < 0.001$	$p = 0.021$	–	$p = 0.038$
SP-C33	9	2.4 (1.6–2.8)	542 (499–566)	1.1 (0.5–1.4)	83 (60–98)
Control	9	1.7 (1.0–1.9)	155 (88–428)	1.5 (0.9–2.6)	50 (5–98)
		$p = 0.002$	$p < 0.001$	$p = 0.038$	$p = 0.049$

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Surfactant Instillation Alone Is as Effective as Surfactant Lavage Followed by Instillation in Experimental Acute Respiratory Distress Syndrome

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Background: We have previously shown that a synthetic surfactant (SP-C33) is effective in experimental acute respiratory distress syndrome (ARDS). **Objective:** It has been suggested that lung lavage with dilute surfactant removes surfactant-inactivating debris from the alveoli, thereby improving the surfactant effect. We tested this hypothesis in a porcine ARDS model using a synthetic surfactant based on SP-C33 [Johansson J et al., *J Appl Physiol* 2003;95:2055–2063]. **Methods:** The lungs of piglets were subjected to saline washings (30 ml/kg × 8) and injurious mechanical ventilation. The ensuing ARDS-like condition was treated by lung lavage with 30 ml/kg of dilute surfactant (2 mg/ml), followed by four 100 mg/kg instillations of undiluted (80 mg/ml) surfactant for a total of 460 mg/kg. Other pigs received surfactant instillations only: 130 + 130 + 100 + 100 mg/kg, or no treatment. Compliance of the respiratory system (C_{rs}), blood gases, ventilatory efficiency index (VEI) [Shashikant B et al., *J Appl Physiol* 2005;99:2204–2211], and protein concentration in a diagnostic lung lavage were measured five hours after the first surfactant dose. P-values for differences between groups were obtained with the Mann-Whitney test. Percent air expanded alveoli (PAEA) was estimated in histologic lung sections. **Results:** PAEA was not significantly different in the two surfactant groups, but greater than in untreated controls. Other findings are shown in the table as median (range). **Conclusion:** SP-C33 surfactant improved lung mechanics and oxygenation, but surfactant lavage did not confer any advantage over instillation alone.

Treatment groups	n	C_{rs} , ml · kg ⁻¹ · cm H ₂ O ⁻¹	PaO ₂ /FiO ₂ mm Hg	VEI	Protein g · l ⁻¹
1: Lavage + instillation	9	1.94 (1.63–2.23)	268 (193–508)	0.10 (0.06–0.11)	1.2 (0.81–1.97)
2: Instillation only	8	2.00 (1.50–2.81)	494 (241–518)	0.11 (0.08–0.16)	1.4 (0.47–1.55)
3: No surfactant	8	1.40 (1.19–1.80)	90 (73–216)	0.08 (0.05–0.09)	1.9 (1.79–1.97)
1 vs. 2		p = 0.945	p = 0.051	p = 0.051	p = 0.945
1 + 2 vs. 3		p = 0.002	p = 0.003	p = 0.030	p = 0.007

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Molecular and Functional Changes of Lung Surfactant in Response to Hyperoxia

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Background: Changes in surfactant function and metabolism in response to injuries depend on their duration and adaptation. While ventilation with high oxygen pressures is used to overcome respiratory insufficiency, no detailed comparison of acute versus prolonged hyperoxic lung injury exists. **Objective:** We investigated the consequences of acute versus prolonged hyperoxic exposure to non-lethal oxygen concentrations with respect to surfactant alterations. **Methods:** Adult rats were exposed to 85% oxygen for 2 or 7 days, and the histological changes were confirmed by light microscopy and immunohistochemistry. Surfactant was isolated from lung lavage fluid and lavaged lung tissue by centrifugation. Functional analysis was performed using a pulsating bubble surfactometer. All surfactant proteins (SP-A, B, C and D) were analyzed by HPLC and immunoblotting, while concentration and metabolism of phospholipids were investigated using isolated perfused lungs, [³H-methyl]choline labelling followed by separation of phospholipid molecular species by HPLC and mass spectrometry. **Results:** Hyperoxia induced progressive inflammation and structural alteration of the lungs. Surfactant function was impaired after 2 days, but normalised with duration of hyperoxia, which was due to inhibition but not decrease of SP-B and SP-C. Phospholipid pools and phosphatidylcholine (PC) synthesis were unchanged after 2 days, but elevated 1.7-fold after 7 days. Incorporation of ³H-labelled PC into surfactant pools progressively decreased, and arachidonoylated phospholipids increased at the expense of saturated PC. **Conclusions:** Hyperoxic challenge causes a persisting impairment in the trafficking and secretion of newly synthesized PC despite recovery of surfactant function and reactive increase in synthesis. Increased alveolar arachidonoylated phospholipids may contribute to inflammation via eicosanoid generation.

An Open-Label Comparison of Calfactant and Poractant Alfa Administration Traits and Impact on Neonatal Intensive Care Unit Resources

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Background: While available surfactants provide fairly similar clinical outcomes, little is known about possible differences in cost or characteristics of administration of the products. **Objective:** To compare poractant alfa (PA) and calfactant (CA) administration traits, short-term clinical responses, and resource utilization for neonatal respiratory distress syndrome. **Methods:** An open-label series of 271 (209 PA and 62 CA) infants were evaluated for 420 administrations. Respiratory therapists collected patient, surfactant (preparation and delivery time), and post-administration clinical data. An economic analysis involved labor costs of surfactant administration and usage, wastage, and product average wholesale price. Analysis utilized pooled variance t-tests for time measurements, clinical observations and economic comparisons. Chi-square tests were employed for proportional differences. **Results:** PA could be prepared and administered more rapidly than CA (mean 3.8 vs. 5.3 min, $p < 0.001$) and percentage of doses prepared and delivered in less than 5 min was significantly higher (58% vs. 4.4%, $p < 0.001$). Doses administered per patient were similar (1.5 vs. 1.7). PA and CA were similar in time to recovery (81 vs. 74%), percent desaturation (25 vs. 27%) and bradycardia (3.8 vs. 8.5%). Reflux was significantly higher with CA (13 vs. 3.5%, $p < 0.001$). Economic analyses found the mean administration costs were USD 1.48 for PA and USD 1.98 for CA ($p = 0.002$). Mean wastage costs were USD 143 for PA and USD 341 for CA ($p < 0.001$). **Conclusion:** PA appeared to involve less neonatal intensive care unit (NICU) resources than CA due to the reduced administration time and less wastage of drug product. Administration of PA resulted in fewer episodes of reflux of product than CA, and tended to result in fewer bradycardia episodes. Future studies should more closely evaluate time, resource, wastage, and post-administrative clinical effects to fully assess the impact of surfactant products in the NICU.

All-Trans Retinoic Acid Does Not Modulate Intra-Amniotic Endotoxin Mediated Pulmonary Inflammation in Fetal Sheep

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Background: Chorioamnionitis, frequently associated with preterm birth, induces fetal lung inflammation and improves pre-

term lung function at the cost of alveolar simplification. However, endotoxin-induced chorioamnionitis reduces all-trans retinoic acid (RA, a potent inducer of genes involved in postnatal lung development) in the fetal lung to 16% of control values. **Objective:** We speculated that administration of RA to the fetus before induction of chorioamnionitis would prevent alveolar simplification. **Methods:** Time-mated ewes with singletons were assigned to groups of 5–7 animals to receive a depot treatment with 20,000 IE of RA in olive oil (or olive oil alone) intra-muscularly 3 h prior to intra-amniotic injection of endotoxin (20 mg, *E. coli* 055:B5) or saline, 7 days before delivery at 124 days gestational age. The right upper lung lobe was processed for morphometric analysis. **Results:** RA administration did not affect chorioamnionitis-induced fetal and systemic inflammation as indicated by interleukin-8 concentrations in lung tissue. RA administration alone did not alter lung maturation. Endotoxin-induced chorioamnionitis increased lung gas volume on the deflating limb of a pressure-volume curve at 40 cm H₂O (22 ± 4 ml/kg; mean \pm SEM) relative to control (5 ± 3 ml/kg, $p < 0.05$) and 20 ± 3 ml/kg after RA pretreatment ($p < 0.05$ vs. control). Alveolar wall thickness was 4.2 ± 0.3 μ m after endotoxin-induced chorioamnionitis and 6.0 ± 0.4 μ m in controls ($p < 0.05$ vs. control) and 5.5 ± 0.2 μ m after RA pretreatment ($p < 0.05$ vs. endotoxin, $p > 0.05$ vs. control). The ratio of airspace versus tissue was 2.1 ± 0.3 in controls and 4.6 ± 0.3 in endotoxin-induced chorioamnionitis and 4.1 ± 0.5 after RA pretreatment (both $p < 0.05$ vs. control). RA pretreatment in utero did not affect lung maturation, pulmonary inflammation or systemic inflammation after endotoxin-induced chorioamnionitis. **Conclusion:** Fetal treatment with RA does not prevent the development of alveolar simplification.

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Intravenous Endotoxin Results in Pulmonary Inflammation and Lung Remodeling in Preterm Fetal Sheep

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Background: Chorioamnionitis and fetal systemic inflammatory response syndrome are associated with adverse outcome. Interestingly, chorioamnionitis is associated with reduced risk for respiratory distress syndrome but increased risk for bronchopulmonary dysplasia (BPD). BPD has been associated with impaired lung development marked by a rearrangement of elastin deposition. **Objective:** We hypothesized that elastin is part of the structural changes induced by inflammation in an experimental model of intravenous *E. coli* endotoxin injection into fetal sheep. **Methods:** Thirteen fetal sheep were chronically instrumented at 107 days gestational age (term is 147 days). 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 (lipopolysaccharide, LPS; *E. coli*, 0127:B8) intravenously. Animals were deliv-

ered after an additional 7 days. Paraffin embedded lung tissues were stained for nuclear factor (NF)-kappa B for proinflammatory gene transcription, surfactant protein B for lung maturation and elastin for structural remodeling. Staining intensity was analyzed in a 4-step semi-quantitative scale. Results are presented as median and interquartile range. **Results:** NF-kappa B was detected in the nucleus of endothelial cells and alveolar macrophages (3; 2.7–3.5 vs. 1; 0.4–1.6 in controls, $p < 0.05$). Surfactant protein B was detected in alveolar type II cells in the study group (3; 2.3–3.6 vs. 1; 0.6–2.2 in controls, $p < 0.05$). Elastin was detected around pulmonary blood vessels in both control and study group. Elastin fibers were detected in alveolar crests after intravenous endotoxin but not in the control group. **Conclusion:** Intravenous injection of endotoxin causes fetal pulmonary inflammation and maturation. Within 7 days of exposure to intravenous endotoxin, elastin fiber deposition in the fetal lung indicated fetal lung injury and remodeling. These early, strictly intra-uterinely induced changes may be an important predisposing step to the postnatal development of BPD.

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Transforming Growth Factor-Beta Reprograms Cell Fate Determination in Pulmonary Endodermal Cells

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Background: We have previously shown that bioactive transforming growth factor-beta (TGF-beta) can be recovered from the lungs of prematurely born neonates who are at risk for development of bronchopulmonary dysplasia (BPD). In these neonates, higher levels of bioactive TGF-beta on day 1 of life correlated significantly with severity of BPD. The mechanism by which TGF-beta adversely affects pulmonary outcome of the neonate remains unknown. **Objective:** To use a mesenchyme-free mouse endodermal explant culture to examine the fundamental mechanism by which TGF-beta may affect morphogenesis and cell behavior. **Methods:** Mesenchyme-free endodermal tissue from embryonic lungs of wild type and transgenic mice were explanted in Matrigel and treated with various growth factors, with and without TGF-beta. Both morphology as well as expression of key genes such as the transcription factor *Nkx2.1* and the surfactant protein gene *SpC* were examined. **Results:** TGF-beta strongly inhibited branching behavior of embryonic lung endoderm in explant culture. Inhibitory activity of TGF-beta was mediated through Smad3, which is also known to inhibit *SpB* gene expression. TGF-beta also repressed *Nkx2.1* and *SpC* gene expression, suggesting that it alters cell fate determination. **Conclusions:** Deleterious effects of TGF-beta may be related to its ability to abrogate lung morphogenesis and function through repression of key genes such as *Nkx2.1* and *SpC*.

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Modest Improvement in Lung Function with Synthetic Surfactant in Experimental Acute Respiratory Distress Syndrome Induced by Instillation of HCl and Injurious Ventilation

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Background: We have previously shown that a synthetic surfactant based on SP-C33 is effective in experimental acute respiratory distress syndrome (ARDS) induced by surfactant depletion and injurious ventilation. This finding is perhaps to be expected, since the surfactant is effective in another condition with surfactant deficiency, namely lung immaturity [Johansson J et al., J Appl Physiol 2003;95:2055–2063]. **Objective:** In order to further assess the potential value of this surfactant in ARDS, we tested it in a model not involving direct removal of surfactant. **Methods:** 16 ml/kg of HCl (pH 1.5) was infused into the lungs of 9 piglets and remained there for about 30 s. The piglets then underwent 45 min of injurious mechanical ventilation (inspiratory pressure 40 cm H₂O, end-expiratory pressure –5 cm H₂O). Surfactant was instilled intratracheally in four divided doses for a total of 460 mg/kg. Four sham controls were not subjected to lung damage or surfactant. Outcome was assessed after 4.5 h with four measures reflecting lung mechanics, oxygenation, carbon dioxide elimination, and alveolar protein leak, measured as protein concentration in a diagnostic lavage. The lungs were examined by light microscopy. **Results:** Surfactant, compared to placebo, improved ventilatory efficiency index (VEI) (for definition see Shashikant B et al., J Appl Physiol 2005;99:2204–2211). There was also a trend towards improved compliance of the respiratory system (C_{rs}) and oxygenation (table). Lungs of experimental animals had irregular alveolar expansion, recruitment of granulocytes to the alveolar spaces and widespread laminar necrosis of airway epithelium probably induced by aspirated HCl. **Conclusion:** There was a modest effect of treatment with the synthetic surfactant, less pronounced than in the other ARDS model.

	n	C_{rs} , ml · cm H ₂ O ⁻¹ · kg ⁻¹	PaO ₂ /FiO ₂ mm Hg	Protein in lavage fluid, g · l ⁻¹	VEI
Surfactant	9	1.28 (0.86–1.83)	377 (180–561)	1.49 (0.27–1.84)	0.10 (0.06–0.15)
Placebo	10	1.02 (0.57–1.45)	212 (58–551)	1.67 (0.75–2.42)	0.07 (0.02–0.14)
P surfactant vs. placebo		0.11	0.11	0.27	0.016
Sham	4	3.48 (2.83–3.92)	486 (241–545)	0.67 (0.17–1.36)	0.23 (0.14–0.28)

Natural History of Patent Ductus Arteriosus, Spontaneous Closure Rate and Response to Indomethacin Treatment in Very Low Birth Weight Infants Documented by Serial Echocardiography

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Background: Patent ductus arteriosus (PDA) continues to play a significant role in the morbidity among very low birth weight (VLBW) infants. Data on early spontaneous closure and reopening after initial closure are not well described, especially in extremely low birth weight (ELBW; <1000 g) infants. **Objective:** To follow the course of PDA in VLBW infants by serial echocardiography. **Design/Methods:** Prospective observational study using serial echocardiography starting at 12–48 h of age in VLBW infants admitted to the neonatal intensive care unit (NICU) at Good Samaritan Hospital between January 2000 and October 2005. Infants with chromosomal anomalies, major congenital heart disease or indomethacin (INDO) treatment prior to the first echocardiogram were excluded. All infants had serial echocardiograms until permanent closure of the PDA was documented. **Results:** A total of 145 VLBW infants were studied with serial echocardiography. Fifty-five out of the 145 (38%) infants showed spontaneous closure of the PDA on the first study, and 11/55 (20%) had a very small PDA that closed spontaneously later. Two out of the 55 (4%) ELBW patients reopened after initial spontaneous closure. Initial closure after INDO was high (80% for all VLBW infants). However, the rate of reopening (25%) and the need for surgical ligation (18%) remained high and affected primarily the ELBW infants. Data in the table are given as N/Total and (%). **Conclusions:** Serial echocardiography commencing in the first 12–48 h of age enables accurate description of rates of spontaneous closure of PDA, response to INDO, and reopening after initial closure. Our data indicate that reopening after spontaneous closure is rare even in the ELBW infant.

Birth weight, g	501–750	751–1000	1001–1250	1251–1500	501–1500
Spontaneous closure	9/36 (25)	14/39 (36)	13/35 (37)	19/35 (54)	55/145 (38)
Closed after INDO	19/27 (70)	18/25 (72)	20/22 (91)	15/16 (99)	72/90 (80)
Reopening after INDO	8/19 (47)	6/18 (33)	3/20 (15)	1/15 (7)	18/72 (25)
PDA ligation	13/36 (36)	12/39 (31)	1/35 (3)	0/35 (0)	26/145 (18)

A Physiologic Low Oxygen Protocol Significantly Reduces the Incidence of Threshold Retinopathy of Prematurity in Very Low Birth Weight Premature Infants

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Background: Retinopathy of prematurity (ROP) is a major cause of blindness in very low birth weight (VLBW) infants and is also associated with significant long term visual problems. Exposure of the immature retina to high levels of oxygen leads to vaso-obliteration followed by vaso-proliferation. Oxygen regulated growth factors such as vascular endothelial growth factor (VEGF) as well as non-oxygen regulated factors such as insulin-like growth factor-1 (IGF-1) have been shown to play a critical role in ROP. VEGF up-regulation with a concomitant increase in IGF-1 during the neovascularization phase leads to proliferative ROP. Observational studies have reported that use of a physiologic low oxygen level is associated with a significant reduction in ROP. **Methods:** Prospective observational study of the effects of physiological low oxygen protocol (PLOP) on threshold ROP (PLOP-ROP) requiring laser photocoagulation in VLBW infants admitted to three institutions (Good Samaritan Hospital (GSH), Los Angeles, Cedars-Sinai Medical Center (CSMC), Los Angeles, and National University Hospital, Singapore (NUHS)). PLOP protocol was implemented to keep oxygen saturation by pulse oximeter between 83 and 93% in VLBW infants receiving supplemental oxygen as previously published in Pediatrics 2003;111:339–345. **Results:** The incidence of threshold ROP before and after implementation of the PLOP-ROP protocol was compared. The transition year was not included. The incidence of threshold ROP decreased in each center: GSH: 14.8 to 4.9% (8/54 to 2/41); CSMC: 4.4 to 0% (3/68 to 0/72); NUHS: 14.0 to 0% (6/43 to 0/29). The p value for the combined results (17/165 to 2/142) is 0.0006. No increase in necrotizing enterocolitis or neonatal mortality was noted during this period. **Conclusions:** A physiologic low oxygen protocol combined with strict control of fluctuations in oxygen saturations significantly reduces the risk of threshold ROP in VLBW infants. Long-term follow-up studies are ongoing to evaluate the neurodevelopmental outcome in these patients.

Surfactant Therapy in Very Low Birth Weight Infants during Nasal Ventilation: A Feasibility Study

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Background: Incidence of bronchopulmonary dysplasia (BPD) in very low birth weight (VLBW; <1,500 g) infants continues to remain unacceptably high, despite advances in perinatal care, neo-

natal ventilation and surfactant therapy. Nasal ventilation using nasal continuous positive airway pressure (NCPAP) or nasal intermittent positive pressure ventilation (NIPPV) is being used increasingly as a primary mode of respiratory support or following extubation from endotracheal intubation. Centers that use NCPAP often do not use surfactant to avoid intubation, mechanical ventilation and subsequent lung injury. Lowest rates of BPD have been reported from these centers. Clinicians are reluctant to extubate VLBW infants in the immediate postnatal period due to lack of experience with NCPAP or NIPPV, perceived need for additional doses of surfactant based on the clinical course, and lack of randomized trials demonstrating the superiority of NCPAP or NIPPV. **Objective:** To describe our experience of administering surfactant to VLBW infants on NCPAP or NIPPV for the first or subsequent doses. **Methods:** VLBW infants who were receiving NCPAP or NIPPV as a primary mode of respiratory support or following extubation after initial dose of surfactant (Poractant alfa 200 mg/kg) during the first 48 h of age were included. First dose was given as an early rescue mode if infants were intubated for respiratory distress and required 30% oxygen. Additional doses of surfactant may be given if they continue to require 30% oxygen. **Results:** Ten VLBW infants received Poractant alfa while they were on NCPAP or NIPPV. Patients were briefly intubated, given surfactant, and immediately extubated to NCPAP or NIPPV. None of the patients had desaturation episodes or bradycardia during surfactant administration. Six patients received their first dose of surfactant while on NCPAP or NIPPV and the remaining 4 patients received their second dose of surfactant after they had been extubated following the first dose of surfactant. **Conclusions:** Our experience indicates that it is feasible and safe to give surfactant to infants on NCPAP or NIPPV. We are currently evaluating the long-term outcome of this practice on the incidence of BPD in VLBW infants.

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Inflammatory Aspects of Meconium Aspiration Syndrome

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Background: Meconium aspiration syndrome (MAS), an important cause of respiratory distress in the term newborn, is a serious condition with high morbidity and mortality. Current therapies are still supportive. The pathophysiology is complex and not well defined including airway obstruction, epithelial injury, surfactant inactivation, inflammation and pulmonary hypertension. We have previously shown that meconium is a potent activator of the complement system in vitro and in vivo in a newborn pig model of MAS, the latter associated with a systemic inflammatory response reflected by cytokine production. **Objective:** We hypothesize that complement contributes to the inflammatory reaction in MAS. Furthermore, since MAS may be associated with infection in utero and the meconium used for in vitro experiments contains endotoxin, CD14-dependent signaling may contribute to meconium-induced cytokine production. **Methods:** Human whole blood from 6 different donors was incubated with meconium for 4 h in the presence or absence of

monoclonal antibodies blocking complement activation (anti-factor D and anti-C2) and/or CD14. The terminal complement complex (TCC) and proinflammatory cytokines were measured. **Results:** Complement inhibition completely blocked meconium-induced TCC formation whereas inhibition of CD14 had no effect on TCC. TNF alpha production was markedly inhibited (>50%) by blocking either complement or CD14. IL-6 and IL-1 beta production was only slightly reduced by complement inhibition but markedly reduced by CD14 inhibition. Interestingly, the combined inhibition of complement and CD14 completely abolished the production of all three cytokines. **Conclusion:** Meconium-induced cytokine production is mediated by both complement and CD14. Thus, a combined inhibition of these two effector mechanisms may be an interesting therapeutic approach to reduce the inflammatory reaction in MAS associated with endotoxin-containing amniotic fluid.

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Novel Mutations in the Gene Encoding ABCA3 Resulting in Fatal Neonatal Lung Disease

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Background: ABCA3 deficiency is a newly described genetic lung disorder with clinical features similar to surfactant protein B deficiency. **Objective:** We investigated whether ABCA3 was the cause of neonatal lung disease in three patients with fatal neonatal respiratory distress. **Methods:** DNA sequence analysis of the ABCA3 gene was performed in the parents of a child who died and whose lung histology was suggestive for a surfactant genetic disorder, and in two full-term infants with fatal neonatal lung disease. Lung tissue was examined by routine histology, immunohistochemical staining and electron microscopy. **Results:** Three Norwegian patients with ABCA3 deficiency were identified. One patient had a phenotype that differed from previous descriptions of this disease since the initial neonatal period was uneventful. The diagnosis was established 19 years after death by analyzing DNA material from the parents, with novel ABCA3 mutations identified on one allele in each parent. The other two patients had more typical courses with the onset of respiratory symptoms immediately after birth. ABCA3 mutations were identified on both alleles from the two infants, and electron microscopy of type II cells revealed abnormal lamellar body formation characteristic of this disorder. **Conclusion:** ABCA3 deficiency is a cause of lung disease in the Norwegian population, and the finding of five novel mutations indicates allelic heterogeneity for ABCA3 mutations. A phenotype with delayed onset of respiratory symptoms has also been recognized.

Hypoxia and Reoxygenation with Different Oxygen Concentrations. A Study of Gene Regulation in Newborn Piglets

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Background: Pulmonary damage, as a result of oxygen exposure, is an important clinical complication in infants who require oxygen as treatment. Resuscitation at birth with pure oxygen is associated with an increased level of childhood cancer. **Objective:** To study gene expression we used GeneChip[®] Porcine Genome Array from Affymetrix. The microarrays were performed through isolation of RNA from snap frozen lung tissue. **Methods:** Piglets (12–36 h) underwent hypoxia until base excess was -20 mmol/l or blood pressure <15 mm Hg, and were then resuscitated for 15 min by ventilation with 21%, 40%, 60% and 100% oxygen ($n = 12$, $n = 10$, $n = 10$, $n = 10$, baseline $n = 10$) and thereafter observed for 1 h. **Results:** The 100%, 60%, 40% and room-air group had a mean arterial pO_2 value of respectively 41.7, 26.1, 20.0 and 12.4 kPa after 15 min of resuscitation ($p < 0.001$). In the 100% oxygen group it took significantly longer to achieve normalisation of pH and SaO_2 compared to room-air. The microarray study in the room-air and 100% oxygen group showed significant difference in gene expression profiles for genes related to cell-replication and inflammatory response. We will explore this further with RT-PCR. **Conclusion:** Hypoxia and subsequent resuscitation for 15 min with high percentage oxygen gives a dose-dependent hyperoxia with increased oxidative stress and risk of cell damage and long term consequences. Hyperoxia leads to changes in expression of genes related to cell-replication and inflammatory response compared to room-air resuscitation.

Silicone Oil Interferes with Surfactant Protein (SP)-B but Not with SP-C

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Background: After contact with silicone oil, surface activity of pulmonary surfactant is impaired. Silicone oil is used as a lubricant in syringes containing rubber coated plungers. The hydrophobic surfactant proteins SP-B and SP-C are essential for formation of the phospholipid monolayer at the air-liquid interface and for reduction of surface tension during film compression. **Objective:** To investigate whether impairment of surface activity might be related to effects on the hydrophobic surfactant proteins. **Methods:** Silicone oil and Curosurf were mixed at weight ratios of 0, 0.13, 0.38 and 1.3 mg silicone oil/mg phospholipids and aliquots thereof were

extracted with organic solvents and evaporated. The hydrophobic proteins in the original and extracted samples were separated by gel electrophoresis and determined by Western blot analysis using antibodies against SP-B and SP-C (derived from rabbits, kind gift of T. Weaver, Cincinnati, USA). **Results:** Anti-SP-B blots of extracted material showed significant reduction of SP-B for samples containing ≥ 0.38 mg silicone oil/mg phospholipids. In the original samples neither SP-C nor SP-B were reduced by addition of silicone oil. **Conclusions:** The results indicate specific interference of silicone oil with SP-B, but not with SP-C as a possible mechanism for impairment of surface activity. To preserve surface properties of pulmonary surfactant, exposure to silicone oil should be avoided.

'Watterberg Effect' in Infants Less than 30 Weeks Gestation at Birth

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Background: Although the 'Watterberg effect' was confirmed in animal experiments lately published clinical studies showed that the association between chorioamnionitis and bronchopulmonary dysplasia (BPD) is more complicated. **Objective:** To examine the association between chorioamnionitis, concentration of IL-6 and IL-8 in umbilical blood, gastric aspirate (GA) at the time of birth and tracheal aspirate (TA) before surfactant treatment and the occurrence of BPD in infants less than 30 weeks' gestation at birth. **Methods:** 138 infants with a gestational age less than 30 weeks were prospectively enrolled during a period of 22 months. Placental pathology was reviewed for the presence of chorioamnionitis. Commercial ELISA kits were used for determination of IL-6 and IL-8. BPD was defined as any supplemental oxygen requirement at 36 weeks' postmenstrual age. **Results:** BPD developed in 33 infants, 51 had uncomplicated respiratory distress syndrome (RDS), while the others served as a control group. The concentrations of IL-6 in umbilical vein, IL-8 in GA and TA were significantly higher in the BPD group compared to the uncomplicated RDS group; however, no difference was found when compared to the control group, in which the gestational age was significantly higher than in the BPD group. The concentration of IL-8 in TA was positively correlated with the concentration of IL-8 in GA ($r = 0.72$) and oxygenation index (OI) at maximal ventilatory support (MVS; $r = 0.59$). MVA (corrected for gestational age) showed that elevated OI (OR 1.2, 95% CI 1.1–1.4; $p = 0.004$) and an increase of Apgar score between 5th and 1st minute of more than 2 points (OR 19.6, 95% CI 1.7–58.7; $p = 0.02$) were independent risk factors for BPD. The MVA model was similar when OI was substituted by the combined variable of maximal airway pressure (MAP) >8 cm H_2O and the concentration of IL-8 in GA above 75th percentile (OR 4.1, 95% CI 1.2–14.1; $p = 0.02$), but in this case the correlation coefficient was lower. **Conclusions:** 'Watterberg effect' was dependent on gestational age. Although no marker of prenatal inflammation was shown to be an independent risk factor for BPD, IL-8 in GA was associated with increased risk for BPD when combined with MAP.

Reduced Surfactant Disaturated Phosphatidylcholine Pool Size Is Associated with Extubation Failure in Preterm Infants Recovering from Respiratory Distress Syndrome

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Background: Extubation failure (EF) occurs in 2–25% of preterm infants recovering from respiratory distress syndrome (RDS) [Rothaar RC, Epstein SK, Curr Opin Crit Care 2003;1:59–66] and its pathophysiology is multifactorial. **Objective:** To assess if preterm babies recovering from RDS and who fail extubation have reduced amounts of pulmonary disaturated phosphatidylcholine (DSPC). **Methods:** DSPC pool size and half-life were measured by stable isotope technology [Cogo PE et al., Crit Care Med 2003;5:1532–1538] in preterm infants recovering from RDS. Inclusion criteria: birth weight (BW) <1,500 g with RDS requiring surfactant therapy and mechanical ventilation, planned extubation after 72 h of life and after at least 36 h from the last treatment dose of exogenous surfactant. Infants were extubated and re-intubated according to well defined criteria. Clinical variables were recorded at least hourly throughout the study. Infants who, after extubation, needed re-intubation within 48 h from extubation or experienced significant worsening of the respiratory status (high CPAP settings with $FiO_2 > 0.40$ and > 5 cm H_2O to obtain $SaO_2 > 88\%$) were grouped in the EF group. All other infants who were successfully extubated were considered as extubation successes (ES). DSPC kinetics were measured by stable isotope tracing and mass spectrometry of DSPC from sequential tracheal aspirates. Results are given as mean (SD) and compared by unpaired t test; $p < 0.05$ was regarded as significant. **Results:** Twenty-three infants fell in the EF and 29 in the ES group. 22 infants were not extubated as planned for worsening of the respiratory status and were not included in the analysis. Clinical characteristics and demographics (BW, gestational age, weight at the study, prenatal steroids, cumulative dose of surfactant before extubation and days of mechanical ventilation) were similar between EF and ES groups. DSPC pool size was 26.4 (19.7) mg/kg and 42.1 (31.8) mg/kg in the EF and the ES groups respectively ($p = 0.034$), whereas half-life was 19.6 (16.9) h and 21.3 (16.9) h respectively (NS). **Conclusions:** Preterm infants who fail extubation or experience significant worsening of respiratory status after extubation had much lower DSPC pool size than infants who were successfully extubated (ES). The cause of this persisting surfactant deficiency in EF needs to be investigated. Supplementation studies with exogenous surfactant should be considered for EF patients.

Alveolar Morphometry in Rat Lungs with Surfactant Deficiency

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Background: There is no consensus regarding shape and size changes of lung alveoli during the respiratory cycle in normal subjects and under conditions of surfactant deficiency. Pulmonary surfactant, which is present at the alveolar air-liquid interface reduces surface tension and stabilizes the alveoli. Deficiency of surfactant, as in neonatal respiratory distress syndrome (RDS), impairs lung function. **Objective:** To study the single alveolar dynamic morphometry during the respiratory cycle in rats at baseline conditions, following bronchoalveolar lavage (BAL) and after surfactant replacement. **Methods:** We investigated alveolar morphometry in normal and surfactant-deficient rat lungs using video-microscopy. The alveoli were pictured through the pleura by means of a video camera, connected to a personal computer. The rat was intubated and ventilated, and tracheal pressure was measured. Image analysis provided size and shape distribution of alveoli as well as pressure versus average alveolar dimension (P-AD) curve in normal lungs, after surfactant depletion by BAL and in lavaged lungs that were treated with exogenous surfactant (SRT lung). Histological sections were taken from the lungs of the different study groups at various intratracheal pressures. **Results:** Alveoli undergo substantial size and shape changes during the respiratory cycle. P-AD curves of normal lungs show a hysteresis that almost disappears in BAL lungs (inflation and deflation curves overlap) and is restored to some extent in SRT lungs. Alveolar sizes and shapes in BAL lungs were more dispersed than in normal and SRT lungs. The mean values of the alveolar aspect ratio (the ratio between the maximal and the minimal alveolar dimension) as well as the width of the histogram were greater in BAL lungs compared to normal lungs. The SRT lungs with the partially recovered surfactant are characterized by a mean aspect ratio and width of distribution that lie in between normal and BAL lungs. In BAL lungs there is no relationship between pressure and mean aspect ratio, meaning that crumbling and folding of the tissue may happen also at higher pressures. The SRT lungs lie between the BAL and normal lungs regarding crumbling and folding. **Conclusions:** Surfactant deficiency reduces the number of open alveoli (and alveolar ducts), increases their size and causes the small alveoli to collapse into clusters. Total alveolar surface area for gas exchange is reduced as many small alveoli presumably collapse. It is also possible that the enlarged cavities in the video-microscopy of BAL lungs are generated when some alveoli collapse around the alveolar duct – which in turn is stretched wide open. The effect of surfactant replacement therapy reverses the alterations caused by the BAL procedure and pulls the lung partially back towards 'normal' in terms of shape of the P-AD curve, and shape of alveoli.

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