

Abstracts

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1

Nasal Ventilation in Preterm Infants: A Novel Means of Delivering Time-Cycled Pressure and Flow-Limited Intermittent Mandatory Ventilation via Nasal Cannula in Newborn Infants

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Background: Nasal interfaces to provide nasal ventilation (bi-nasal or nasopharyngeal prongs and variable flow devices) are cumbersome and may cause nasal injury and nasal obstruction due to secretions. We describe the use of novel means of delivering time-cycled pressure and flow-limited (TCPFL) mode via nasal cannula (NC-IMV) in neonates requiring respiratory support as a primary mode of support of post-extubation or for treatment of apnea of prematurity. **Objective:** To describe our experience of delivering NC-IMV in neonates requiring respiratory support in this prospective observational study. **Methods:** Preterm infants intubated for respiratory distress syndrome and given poractant alfa were extubated to NC-IMV. Neonates with respiratory distress or moderate-to-severe apnea were also started on NC-IMV. Peak inspiratory pressures up to 30 cm H₂O were used. Flow rate was limited to 6–7 LPM. Inspiratory time, positive end expiratory pressure, and IMV rate were set at 0.5 seconds, 5 cm H₂O, and 30–40 breaths per minute, respectively. **Results:** Seventy infants were treated with NC-IMV. Birth weights and gestational ages ranged from 505 to 4,167 g and 23 to 41 weeks, respectively. Age at the time of starting NC-IMV ranged from 1 to 81 days. NC-IMV was used from 1 to 39 days for a total of 652 days. All infants tolerated NC-IMV. No cases of nasal injury, air leaks, gastric or eardrum perforation were seen. NC-IMV failure rate requiring reintubation in our study population was only 8%. **Conclusions:** NC-IMV is feasible and well tolerated. TCPFL NC-IMV allows clinicians to limit pressures and can be delivered safely in neonates.

2

Surfactant via Gastric Tube in Spontaneously Breathing Very Low Birth Weight Infants on Nasal CPAP Prevents Mechanical Ventilation

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Background: Nasal continuous positive airway pressure (CPAP) is a strategy to reduce ventilator-induced lung injury. Infants on CPAP with respiratory distress syndrome (RDS) may benefit from surfactant. **Objective:** To evaluate effectiveness/safety of surfactant instillation via a gastric tube. **Methods:** Randomized multicenter study in Germany. Entry criteria: gestational age 26+0 to 28+6 weeks, age: <12 h, RDS with FiO₂ >0.30, no malformations. Intervention: surfactant by gastric tube as previously described. Endpoint: rate of mechanical ventilation between 24 and 72 h. **Results:** 108 infants received the intervention, 112 controls received standard treatment, i.e. continuation on CPAP and ventilation/rescue surfactant if indicated. 51/112 (45%) infants were put on mechanical ventilation in this group as opposed to only 30/108 (28%) in the intervention group ($p < 0.05$). No differences were observed in the rate of adverse events, including pneumothorax and intracerebral hemorrhage. **Conclusions:** This study demonstrates a reduced need for subsequent mechanical ventilation following surfactant administration via a gastric tube while the infants are breathing on CPAP.

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3

Surfactant Replacement Therapy in Preterm Infants: A European Survey

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Background: International guidelines provide recommendations on the optimal surfactant replacement strategy in preterm infants. **Objective:** To determine if these guidelines are implemented in clinical practice. **Methods:** Data on surfactant treatment were collected in 173 European neonatal intensive care units (NICUs) by questionnaire and patient data collection. **Results:** All but 2 NICUs used exogenous surfactant in the treatment of respiratory distress syndrome. NICUs used animal-derived surfactant with poractant alfa being most widely used (86%). 39% of the NICUs claimed to use prophylactic treatment (<15 min of life). Data on surfactant treatment were collected in 460 infants, with a median gestational age of 27 weeks and a birth weight of 860 g. The median age of surfactant administration was 60 min (IQR 30–180). Prophylactic treatment was used in 23% of the infants, and 28% of the infants received surfactant >2 h after birth. Poractant alfa was used in 77% of the infants. The median first dose was 148 mg/kg and 43% of the infants received multiple doses. **Conclusions:** With the exception of surfactant timing, guidelines on surfactant replacement therapy seem to be implemented in European NICUs.

4

Surfactant plus Polymyxin B May Prevent Bacterial Translocation by the Air-Liquid Barrier

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Background: Microorganisms may pass by the air-liquid interface in clinical conditions, when the surface activity of pulmonary surfactant is impaired. Such conditions include neonatal pneumonia, neonatal respiratory distress and meconium aspiration syndrome. To prevent bacterial translocation, surfactant treatment seems to be less effective than open lung ventilation. However, the properties of surfactant can be expanded by addition of the antimicrobial peptide polymyxin B (PxB). Mixtures of the modified natural porcine surfactant (pSF) and PxB prevent proliferation of Gram-negative bacteria in ventilated neonatal rabbits. **Objective:** To investigate bacterial translocation in ventilated immature neonatal rabbits treated with pSF ± PxB. **Methods:** Neonatal near-term rabbits were treated with pSF, pSF + PxB (0.25–1%) or saline (control). Animals were ventilated with stan-

standardised tidal volumes and received ~10⁷/E. coli intratracheally. After 240 min, animals were killed, the right lung and left kidney were excised and bacterial growth was determined. The left lung was used for histological analysis. **Results:** We found E. coli in the left kidney in 7 of 10 control animals and in 3 of 9 pSF-treated animals, but we found no bacterial contamination in any animal treated with pSF + PxB ($p < 0.01$). Bacterial growth was significantly reduced in animals treated with PxB ± pSF compared to control animals or animals receiving only pSF. In histology, this was accompanied by a reduction of severe inflammatory tissue destruction. **Conclusions:** Mixtures of PxB and pulmonary surfactant show antimicrobial effects in neonatal rabbits and prevent systemic spreading of E. coli.

Ethical Approval: Stockholms norra djurförsöksetiska nämnd.

5

Resuscitation with 100% Oxygen Gives Increased Oxidative Stress in Lung Tissue and Influences the Capacity to Repair Base Lesions on DNA

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Background: Newborn resuscitation with pure oxygen may induce short- and long-term pathological changes via oxidative stress. The lungs are in direct and continuous communication with the outside environment and are exposed directly to the highest partial pressure of inspired oxygen. **Objective:** To study antioxidant capacity and DNA repair activities in lung tissue from newborn piglets 1 and 9 h after preceding hypoxia and reoxygenation. **Methods:** Hypoxemia was induced by ventilation with 8% oxygen in nitrogen and maintained until base excess reached -20 mmol/l or mean arterial blood pressure decreased below 20 mm Hg. The piglets, $n = 5-10$ in each group, were resuscitated for 15 or 30 min by ventilation with 21 or 100% oxygen and observed for 1 or 9 h before lung tissue samples were removed and snap-frozen. Total antioxidant capacity and capacity to repair base lesions on DNA were measured. Controls underwent surgery, stabilization and ventilation, but were not exposed to hypoxia and reoxygenation. **Results:** One hour after the end of resuscitation the total antioxidant capacity in lung tissue was significantly lower in the group resuscitated with 100% oxygen compared to 21% ($p < 0.02$), but after 9 h there was no difference. DNA repair activity was significantly reduced 1 h after resuscitation and was lowest in the 100% oxygen group ($p < 0.05$). After 9 h there was still a tendency to reduced base lesion repair on DNA. **Conclusions:** Hypoxia and subsequent resuscitation with 100% oxygen causes increased oxidative stress and risk of cell damage and long-term consequences such as accumulation of mutations in the genome.

6

Variation in Phosphatidylcholine Synthesis in Children with Acute Lung Injury: An in vivo Stable Isotope Label Approach

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Background: Acute lung injury (ALI) is an inflammatory lung condition with significant morbidity and mortality. The lipid profile of surfactant is significantly altered in patients with ALI, but it is not clear whether this is due to decreased surfactant synthesis or increased degradation. **Objective and Methods:** This study employs a novel method to quantify surfactant kinetics in children in vivo. Five children, aged 2 months to 3 years, admitted to the Paediatric Intensive Care Unit (PICU) with a clinical diagnosis of ALI, were infused with *methyl*-D9 choline chloride. Synthesis of phosphatidylcholine (PC) in sequential lung bronchoalveolar lavage (BAL) and serum samples were analysed by electrospray ionisation tandem mass spectrometry (ESI-MS/MS). **Results:** Measurable incorporation of D9 choline into both surfactant and serum PC was demonstrated, with peak incorporations of $0.7 \pm 0.08\%$ in BAL fluid and $3.0 \pm 0.8\%$ in serum. The rate of surfactant synthesis varied widely and did not correlate with respective measurement of serum PC synthesis, highlighting lung and systemic effects of ALI. **Conclusion:** This study demonstrates the feasibility of D9 choline labelling to quantify kinetics of surfactant and serum PC synthesis in vivo. The variation in BAL PC synthesis indicates a wide range of surfactant synthesis/secretion, while comparison with serum PC synthesis suggests that much of this variation is lung specific.

Ethics: Approved by Oxford Research Ethics Committee: Reference 07/H0606/125.

7

Increased Expression of Surfactant Protein A in the Fetal Gut during Endotoxin-Induced Chorioamnionitis

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Background: Chorioamnionitis is the most significant cause of prenatal inflammation and preterm delivery. Both prematurity and prenatal inflammation have been strongly linked with compromised postnatal developmental outcomes of the lung and brain. Recently, we showed for the first time that prematurity and inflammation in utero were associated with a disturbed intesti-

nal immune defence, gut barrier function and the vascular system. **Objective:** To investigate whether the expression of endotoxin-binding proteins which possess innate immune functions, including Toll-like receptor (TLR)4, myeloid differentiation (MD)-2, and surfactant protein A (SP-A), are altered following endotoxin-induced chorioamnionitis. **Methods:** Chorioamnionitis was induced at different gestational ages (GA). Animals were killed at low GA after 2 days or 14 days exposure to chorioamnionitis. Long-term effects of 30 days exposure to chorioamnionitis were studied in near-term animals after induction of chorioamnionitis. **Results:** In preterm animals, TLR4 and MD-2 mRNA levels were decreased by 55% within 2 days after endotoxin-induced chorioamnionitis compared to saline-treated animals ($p < 0.05$). In saline-treated animals and in lambs exposed to endotoxin for 2 days, SP-A was not detected. However, 14 days after endotoxin administration preterm animals had increased expression of SP-A which lasted till 30 days after induction of chorioamnionitis in the near-term animals. **Conclusions:** Our results indicate that the fetal gut responded with increased expression of SP-A which is binding and inactivating endotoxin. The decreased levels of TLR4 and MD-2 and the increased SP-A levels suggest an endotoxin tolerance phenotype which merits further study.

8

Global Gene Expression in Newborn Mouse Lung Tissue after Hypoxia and Reoxygenation

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Background: Perinatal hypoxia and resuscitation can be detrimental and the optimal inspired oxygen concentration during resuscitation is important to define as 5–10% of newborns require resuscitation to some degree. Hypoxia starts a cascade of biochemical alterations that proceeds into the recovery period after resuscitation. Immature oligodendrocytes, retinal cells and the developing lungs are especially vulnerable to free radical-induced injury. **Objective:** To study whole genome expression alterations induced by supplementary oxygen in vulnerable tissues in a newborn mouse model of hypoxia and reoxygenation. Microarray technology was used as a hypothesis generating tool to identify the most differentially expressed genes. **Methods:** 25 C57BL/6 mice on postnatal day 7 were randomized to hypoxia (8% O₂, $36 \pm 0.5^\circ\text{C}$) for 120 min and reoxygenation in room air (H21, n = 6), 40% (H40, n = 6), 60% (H60, n = 7) or 100% (H100, n = 6) O₂ for 30 min. Mice were then killed and rapidly dissected on ice after a 150-min recovery period. Two control groups not exposed to hypoxia, but either room air (C21, n = 5) or 100% O₂ (C100, n = 8) for 30 min were used as com-

parison. GeneChip Mouse Gene ST 1.0 Arrays (Affymetrix) were used to analyze whole genome expression. Statistical analysis was performed by using BAMarray software. The Gene Ontology and DAVID Bioinformatic Resources 2008 databases were used for functional analysis. **Results:** We found 1,185 of the 34,760 probe sets on the microarray chip to be significantly differentially expressed in lung tissue between group C21 and either of the four intervention groups (H21; 103, H40; 280, H60; 643, H100; 280). Among the 19,461 probe sets with known gene ID, 560 genes were significantly differentially expressed (H21; 39, H40; 148, H60; 343, H100; 145). Preliminary gene ontology analyses of the genes in the H100 show that genes involved in cell growth and differentiation, energy metabolism, inflammation, apoptosis and repair mechanisms are represented. **Conclusion:** In the current model, hypoxia and reoxygenation with inspired oxygen concentrations from 21–100% induce significant changes in 1,185 probe sets and 560 genes in the lung tissue of newborn mice. The impact of these gene expression alterations needs further analysis.

9

Glucocorticoids Potentiate Interleukin 6-Induced Surfactant Protein-B Expression in H441 Cells by Enhancing the JAK/STAT Signaling Pathway

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Background: The respiratory distress syndrome (RDS) contributes considerably to perinatal morbidity and mortality of preterm infants. Concentration of surfactant protein B (SP-B) is decreased in RDS. Both maternal antenatal steroid administration and chorioamnionitis reduce the incidence and severity of RDS. An important mediator in chorioamnionitis is interleukin-6 (IL-6) using the Jak-Stat signaling pathway for signal transduction. **Objective:** We hypothesized that betamethasone (BTM), dexamethasone (DXM) and IL-6 have synergistic effects on SP-B gene expression and Stat3 phosphorylation in H441 cells. **Methods and Results:** DXM and BTM increased SP-B mRNA levels by 16.5 (13.3)-fold and IL-6 alone by 2.3-fold. After 48-hour exposure of cells to DXM or BTM, IL-6 caused a significantly greater increase in SP-B mRNA levels (28.1-fold) compared to IL-6 or glucocorticoids alone. While IL-6 stimulated tyrosine phosphorylation of Stat3 in a time- and dose-dependent way, DXM and BTM alone had no effect on Stat3 phosphorylation. Both DXM and BTM could potentiate IL-6 induced phosphorylation of Stat3. The synergism of glucocorticoids and IL-6 on SP-B gene expression and the effect of glucocorticoids on IL-6 induced Stat3 phosphorylation could be blocked by a specific Jak-inhibitor. Expression level analysis showed that glucocorticoids increased the expression of the IL-6 binding alpha-subunit receptor (IL-6R). **Conclusion:** Our findings represent an example of a pulmonary regulatory system in which steroids increase the effect of the proinflamma-

tory cytokine IL-6 by up-regulation of its receptor. The described interaction of IL-6 and glucocorticoids helps to explain why prenatal inflammation and antenatal steroid administration can attenuate severity of RDS in preterm infants.

10

Surfactant Protein A Predisposes to Preterm Labor

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Background: Surfactant protein A (SP-A) in amniotic fluid predicts the risk of respiratory distress syndrome (RDS). The genotypes of SP-A influence the susceptibility to RDS. SP-A binds microbes and serves as either a pro- or anti-inflammatory ligand, influencing the expression of cytokines in vitro. **Objective:** To study whether SP-A influences pulmonary and extrapulmonary inflammatory responses in intrauterine infection (IUI), and whether SP-A affects the susceptibility to endotoxin (LPS)-induced spontaneous preterm birth (SPB). **Methods:** Transgenic (tg) mice expressing rSP-A by the SP-C promoter and wild-type animals were tested in the setting of LPS-induced preterm birth. We determined SP-A, and the LPS-induced changes: (1) of several cytokines in serum and in amniotic fluid (AF), and (2) of mRNAs of SP-A, TLR2, TLR4 and of several cytokines in fetal lung and in extraembryonic tissues. **Results:** In premature tg-mice the expression of SP-A in fetal lung and in extraembryonic tissue and SP-A contents in AF and in fetal lung were increased by >10-fold. In tg-animals, SPB was induced at lower concentration of LPS than in wild-type animals. Expressions and contents of IL-10 and TNF α were different in tg-animals compared to wild-type animals. **Conclusions:** SP-A participates in the regulation of cytokine responses in lung and in extraembryonic tissue. We propose that high levels of SP-A protein promote preterm labor and delivery.

11

Fibrinogen Counteracts Inactivation of Lung Surfactant

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Background: Plasma proteins are known to interfere with biophysical properties of pulmonary surfactant. **Objective:** To investigate the effect of fibrinogen on exogenous surfactant. **Methods:** Curosurf[®] (80 mg/ml) without (CuroC) or with fibrinogen (CuroCF) (40 mg/ml) was subjected to surface area cycling for 6 days at 37°C. After dilution to 10 mg/ml minimum and maxi-

imum surface tension (γ_{\min} and γ_{\max}), and percent of area compression needed to reach γ_{\min} of 5 mN/m (area%) were evaluated by captive bubble surfactometer. Immature newborn rabbits were treated with Curosurf, CuroC or CuroCF (200 mg/kg), or no material (Control) and ventilated with a standardized sequence of peak insufflation pressures. **Results:** Area% and γ_{\max} were reduced in CuroCF versus CuroC (both $p < 0.05$). Animals with Curosurf and CuroCF had higher VT and compliance than Control. Lung gas volumes (median and range; ml/kg) were in CuroC 6.4 [3.9–32.0], in CuroCF 12.9 [8.1–34.5] and in Curosurf 17.4 [13.5–29.3]. **Conclusions:** Data indicate that fibrinogen protects Curosurf against inactivation.

Ethics: The study was approved by the Local Ethical Committee for Animal Research.

12

Interaction of Transforming Growth Factor $\beta 1$ with Surfactant Protein A

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Background and Objective: Transforming growth factor $\beta 1$ (TGF- $\beta 1$) was shown to be present in surfactant protein-A (SP-A) preparations. Our aim was to characterise the potential binding between SP-A and TGF- $\beta 1$. **Methods and Results:** We demonstrated the presence of TGF- $\beta 1$ in human as well as porcine surfactants and in purified SP-A. Size-exclusion chromatography indicated that TGF- $\beta 1$ was bound to SP-A. After deglycosylation of SP-A with N-glycosidase F, TGF- $\beta 1$ is released from SP-A indicating a role for the carbohydrate moieties. Intact TGF- $\beta 1$ free SP-A can be obtained by incubating SP-A with 5 mM deoxycholate at pH 9.4 and size-exclusion chromatography. Binding studies with immobilized SP-A and 125I-TGF- $\beta 1$ indicated that the binding is reversible, time- and concentration dependent. The apparent dissociation constant K_d is 53 ± 18 (SEM) pM indicating high affinity binding. Addition of excess of the latency associated peptide (LAP) prevented the binding of 125I-TGF- $\beta 1$ to SP-A. LAP added after the binding of 125I-TGF- $\beta 1$ to SP-A liberated the bound 125I-TGF- $\beta 1$. These observations suggest that latent TGF- $\beta 1$, which is formed by incubation of LAP with 125I-TGF- $\beta 1$ to 125I-latent TGF- $\beta 1$, does not bind to SP-A. This hypothesis was further strengthened by the observation that latent TGF- $\beta 1$ cannot compete with 125I-TGF- $\beta 1$ for SP-A binding. **Conclusions:** These results demonstrate binding of TGF- $\beta 1$ to SP-A which may have implications for the biological activities of these proteins in the lung.

13

Total Liquid Ventilation in Meconium Aspiration Syndrome

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Background: Total lung ventilation (TLV) is considered a promising therapy for bronchoalveolar lavage (BAL) with perfluorocarbon in severe meconium aspiration syndrome (MAS). **Objective:** To compare the efficacy of TLV-BAL with BAL alone using a dilution of surfactant (S-BAL) in an ovine model of MAS. **Methods:** Once intubated, anesthetized and paralysed, 20 newborn lambs underwent surgery to monitor blood gases and hemodynamic measures using thermodilution with PICCOplus and VoLEF devices (Pulsion Medical System, Germany). Severe MAS was achieved with instillation of 2×1 ml/kg of a 25% dilution of human meconium. Animals were then randomized into 2 groups of 10: (1) TLV-BAL, liquid-ventilated using a specially designed TLV device (INOLIVENT 4; Sherbrooke University, Canada) with perfluorodecalin (PFDEC; F2 Chemicals, UK) and (2) S-BAL, gas-ventilated and lavaged with 2×15 ml/kg of a 5 mg/ml surfactant dilution (BLES biochemicals Inc., Canada). Both groups were ventilated for a total of 4 hours and then killed. **Results:** TLV-BAL was associated with better short-term toleration of the lavage procedure, a significantly higher arterial oxygen tension and lower ventilatory support than S-BAL. Other outcomes were comparable except for a slight increase of mean pulmonary arterial pressure during TLV. There was better meconium recovery with TLV-BAL (43 ± 14 vs. $28 \pm 10\%$; $p = 0.022$). Lung histological analysis showed no difference between total scores. **Conclusions:** TLV using an advanced TLV device enables a more effective and better tolerated short-term lavage procedure than BAL with a diluted surfactant (S-BAL) in a severe acute MAS newborn lamb model. These results open the way for a clinical trial.

14

First Report of a Newborn Rat Ventilation Model for Bronchopulmonary Dysplasia Permitting Evaluation of Long-Term Outcome

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Background: Bronchopulmonary dysplasia (BPD) remains the leading cause of chronic pulmonary morbidity among pre-term neonates. However, the exact pathophysiology is still unknown. Here we present the first results from a new model inte-

grating the most common risk factors for BPD (lung immaturity, inflammation, mechanical ventilation (MV), oxygen), which allows long-term outcome evaluation due to a non-traumatic intubation procedure. **Objectives:** To test the feasibility of a new rat model by investigating effects of MV, inflammation and oxygen applied to immature lungs after a ventilation-free interval. **Methods:** On day 4, 5, or 6 newborn rats were given an intraperitoneal injection of lipopolysaccharides to induce a systemic inflammation. 24 h later they were anesthetized, endotracheally intubated and ventilated for 8 h with 60% oxygen. After weaning of anesthesia and MV the newborn rats were extubated and returned to their mothers. Two days later they were killed and outcome measurements were performed (histology, quantitative RT-PCR) and

compared to animals investigated directly after MV. **Results:** Directly after MV, histological signs of ventilator-induced lung injury were found. After 48 h, the first signs of early BPD were seen with delayed alveolar formation. Expression of inflammatory genes was only transiently increased. After 48 h genes involved in alveolarization, such as matrix metalloproteinase-9 and tropoelastin, showed a significant change of their expression. **Conclusion:** For the first time we can evaluate in a newborn rat model the effects of MV after a ventilation-free interval. This allows discrimination between immediate response genes and delayed changes of expression of more structural genes involved in alveolarization.