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Abstracts

1
Surfactant Treatment during Continuous Positive Airway Pressure – A Safe Alternative for Moderately Preterm Infants

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Background: Mechanical ventilation (MV) is one of the cornerstones in the treatment of preterm infants with respiratory distress syndrome (RDS). However, MV also contributes to lung injury and is one of the major risk factors for later development of bronchopulmonary dysplasia (BPD). A treatment strategy with surfactant administration during nasal continuous positive airway pressure (nCPAP) significantly reduces the number of preterm infants with RDS requiring MV. **Objective:** To evaluate the effects of implementing surfactant administration during a short intubation, INSURE (i.e. INtubation SURfactant Extubation) in combination with nCPAP for moderately preterm infants with RDS. **Methods:** We conducted a retrospective, descriptive study of two neonatal units in Stockholm, Sweden (Karolinska Solna, KS and Karolinska Huddinge, KH) over a 10-year period (1993–2002) during which the INSURE-strategy was implemented at one unit (KH) in 1998. All inborn infants during 1993–2002 with gestational age ≥ 27 to <34 weeks and RDS (n = 420) were included in the study. During 1998–2002, INSURE-treated infants receiving Curosurf® (n = 42) were compared to infants receiving surfactant in conjunction with MV (Surf + MV, n = 67). **Results:** Following the implementation of INSURE at KH the number of infants requiring MV was reduced by 50% ($p < 0.01$). At KS, the rate of MV remained unchanged over the same time period. During 1998–2002, only 17% of INSURE-treated infants required more than 1 dose of surfactant compared to 58% of infants receiving Surf + MV ($p < 0.01$). Improvement in oxygenation (a/A PO_2 ratio) was more pronounced and sustained after INSURE compared to the Surf + MV group. The incidence of BPD was similar in both groups. **Conclusion:** The need for mechanical ventilation can be effectively reduced by implementing a treatment strategy of surfactant administration during a brief intubation in moderately preterm infants on nCPAP and is associated with a decreased number of surfactant doses and no adverse effects on outcome.

2
Unexplained Respiratory Distress in Term Neonates Caused by Hereditary Abnormalities of Pulmonary Surfactant

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Background: Progressive respiratory distress in full-term newborn infants may be due to surfactant protein B (SP-B) deficiency, an autosomal recessive disease caused by mutations of the gene encoding SP-B *(SFTPB)*. Mutations in the gene encoding ABCA3 *(ABCA3)* have recently been recognized to result in the same phenotype of fatal respiratory distress in term newborns. **Objective:** To determine the relative contributions of *SFTPB* and *ABCA3* mutations causing unexplained, fatal respiratory distress in term or near term neonates. **Methods and Patients:** 12 term newborn infants with severe unexplained respiratory distress were evaluated from 1998 through 2004. All died within two months despite maximal supportive therapy, including mechanical ventilation, supplemental surfactant, high frequency oscillatory ventilation, and inhaled nitric oxide. Two infants had a family history of fatal neonatal lung disease. DNA analysis was performed with a DHPLC (Denaturing High-Performance Liquid Chromatography) and di-

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rect sequencing protocol for *SFTPB* and by direct sequencing for *ABCA3*. When possible, light microscopy, immunohistochemical staining for SP-B and electron microscopy were performed on lung tissue. **Results:** Mutations in *SFTPB* were found in 2 infants and mutations in *ABCA3* in 5, with novel *ABCA3* mutations identified. The 2 infants with SP-B deficiency showed alveolar proteinosis at lung optical microscopy and the absence of SP-B at immunohistochemistry of the lung. Lung of infants with ABCA3 deficiency showed type II pneumocytes containing altered lamellar bodies and dense inclusions at electron microscopy. **Conclusions:** 58% term newborn infants with irreversible lung disease of unknown etiology in this series had genetic abnormalities of pulmonary surfactant identified. *ABCA3* mutations were a more frequent cause of this phenotype than *SFTPB* mutations. We speculate that other as yet unidentified genetic mechanisms may be responsible for the fatal lung disease in the infants in whom *SFTPB* and *ABCA3* mutations were not found.

3

High Frequency Oscillatory Ventilation vs. Conventional Ventilation: Effect on Pulmonary Surfactant Disaturated Phosphatidylcholine Kinetics in Preterm Infants with Respiratory Distress Syndrome

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Background: Early high frequency oscillatory ventilation (HFOV) in comparison with conventional ventilation (CV) decreases exogenous surfactant requirements [Plavka et al., Intensive Care Med 1999;25:68–75; Moriette et al., Pediatrics 2001;107: 363–372]. This clinical finding can be explained by one, or by a combination of the following: (a) enhanced synthesis, (b) reduced catabolism, (c) surfactant deficiency masked by the high mean airway pressure of HFOV. Disaturated phosphatidylcholine (DSPC) synthesis was found not to be different during HFOV and CV in neonatal respiratory distress syndrome (RDS) using ^{13}C-glucose as tracer [Merchak et al., J Pediatr 2002;140:693–698]. Hypotheses (b) and (c) remain untested. **Objective:** Using a newly developed method [Torresin et al., Am J Respir Crit Care Med 2000;161: 1584–1589] employing the stable isotope dipalmitoylphosphatidylcholine (^{13}C-DPPC) as intra-tracheal tracer, we studied surfactant DSPC half life (HL) in preterm infants with RDS, on HFOV or CV. **Methods:** We studied 18 preterm infants all treated with exogenous surfactant soon after birth, and randomly assigned to elective HFOV (n = 8, birth weight (BW) 692 ± 223 g, gestational age (GA) 26 ± 2.8 weeks) or CV (n = 10, BW 772 ± 165 g, GA 26 ± 1.9 weeks). Ventilator settings and gas exchange indices were recorded hourly. DSPC kinetics were calculated from the ^{13}C enrichment curves of DSPC-palmitate from serial tracheal aspirates, measured by gas-chromatography mass spectrometry. Study variables are given as mean and SD and compared by t test and non-parametric tests where appropriate. $p < 0.05$ was regarded as statistically significant. **Results:** The two groups were similar with regards to all demographic and clinical variables, including severity of respiratory status, prenatal steroids and amount of exogenous surfactant. DSPC HL was computable in all study infants except one in the HFOV group who was extubated 18 h after start of the study. Mean (± SD) DSPC HL was significantly different between HFOV (59 ± 25 h) and CV (40 ± 13 h) $p = 0.03$. **Conclusion:** HFOV in preterm infants with RDS resulted in significantly longer DSPC HL compared to infants treated with CV. We speculate that HFOV spares surfactant in neonatal RDS. Hypothesis (c) remains to be tested.

4

Resuscitation with 100% O_2 Increases Pulmonary Matrix Metalloproteinases and Interleukin-8 in Newborn Hypoxic Piglets

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Background: Pulmonary damage, as a result of oxygen exposure, is an important clinical complication in infants who require hyperoxia as treatment. Matrix metalloproteinases (MMPs) play a role in pulmonary extracellular matrix remodelling and degradation after ischemia-reperfusion. **Objective:** To assess pulmonary injury in newborn piglets undergoing global hypoxia and subsequent resuscitation with 21 or 100% O_2 by MMP activity, oxidative stress and interleukin (IL)-8 2.5 h after resuscitation. **Material and Methods:** Hypoxic newborn piglets (12–36 h of age) were resuscitated for 30 min by ventilation with 21 or 100% O_2, and thereafter observed for 150 min. In pulmonary tissue extracts, MMP activity was analysed by broad matrix-degrading capacity (total MMP) and zymography. MMP-2 mRNA expression was evaluated by quantitative real-time PCR. Total endogenous antioxidant capacity was measured by the oxygen radical absorbance capacity (ORAC) assay, and IL-8 was analysed by ELISA technique. In bronchoalveolar lavage (BAL) fluid, MMPs were analysed by zymography. **Results:** In pulmonary tissue, pro- and active-MMP-2 were increased in piglets resuscitated with 100% O_2 compared to 21% O_2 ($p < 0.05$). Pro-MMP-9, total MMP activity and MMP-2 mRNA levels were significantly increased in resuscitated piglets compared to baseline ($p < 0.05$). Net gelatinolytic activity increased in submucosa and blood vessels after 100% O_2 ($p < 0.05$), and only in the blood vessels after 21% O_2. Compared to baseline, ORAC values were considerably lowered in the resuscitated piglets ($p < 0.05$) and significantly reduced in the 100% O_2 versus ambient air group ($p < 0.05$). In BAL fluid, pro-MMP-9 and pro-MMP-2 increased two-fold in the 100% O_2 group compared to 21% O_2 ($p < 0.05$). IL-8 concentration increased significantly in piglets resuscitated with 100% O_2

compared to ambient air ($p < 0.05$) suggesting a marked pro inflammatory response in the tissue. **Conclusion:** Resuscitation of piglets with 100% oxygen is detrimental to pulmonary tissue compared to ambient air. Care must be taken when giving pure oxygen to newborn infants.

5
Surfactant Associated Protein B Kinetics Studied in vivo in Newborn Infants by Stable Isotopes

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Background: Surfactant protein B (SP-B) is critical to the biophysical function of pulmonary surfactant. No information is available on SP-B synthesis and kinetics in humans. **Objective:** To describe a new method to measure SP-B kinetics in vivo in humans by means of stable isotopes. **Design/Methods:** We administered a 24 h infusion of ^{13}C-valine as a metabolic precursor of SP-B to 6 newborn infants (weight 3.5 ± 0.5 kg and age 12 days, range 1–43 days). Three of the study infants also received ^{2}H-palmitate to label surfactant disaturated phosphatidylcholine (DSPC). SP-B and DSPC were isolated from tracheal aspirates and their respective ^{13}C and ^{2}H enrichments were measured by gas chromatography-mass spectrometry. **Results:** SP-B kinetics were successfully measured in all 6 infants. SP-B median (range) fractional synthesis rate (FSR) was 30% per day (20–78% per day), secretion time 4.5 h (1–9 h), time to peak 24 h (12–36 h) and half-life 21 h (8–35 h). The ascending part of SP-B kinetic curve was similar to the DSPC curve, suggesting similar secretion pathways. SP-B half-life appeared to be shorter than DSPC half-life. These results agree with existing animal data. **Conclusions:** We conclude that the measurement of SP-B kinetics is feasible in vivo in humans by stable isotope technology.

6
Measurement of Surfactant Disaturated Phosphatidylcholine Synthesis in Infants Using Deuterium Incorporation from Body Water

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Background: Synthesis of disaturated phosphatidylcholine (DSPC) has been measured in infants using carbon thirteen (^{13}C) labelled glucose, by measuring the ^{13}C incorporation into DSPC-palmitate from tracheal aspirates (TAs) in infants requiring mechanical ventilation [Bunt et al., Am J Respir Crit Care Med 1998; 157:810–814]. A constant 24 h intravenous (IV) infusion of ^{13}C-glucose is required. Palmitate synthesis has been measured in animals and humans using the incorporation of deuterium from body water [Ajie et al., Am J Physiol 1995;269:247–252]. Body water can be labelled by IV or oral administration of heavy water ($^{2}H_2O$) and the isotopic enrichment measured in urine, thus avoiding blood sampling. **Objective:** to study if $^{2}H_2O$ could be used as a tracer to measure DSPC synthesis in infants. **Methods:** $^{2}H_2O$ was given to 8 infants ventilated for respiratory failure, 4 were term newborns (weight 3.3 ± 0.8 kg, gestational age 38.8 ± 2.1 weeks, postnatal age 7.3 ± 9.9 days) and 4 preterms (weight 1.3 ± 0.6 kg, gestational age 30.3 ± 2.5 weeks, postnatal age 8.8 ± 9.2 h). Dosing of the tracer: 0.44 g $^{2}H_2O$/kg as an IV bolus followed by 0.0125 g q 6 h for 48 h. Urine and TAs were obtained prior to dosing and every 6 h thereafter. Isotopic enrichment of urinary water and of TAs-DSPC were analysed by isotope ratio mass-spectrometry. The enrichments of urinary water *(precursor molecule)* and of DSPC *(product)* were normalized for SMOW (reference standard mean ocean water). DSPC fractional synthesis rate (FSR) was obtained by dividing the slope of the linear portion rise of deuterium in DSPC over time by the plateau enrichment value of urinary water. Secretion time (ST) was defined as the time lag between the first $^{2}H_2O$ dosing and the appearance of deuterium in DSPC. Data are given as mean ± SD. **Results:** In all 8 study infants the isotopic enrichment of deuterium in urine was in steady state. Isotopic enrichment in DSPC-palmitate could be reliably measured in all the TAs. DSPC FSR was 6.5 ± 2.9%/day (range 2.6–10.2). FSR for the term infants was 6.6 ± 3.5%/day and 7.3 ± 2.1%/day for the preterm infants. DSPC-ST was 10 ± 10 h in the term and 31 ± 10 h in preterm infants. The latter were all treated with exogenous surfactant. In none of the 8 infants could a plateau of DSPC be identified within 60 h from the first $^{2}H_2O$ dosing. **Conclusion:** Surfactant synthesis can be measured in humans by the incorporation of deuterium from body water. Preliminary results are in line with previous results of our glucose studies. Measurement of surfactant DSPC by this method may prove to be simpler and less invasive than by previously published methods.

7
Surfactant Prophylaxis in Extremely Low Birth Weight Infants: A Survey of Practice in Italy

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Background: There is a lack of information regarding surfactant prophylaxis and resuscitation practice at birth in extremely low birth weight infants (ELBWI). This high-risk population could have better outcomes if optimally managed. **Objective:** To survey surfactant prophylaxis and resuscitation management of ELBWI in Italian tertiary centres. **Methods:** A structured 73-item questionnaire regarding equipment and practice of the centre was sent to the heads of the 86 Italian neonatal intensive care units with on-site

delivery. **Results:** Information was obtained for 74 (86%) centres, in which a total of 1,294 (median 15; range 1–63%) ELBWI were born in 2002. One hundred percent oxygen resuscitation is used in 41 (55.4%) centres; in the remaining 33 (44.6%), varying oxygen concentrations (median 40%; range 21–70) are provided. Positive pressure ventilation (PPV) is manually administered at 61 (82.4%) centres; at the remaining 13 (17.6%), PPV is administered by using a ventilator. Positive end expiratory pressure (PEEP) is routinely used at 26 (35.1%) centres and delivered tidal volumes are measured at 5 (6.7%). Thirty centres (40.5%) routinely administer surfactant in the delivery room. Among them, 17 (56.7%) administer surfactant therapy to all preterm infants with gestational age <28 weeks (range 26–34); the remaining 13 (43.3%) treat all ELBWI needing intubation for resuscitation. Natural surfactant (Curosurf®) is used in all centres at a dose of 100 mg/kg (26.7%) or 200 mg/kg (73.3%). After surfactant administration, ELBWI remain intubated in the majority of centres (86.7%); the INSURE technique is limited to 13.3% of the centres. During 2002, 791 (73.7%) out of 1072 ELBWI born at the 68 responding centres were intubated at birth. In the same period, 230 (21.6%) and 93 (8.7%) out of 1061 ELBWI born at the 66 responding centres received chest compressions and medications, respectively. **Conclusions:** This survey shows that surfactant prophylaxis and approach to neonatal resuscitation of ELBWI vary considerably, reflecting a paucity of evidence and consequent uncertainty among clinicians. Further prospective research in this field is needed.

8
Changing Pattern of Surfactant Use for Extremely Preterm Infants over the Past Decade in Belfast

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Background: Surfactant administration for extremely preterm babies should be as early as possible and mechanical ventilation must be gentle and for the shortest possible duration. We wanted to examine how effective we are at achieving this in Belfast and to compare practice now with that of 10 years ago. **Objective:** To describe recent practice of surfactant administration in Belfast for extremely preterm babies born in 2004 and admitted to the Regional Neonatal Unit and compare it with what happened a decade previously in 1994. **Methods:** This was a retrospective study collecting data on babies <30 weeks' gestation born between 1st January and 31st December 2004 compared with similar babies born between 1st January and 31st December 1994. Data were collected on numbers receiving surfactant, timing of first surfactant dose, duration of mechanical ventilation, use of continuous positive airway pressure (CPAP) and oxygen supplementation and their durations, length of hospital stay as well as mortality and respiratory outcome. **Results:** There were 412 admissions in 1994 of which 66 (16%) were less than 30 weeks' and in 2004 there were 495 admissions with 88 (18%) less than 30 weeks' gestation (table 1). Mortality of babies <30 weeks' in 1994 was 20 (30%) compared with 18 (20%) in 2004 (p = 0.15). **Conclusion:** In 2004 more very preterm babies received prophylactic or early rescue surfactant than in 1994. Mortality rate has fallen but this has occurred at the expense of more chronic lung disease, although length of stay has not increased.

Table 1.

	1994 (n = 66)	2004 (n = 88)	p value
Received surfactant, n (%)	23 (35)	83 (94)	<0.0001
Time of first dose (min), med (IQR)	180 (122–234)	9 (7–18)	<0.0001
Gestation (weeks), med (IQR)	26 (25–28)	26 (25–28)	<NS
Ventilator days, survivors, med (IQR)	1 (0–4)	7 (3–25)	<0.001
CPAP days, survivors, med (IQR)	5 (1–15)	15 (8–25)	<0.001
Survivors in oxygen at 28 days, n (%)	33 (50)	67 (76)	<0.01
Survivors in oxygen at 36 weeks, n (%)	11 (17)	48 (54)	<0.01
Received postnatal dexamethasone, n (%)	15 (23)	2 (2)	<0.0001
Length of stay (days), med (IQR)	65 (44–73)	52 (32–71)	<NS

9
Changes in Oxygen and Ventilatory Support following Reduced Use of Postnatal Steroids for Chronic Lung Disease in Preterm Infants

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Background: Steroid therapy reduces the incidence and severity of chronic lung disease (CLD) in preterm infants, without affecting mortality. As recent studies have correlated this therapy with increased risk for cerebral palsy and developmental delay, current guidelines favor limiting steroid use to 'exceptional clinical circumstances'. **Objective:** We hypothesized that there has been a significant reduction in steroid use since emergence of the adverse effects (1997–1998) and that this reduction has been associated with changes in the pattern of oxygen and ventilatory support in preterm infants at risk for CLD. **Methods:** The study sample (n = 6443) was taken from the Israel National VLBW Neonatal database. The sample included infants born in 1997–2001, with gestational age (GA) 24–32 weeks, without congenital malformations and who required ventilatory support. The sample was divided into 3 eras for comparison – 1997–1998 (era 1 – peak use), 1999–2000 (era 2) and 2001–2002 (era 3 – expected reduction). Univariate analyses and multivariate logistic regression models were constructed in order to account for variations in confounding variables. **Results:** As expected, there has been a significant reduction in steroid use from 23.4% in 1997–1998 to 13.1% in 2001–2002. After adjustment for relevant confounding variables (GA, antenatal steroids, multiple births, mode of delivery, etc), the odds ratio for O_2 requirement at 28 days in era 3 vs. era 1 was 1.30 (95% CI 1.07–1.58) and for O_2 requirement at 36 weeks 1.45 (95% CI 1.17–1.79).

The mean duration of O_2 therapy increased from a mean of 24.7 days in era 1 to 28.5 days in era 3. There was a trend to increased survival from 81.5% in era 1 to 83.4% in era 3. After adjustment, the requirement for any ventilatory support was unchanged in the 3 eras (1:14.1 days; 2:14 days; 3:14.7 days), while the subset of use of nasal CPAP increased significantly from a mean of 2.8 days in era 1 to 4.3 days in era 3 ($p < 0.05$). **Conclusions:** The use of steroid therapy in preterm infants has fallen significantly since the publication of adverse effects of this therapy. This change has been temporally associated with a modest increase in the duration of oxygen requirement. However, over the same period of time, there has been a marked increase in the use of nasal CPAP and a relative reduction in endotracheal mechanical ventilation. Thus, the concomitant trends of reduced steroids and increased nasal CPAP may interact in determining the duration of oxygen therapy.

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Recruitment Pressures during Open Lung High-Frequency Ventilation in Preterm Infants with Respiratory Distress

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Background: Recruitment and subsequent stabilization of collapsed alveoli (open lung ventilation) seems to be crucial in the attenuation of secondary lung injury during high-frequency ventilation (HFV). Most clinicians use oxygenation as an indirect tool to monitor alveolar recruitment, defining optimal recruitment as a fractional inspired oxygen concentration (FiO_2) below 0.30. Data on the feasibility of this approach and the recruitment pressures needed to optimize lung volume are scarce. **Objective:** To determine recruitment and stabilization pressures of collapsed alveoli and to establish the oxygen need following this procedure in preterm infants with respiratory distress syndrome (RDS). **Methods:** Preterm infants with RDS needing HFV were prospectively included. Prior to surfactant treatment the lung was recruited by stepwise increasing the continuous distending pressure (CDP) until oxygenation no longer improved or the FiO_2 was ≤ 0.25 (CDP_O). Next, the CDP was stepwise decreased until oxygenation deteriorated (CDP_C). The lung was once again opened and the CDP was set 2 cm H_2O above CDP_C (optimal CDP, OCDP). The transcutaneous saturation was kept between 86–94% at all times. Both airway pressures and FiO_2 were recorded. **Results:** A total of 36 preterm infants with a mean gestational age of 28.8 ± 2.3 weeks and a birth weight of 1,206 ± 433 g were included. The mean CDP_O and CDP_C were respectively 21.0 ± 4.5 and 13.0 ± 4.1 cm H_2O, resulting in an OCDP of 15.1 ± 4.6 cm H_2O. Optimizing lung volume resulted in a significant drop in FiO_2 from 0.63 ± 0.26 at the start of recruitment to 0.25 ± 0.03 at OCDP ($p < 0.001$). There was a positive correlation between birth weight and CDP_O ($r = 0.48$, $p < 0.01$). **Conclusion:** This is the first study that provides data on recruitment pressures during HFV of preterm infants with RDS. In addition, this study shows that optimal recruitment results in a FiO_2 below 0.30 in the majority of infants.

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Standard Prenatal Steroid Treatment Does Not Alter Cytokine and Internal Surface Area Measurements in Baboon Lungs of Varying Gestational Ages

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Background: Antenatal glucocorticoid (GC) treatment is the standard of care for women at risk for preterm delivery. In sheep lungs, antenatal GC treatment decreased lung mesenchyme and increased air space volume within 24 h, and decreased saccular septation by 7 days after delivery and ventilation [Jobe AH, Clin Obstet Gynecol 2003;46:174–189]. **Objective:** In the 125-day baboon model of bronchopulmonary dysplasia (BPD), a single course of GC (6 mg × 2) is used to mimic human standard of care. We hypothesized that baboon dams treated at 123 days of gestation (term 186 days) with antenatal GC, and then delivered by caesarean section at 125, 146 and 153–156 days, would yield fetuses with lungs that would show differences in alveolar development when compared to non-treated controls (NT) at the same study times. **Methods:** Four to six lungs per study group were comparably fixed, sectioned and morphometrically assessed for internal surface area (ISA) measurements. Bronchoalveolar lavage fluids (BALF) were obtained at necropsy and IL-6 and IL-8 levels were determined by RIA and ELISA. **Results:** ISA values were not significantly different nor were BALF levels of IL-6 and IL-8 significantly different in the 125 d-NT- vs. 125 d-GC-treated study groups. Similarly, at 146 and 153–156 days gestation, the values for ISA and BAL IL-6 and IL-8 values were again not significantly different between the NT- and GC-treated lungs. Body and lung weights were not significantly different among the fetuses within the paired study time groups. **Conclusion:** A single standard course of GC given to the baboon dam at the equivalent of 25 weeks' human gestation does not result in delayed in vivo fetal ISA expansion during critical lung developmental stages.

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Effects of Silicone Oil on Modified Pulmonary Surfactant in vitro and in vivo

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Background: Silicone oil is used for lubrication of 3 part syringes and up to 69% of it can be dissolved by rinsing the syringes with water or soybean oil [Capes et al., PDA J Pharm Sci Technol 1996;50:40–50]. Surface activity of modified porcine surfactant

(Curosurf®) is reduced after contact with 3 part syringes [Herting et al., Lancet 2003;361:311–313]. **Objective:** To investigate the effects of silicone oil on pulmonary surfactant in vitro and in vivo. **Methods:** Molecular composition of silicone oil (B. Braun, Germany), diluted 800-fold in organic solvents, was analysed using electron-spray time of flight mass spectrometry. 6 surfactant preparations were made up from Curosurf®, 80 mg/ml, by addition of 0, 0.1, 0.3, 1, 3 and 10% silicone oil (vol/vol). In vitro surface activity of 32-fold diluted surfactant containing silicone oil was investigated in a pulsating bubble surfactometer. Surface tension at minimum bubble size (50% surface compression) of preparations containing 0, 1 and 10% silicone was recorded after 5 min of pulsation at 37°C at a frequency of 20/min (n = 5 repeated experiments). In vivo effects of surfactant preparations were investigated in immature newborn rabbits. Animals were obtained by hysterotomy, anesthetised, tracheotomised, paralysed and treated intratracheally with different surfactant preparations. Non-treated animals served as controls. All pups were ventilated in a multiplethysmograph system using an insufflation pressure sequence of 35 cm H_2O for 1 min, 25 cm H_2O for 15 min, 20 cm H_2O for 5 min, 15 cm H_2O for 5 min and finally 25 cm H_2O for 5 min. Individual tidal volume curves were recorded at 5-min intervals. The pups were then killed and lungs were excised for determination of end-expiratory gas volume [Scherle, Mikroskopie 1970;26:57–60]. **Results:** Mass spectra showed peaks of series with m/z differences of 74, which could be related to 2 series of polydimethylsiloxanes (PDMS) and are congruent with linear and cyclic PDMS. In vitro surface activity was significantly reduced after addition of 1% silicone oil. In vivo experiments showed no differences in tidal volumes and compliance (calculated by dividing tidal volume with peak insufflation pressure) between groups treated with surfactant containing 0–10% silicone oil. In contrast lung gas volumes were significantly decreased in surfactant-treated groups with $\geq 3\%$ silicone oil ($p < 0.01$). Macroscopically the lungs treated with silicone oil had multiple atelectatic areas. **Conclusions:** Silicone oil used for lubrication of 3 part syringes is mainly composed of PDMS and can inactivate modified pulmonary surfactant in vitro. In immature animals treated with surfactant containing $\geq 3\%$ silicone oil lungs with multiple atelectatic areas and reduced end-expiratory lung gas volumes were observed.

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Effects of Surfactant, Inhaled Nitric Oxide and Positive End-Expiratory Pressure in Premature Piglets with Hypoxemia

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Background: It is important to use an adequate level of positive end-expiratory pressure (PEEP) in ventilated premature lungs for optimal oxygenation and ventilation efficiency. **Objective:** To compare effects of low and high PEEP in premature lungs treated with surfactant and/or inhaled nitric oxide (iNO). **Methods:** Newborn piglets of 85% gestation (term = 114 days), 0.5–1.0 kg, were born by cesarean section and randomly subjected to mechanical ventilation for 6 h with tidal volume 6–8 ml/kg, with either 5–6 (low, L) or 10–12 (high, H) cm H_2O PEEP, and treated in groups (n = 6) with porcine surfactant (100 mg/kg, S), iNO (10 ppm, NO), both surfactant and iNO (SNO), or not treated as controls (C). Values are given as mean ± SD. **Results:** All animals had hypoxemia (PaO_2/FiO_2, 114 ± 36 mm Hg) and low compliance of the respiratory system (Crs, 0.53 ± 0.13 ml/cm H_2O/kg) before randomization, with no significant difference across the groups. Both L-NO and L-SNO groups had dramatic improvements in PaO_2/FiO_2 (465 ± 142, 434 ± 158) and Crs (0.91 ± 0.30, 0.84 ± 0.13) compared to moderate or no effects in the L-S and L-C groups on PaO_2/FiO_2 (276 ± 274, 95 + 123) and Crs (0.54 ± 0.14, 0.87 ± 0.25), respectively. There was moderate improvement of PaO_2/FiO_2 and Crs in the H-NO and H-SNO groups but not in the H-C and H-S groups. Oxygenation index and ventilation index were better in the L-groups whereas H-groups had lower resistance of respiratory system. Variable but improved alveolar disaturated phosphatidylcholine to total protein ratio, minimum surface tension of lung lavage fluid, alveolar aeration, and histological evidence of alleviated lung injury were found in L-SNO, L-S, H-SNO and H-S groups compared to the L-C, L-NO, H-C and H-NO groups. L-SNO and H-SNO had the best effects on oxygenation, lung compliance and alleviation of lung inflammation and injury. NO synthase activity in the lungs was not affected in any of the groups. **Conclusions:** In the ventilated immature animals blood oxygenation and lung function in the presence of iNO or iNO and surfactant may be optimized by low PEEP, and to a lesser degree with high PEEP.

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Structure-Function Correlation for Mini-B Peptide Based on the N- and C-Terminal Domains of Surfactant Protein B

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Background: Surfactant protein B (SP-B) is important for the normal functioning of lung surfactant. Early theoretical SP-B models predicted that the disulfide cross-linked, N- and C-terminal domains fold as charged amphipathic helices, and suggested that these adjacent helices may participate in critical surfactant activities. **Objective:** Here, this hypothesis is tested using a disulfide-linked construct (Mini-B) based on the primary sequences of the N- and C-terminal domains of SP-B. **Methods:** The oxidized Mini-B peptide consists of 34 residues: residues 1–18 correspond to the N-terminal residues 8–25 of full-length human SP-B, residues 19–34 correspond to the C-terminal SP-B residues 63–78. Structural studies included Fourier Transform Infrared (FTIR) spectroscopy and molecular modeling. Mini-B peptide was compared to porcine

SP-B and Mini-B variants and fragments. For all preparations, 0.1 mmol of protein or peptide was mixed with 35 mg total phospholipids consisting of 16 mg of DPPC, 10 mg of DOPC, 3 mg of POPG, 1 mg of POPE, 3 mg of POPS and 2 mg of cholesterol in 1 ml of PBS. In vitro surface activity was investigated using a modified Langmuir/Wilhelmy balance; in vivo function was tested in surfactant-deficient, lavaged rats. **Results:** Both isotope-enhanced FTIR spectroscopy and molecular modeling indicated charged amphipathic α-helices for the N- and C-terminal domains in the Mini-B peptide. Mini-B exhibits in vitro surface activity and produces alterations in the molecular topography of surfactant-lipid dispersions, similar to those achieved by native SP-B. Moreover, in vivo studies of Mini-B show oxygenation and dynamic compliance that compare favorably with those achieved by full-length SP-B. Either Mini-B variants (i.e., reduced disulfides or cationic residues replaced by uncharged residues) or Mini-B fragments (i.e., unlinked N- and C-terminal domains) produced greatly attenuated in vivo and in vitro surfactant properties. **Conclusion:** These findings indicate that the unique charge distribution in amphipathic helical N- and C-terminal domains is involved in the function of native SP-B in surfactant lipids.

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Effects of Synthetic Pulmonary Surfactant with Different Contents of SP-C33

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Background: We have previously investigated a physiologically active synthetic surfactant containing dipalmitoylphosphatidylcholine (DPPC)/palmitoyloleoylphosphatidylglcyerol (POPG) (68:31 w/w) and 2% (w/w) of the SP-C analog, SP-C33. This surfactant increases lung compliance in preterm rabbit fetuses to levels similar to those obtained with commercially available modified natural surfactant preparations and stabilizes the alveoli in animals ventilated with a positive end-expiratory pressure (PEEP) of about 3 cm H_2O. **Objective:** To investigate how the SP-C33 concentration in the synthetic surfactant influences the in vitro and in vivo activity. **Methods:** Synthetic SP-C33, 0–2% w/w, was mixed with phospholipids in organic solvents, evaporated to dryness under N_2 and resuspended in saline. Dynamic surface properties of the surfactant preparations were measured in triplicate at a concentration of 10 mg/ml using a pulsating bubble surfactometer (PBS) and a captive bubble surfactometer (CBS). The PBS experiments were performed using our conventional protocol except that the sample chamber was first filled with a solution of 10% sucrose in saline whereupon the surfactant suspension was injected into the chamber on top of the sucrose solution. Values for minimum and maximum surface tension (γ_{min}, γ_{max}) were recorded after 5 min of pulsation at 37°C at a frequency of 20/min. In the CBS experiments the chamber was first filled with 10% sucrose solution, followed by injection of approximately 2 μl of surfactant. Physiological properties were studied in immature newborn rabbits. Animals were tracheotomized at birth, treated with surfactant (80 mg/ml, 2.5 ml/kg) and ventilated for 30 min with a standardized sequence of insufflation pressures (P) and a PEEP of about 3 cm H_2O. Lung-thorax compliance was calculated from recordings of tidal volume and P minus PEEP. Lung gas volumes (LGV) were measured after the experiment. Curosurf-treated and non-treated animals were used as positive and negative controls, respectively. **Results:** The in vitro studies with PBS showed that a SP-C33 content of at least 1.0% was required to obtain a γ_{min} of <5 mN/m. Similar results were obtained in the CBS. Thus, artificial surfactants with 0 or 0.5% SP-C33 showed median γ_{min} values of 11 and 13 mN/m, and γ_{max} of 45 and 39 mN/m, respectively. For surfactants with 1.0, 1.5 and 2.0% SP-C33 γ_{min} was 3, 2 and 1 mN/m and γ_{max} 39, 37 and 30 mN/m, respectively. In the in vivo experiments animals treated with 0, 1.0 and 2.0% SP-C33 in DPPC/POPG had median compliance values of 0.37, 0.82 and 0.89 ml/cm H_2O/kg, respectively, after 30 min of ventilation. Animals receiving Curosurf had a median compliance of 0.90 ml/cm H_2O/kg and negative controls 0.04 ml/cm H_2O/kg. Median lung gas volumes of groups treated with 0, 1 and 2% SP-C33 were 3, 16 and 13 ml/kg, respectively. Corresponding values for Curosurf-treated and non-treated animals were 28 and 0 ml/kg. **Conclusions:** The presence of SP-C33 is required to obtain a physiologically active synthetic surfactant. Nearly equivalent improvement in compliance and LGV could be reached with 1 and 2% of SP-C33. However, we speculate that a peptide content of 2% may provide a safety margin that could be required for treatment of conditions with inactivation of surfactant caused by large leak of inhibitory plasma proteins into the airspaces.

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Dynamic and Spatial Behavior of Synthetic Phospholipids and Commercial Lung Surfactants Investigated in Repeated Compression – Expansion Cycles of a Langmuir Trough

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Background: Pulmonary lung surfactant (LS) is an essential substance in the mechanics of the breathing process. LS is considered to spread at the air-liquid interface of the alveoli as a mixed monolayer of phospholipids and protein molecules. Our observations indicate that the supramolecular structure of LS at the interphase might be more complex. **Objective:** To study supramolecular, in- and out-of-plane, organization of LS and synthetic surfactant phospholipids at the air-liquid interphase along compression-expansion cycles. **Methods:** The dynamic surface properties of LS and a 4:1 mixture of dipalmitoylphosphatidylcholine and palmitoyloleoylphosphatidylglycerol (DPPC:POPG) were studied on a Langmuir balance and film organization was further analyzed with a Brewster Angle Microscope (BAM) and with atomic force microscopy (AFM). **Results:** AFM analysis of lung surfactant and its artificial analogs indicates that during the compression-expan-

sion cycles that simulate the respiratory cycle, the phospholipid mixture behaves in a different manner from exogenous LS. DPPC:POPG is compressed into several organized patterns: at low surface pressure the DPPC:POPG film is in a coexistence of 'liquid expanded' (LE) phase with higher density regions, the 'liquid condensed' (LC) phase. Upon compression the LC regions grow, and eventually at 40 mN/m the LC monolayer islands coalesce until they collapse and form multilayers. This conformation is partly irreversible upon expansion. AFM observations suggest that the LS (Curosurf®, Infasurf®) behave differently and LC islands that form at low surface pressure do not coalesce to form a continuous monolayer and multilayers. Rather, a discontinuous LS layer or islands form extrusions at different scales at the center and near the perimeters of the liquid domains. Similar phenomena at different scale (~mm) are also observed by BAM. **Conclusions:** The observed extrusions from the ideal monolayer organization may be interpreted as the center for phospholipid aggregation, which prevent the formation of stable multi-layers and enable a reversible expansion of the layer conformation. The differences between the organization traits of the phospholipid mixtures and the natural LS are attributed to the presence of LS proteins SP-B and SP-C. These observations suggest that LS organizes at the air-water interface by several different mechanisms that together contribute both to the efficient performance in surface pressure reduction and economically efficient redistribution of the surfactant.

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Long-Term Neurodevelopmental and Respiratory Outcomes of Children from UK and Ireland Enrolled in a Trial of Early and Late Postnatal Steroids: Systemic and Inhaled

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Background: Corticosteroids given to preterm babies to treat or prevent chronic lung disease have been associated with negative long-term outcomes, including cognitive deficits and cerebral palsy, but differences between methods or timing of administration have yet to be adequately evaluated, particularly in the long-term. **Objective:** To compare early school-age neurodevelopmental and respiratory outcomes of children, from the UK and Ireland who were treated with either early (<3 days) or late (>15 days) postnatal corticosteroids and to compare corticosteroids administered systemically (dexamethasone) with those given by inhalation (budesonide). **Methods:** One hundred and twenty six survivors from the UK and Ireland (84%) who had been recruited to the OSECT study [Halliday et al., Pediatrics 2001;107:232–240] were assessed at a mean age of 7 years. The factorial design of the original study allowed for comparison of timing and method of administration. The outcome measures assessed were level of disability, presence of cerebral palsy, general cognitive ability, behavioural difficulties and competencies, growth, blood pressure and respiratory symptoms. **Results:** There were no significant differences among the treatment groups for cognitive ability scores, behavioural competencies or difficulties, overall disability rates, cerebral palsy, growth, respiratory morbidity and diastolic blood pressure. Those treated late had a higher proportion of children with systolic blood pressure >95th centile than those treated early (26 vs. 6%; p = 0.006). Timing of postnatal dexamethasone on an 'as-treated basis' was associated with altered growth; those treated late were 3 cm shorter (p = 0.008), 1.6 kg lighter (p = 0.005) and had head circumferences that were 0.5 cm smaller (p = 0.013) at follow-up than those treated early. **Conclusions:** This study indicates that although postnatal steroids may be associated with poor long-term outcomes, altering the method or timing of administration may not significantly affect long-term outcomes.

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The Effect of Bronchoalveolar Lavage plus Supplementary Surfactant in a Piglet Model of Meconium Aspiration

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Background: Meconium aspiration causes obstruction and inflammation of the airways and affects surfactant function. Surfactant administration and brochoalveolar lavage (BAL) are under evaluation. **Objective:** To evaluate the effects of BAL with normal saline followed by supplementary surfactant in a piglet model of meconium aspiration syndrome (MAS). **Design/Methods:** Fifteen newborn piglets were ventilated with fixed settings (FiO_2 1.0, pressure 20/5 cm H_2O, frequency 40/min and inspiration time 0.4 s). After instillation of 4 ml/kg of 20% meconium in normal saline, the piglets were randomized to: group I (n = 5) tracheal aspiration without BAL; group II (n = 5) BAL with 15 ml/kg normal saline; group III (n = 5) BAL with normal saline followed by supplementary surfactant (Curosurf®, 100 mg/kg). Arterial blood gases were obtained prior to and after meconium instillation, 30 min and 6 h after therapeutic intervention. Bronchoalveolar fluid was obtained for total cell count at the end of the study. **Results:** PaO_2 and hemoglobin saturation were significantly higher at 6 h after intervention in groups I and III compared to group II (PaO_2 93 ± 60, 189 ± 115 and 37 ± 11 mm Hg) (p = 0.023), and hemoglobin saturation 77 ± 30%, 95 ± 8% and 41 ± 23%, respectively (p = 0.008). Group III showed a trend to have a higher PaO_2 than group I (p = 0.07). Total cell count was 3,102 ± 784 in group I, 1,266 ± 300 in group II, and 1,452 ± 211 in group III. There was a trend for lower counts in the 2 groups in which BAL was performed (p = 0.06). **Conclusions:** BAL with normal saline followed by supplementary surfactant seems to improve oxygenation in an experimental piglet model of MAS.

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Lung Expression of *Hox* Genes in Nitrofen-Induced Congenital Diaphragmatic Hernia and the Effects of Antioxidant Vitamins

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Background: Exposure of fetal rats to the herbicide nitrofen induces congenital diaphragmatic hernia (CDH) with pulmonary hypoplasia which can be partially prevented by antioxidant vitamins. The *Hox* genes code for a family of 39 transcription factors within four gene clusters. *Hox* genes from A and B clusters have been demonstrated to have roles in regulating lung morphogenesis and abnormal expression of *Hox* genes has been demonstrated in several congenital lung malformations and disorders of lung development. It has been postulated that abnormal expression of *Hox* genes may be involved with the pathogenesis of CDH. **Objective:** To determine the patterns of *Hox* gene expression in nitrofen-exposed fetal rat lungs and to see if antenatal antioxidant vitamins alter this expression. **Methods:** Rat fetuses were exposed to either nitrofen or oil (control) on e9.5 and were subsequently exposed antenatally to saline, vitamin A, vitamin C or vitamin E on e16 to e20. The lungs were recovered on e21 and were processed for measurement of *Hoxa2*, *Hoxa3*, *Hoxa4*, *Hoxa5*, *Hoxa6*, *Hoxb2*, *Hoxb3*, *Hoxb4*, *Hoxb5* and *Hoxb6* by quantitative PCR using validated TaqMan™ primers and probes. **Results:** Nitrofen reduced the expression of *Hoxa2*, *Hoxa4*, *Hoxa6* and *Hoxb3* in comparison with controls (all $p < 0.01$). Treatment with each of the antioxidant vitamins restored expression of *Hoxa2* and *Hoxa4* in nitrofen-exposed fetal rat lungs to the levels of non-exposed lungs. Vitamin treatment had no effect on the reduced expression of Hoxa6 and only vitamin E restored the reduced expression of Hoxb3 in nitrofen-exposed lungs. **Conclusions:** Nitrofen-induced lung hypoplasia in rats with CDH is associated with down-regulation of several *Hox* genes. Prenatal antioxidant vitamin treatment may improve lung hypoplasia by increasing the expression of some of these down-regulated *Hox* genes.

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Curosurf® Increases Phagocytosis of Apoptotic Neutrophils by Alveolar Macrophages in vivo

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Background: Phagocytosis of apoptotic neutrophils by alveolar macrophages is an essential mechanism of the clearance of neutrophils in the lung. Phagocytosis by alveolar macrophages is mediated by annexin V which is expressed by apoptotic neutrophils. Apoptotic neutrophils that are not phagocytosed in a timely fashion become necrotic and are a proinflammatory stimulus themselves. Phospholipids and hydrophobic surfactant proteins have been shown to increase the phagocytosis of apoptotic neutrophils by macrophages in vitro. **Objective:** In this study we tested the effect of Curosurf® on phagocytosis of apoptotic neutrophils by alveolar macrophages in lungs of adult mice. **Methods:** Neutrophils were isolated by Percoll gradient centrifugation. Red blood cells were removed by osmotic lysis. Purity was assessed by staining for myeloperoxidase (MPO) which is expressed in neutrophils. Apoptosis was induced in neutrophils by UV radiation (302 nm) for 30 min. Apoptosis was confirmed by flow cytometry staining for Annexin V and propidium iodide counterstaining. Three-month-old adult male C57/Black 6 mice were anesthetized and intubated. Curosurf in a dose of 200 mg/kg or 50 μl saline was instilled. Subsequently, 10^6 apoptotic neutrophils suspended in 100 μl of saline were injected via tracheal intubation in each mouse ($n = 9$ in each group). Bronchoalveloar lavage (BAL) was performed 30 min after instillation. Alveolar macrophages and neutrophils were counted. Cytospins were stained for MPO to identify phagocytosed apoptotic neutrophils. Phagocytosis of apoptotic neutrophils was quantified by counting the number of MPO positive vesicles in alveolar macrophages and determining the percentage of MPO positive alveolar macrophages. A phagocytic index was calculated by multiplying the percentage of MPO positive macrophages by the average number of phagocytosed vesicles. **Results:** Less than 10% ($3.1 \pm 0.2 \times 10^4$) of the initial number of apoptotic neutrophils were recovered after 30 min by BAL. The phagocytosis index after instillation of apoptotic cells was 3.8 ± 0.9 after saline injection and 7.5 ± 1.1 after Curosurf instillation ($p < 0.05$ vs. saline injection). The number of alveolar macrophages recovered from BAL fluid after instillation of apoptotic neutrophils was $2.4 \pm 0.1 \times 10^5$/kg after saline injection and $20.1 \pm 4.1 \times 10^5$/kg in Curosurf-treated animals ($p < 0.05$ versus saline). **Conclusion:** Exogenous surfactant increases the number of alveolar macrophages and the phagocytosis of apoptotic neutrophils in vivo. Anti-inflammatory properties of surfactant therapy may be mediated in part by increased number of alveolar macrophages and increased phagocytosis of apoptotic neutrophils by these macrophages.

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Surfactant Protein B Gene Polymorphism: Comparison between Healthy Term and Preterm Newborns with Respiratory Distress Syndrome

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Background: Surfactant protein B (SP-B) is essential for normal lung function. The human SP-B gene is located on the short arm of chromosome 2 (2p12→p11.2), encompasses approximately 9.5 kilobases and has 11 exons. Polymorphism and mutations in

the genes that encode the surfactant components, particularly the SP-B gene, have been associated with the development of respiratory distress syndrome (RDS). **Objectives:** To analyze SP-B gene polymorphism frequencies in preterm babies with RDS and healthy term newborns, to compare the polymorphism frequencies between both groups and to evaluate if there are differences related to sex, race and RDS. **Methods:** We included 150 Brazilian neonates, 50 preterm with RDS and gestational ages ranging from 28 weeks to 33 weeks and 6 days, and 100 healthy term newborns with gestational ages ranging from 37 weeks to 41 weeks and 6 days, admitted to our unit from June 2001 to July 2004. Four SP-B gene polymorphisms were analyzed: A/C at ¨C 18, C/T at 1580, A/G at 9306 and G/C at nucleotide 8714. The polymorphisms were detected by PCR amplification of genomic DNA and genotyping. The genotypes were determined using PCR-based converted restriction fragment length polymorphism (cRFLP). **Results:** The control group comprised 100 apparently healthy term newborns; 42 (42%) were female and 58 (58%) male; 39 (39%) were whites and 61 (61%) non-whites. Weight ranged from 2,280 to 4,740 g (mean 3,240 g); gestational age ranged from 37 weeks to 41 weeks and 6 days (mean 39 weeks and 3 days). The RDS group comprised 50 preterm neonates, 21 (42%) female and 29 (58%) male; 28 (56%) were whites and 22 (44%) non-whites. Weight ranged from 640 to 2,080 g (mean 1,273 g); mean gestational age was 31 weeks and 2 days (range, 28–33 weeks and 6 days). All genotype frequencies were similar in both groups when sex and race were analyzed together. When race was analyzed separately, there was a statistically significant difference between both groups in the polymorphism G/C at 8714 ($p = 0.028$). There was no difference between both groups in all polymorphisms when sex was analyzed separately. **Conclusions:** The analysis of the SP-B polymorphism G/C 8714 showed that in white neonates the genotype GG was only found in the RDS group and the genotype GC was more frequently found in controls. This suggests that genotype GG could be associated with RDS and reinforces the need of other studies to address this issue.

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Hydrocortisone Supplementation Shortly after Birth for Prevention of Bronchopulmonary Dysplasia: Pretreatment Cortisol Values May Predict the Response

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Background: Dexamethasone given shortly after the birth decreases the incidence of bronchopulmonary dysplasia (BPD) in very premature infants (Halliday et al., Cochrane Database Syst Rev 2001;CD00146). However, early dexamethasone treatment increases the risk of adverse neurological outcome [Yeh et al., N Engl J Med 2004;350:1304–1313]. On the other hand, administration of a low-stress dose of hydrocortisone (HC) shortly after birth has increased survival without BPD of small preterm infants [Watterberg et al., Pediatrics 1999;104:1258–1263]. This result has not been confirmed. **Objectives:** The aim of this trial was to study the effect of HC treatment shortly after birth on survival without BPD at postmenstrual age of 36 weeks. Further aim was to investigate whether serum cortisol concentrations predict the response. **Study Design:** We performed a randomized, blinded, placebo-controlled trial on infants with gestation ≤ 30 weeks, birth weight 501–1,250 g and respiratory failure. HC was started before 36 h of age at 2.0 mg/kg i.v. for 2 days, 1.5 mg/kg i.v. for 2 days, followed by 0.75 mg/kg i.v. for 6 days. Shortly before the HC treatment was started, basal and stimulated serum cortisols (before and 30 min after i.v. infusion of ACTH, 0.1 μg/kg) were measured. **Results:** The study was discontinued early because of gastrointestinal perforations in the HC group (4/25 vs. 0/26, $p = 0.05$); three of the four had been treated with indomethacin/ibuprofen. The incidence of BPD (28% vs. placebo 42%, $p = 0.28$) tended to be lower and that of patent ductus arteriosus (36 vs. 73%, $p = 0.01$) was lower in the HC group. The HC-treated infants with serum cortisol concentrations above the median had a high risk of gastrointestinal perforation. In infants with cortisols below the median, the HC treatment increased survival without BPD. **Conclusion:** Low serum cortisol concentrations shortly after birth identify the very high-risk preterm infants who may benefit from HC supplementation. Further randomized trials including long-term follow-up are required to substantiate this proposal.

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