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Surfactant kinetics in newborn infants with pneumonia and Respiratory Distress Syndrome

Cinetica del surfattante nel neonato con polmonite e con sindrome da distress respiratorio

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Summary

The role of pulmonary surfactant in neonatal pneumonia is largely unknown and treatment with exogenous surfactant remains controversial.

Objective. To measure (a) the amount of disaturated phosphatidylcholine surfactant recovered from tracheal aspirates and (b) its half-life in 4 groups of newborns: full-terms with pneumonia, preterms with pneumonia, full-terms with normal lung, and preterms with Respiratory Distress Syndrome.

Methods. All infants received an intratracheal tracer dose of ^{13}C labeled disaturated phosphatidylcholine. In full-terms with pneumonia, preterms with pneumonia, and preterms with Respiratory Distress Syndrome tracer was added to exogenous surfactant. Amount of desaturated-phosphatidylcholine and isotopic enrichments were measured from serial tracheal aspirates by gas chromatography and gas chromatography-mass spectrometry.

Results. The amount of desaturated-phosphatidylcholine was not different in the four study groups whereas its half-life was significantly shorter in full-terms with pneumonia (19.3 ± 7.3 h), preterms with pneumonia (34.8 ± 9.4 h), and preterms with Respiratory Distress Syndrome (28.7 ± 15.9 h) compared to full-terms with normal lung (61.8 ± 28.6 h), $p = 0.002$. By multiple regression analysis the cumulative dose of exogenous surfactant and the oxygenation index were significant predictors of disaturated phosphatidylcholine half-life ($R^2 = 0.5$ $p = 0.03$).

Conclusion. Term and preterm newborns with pneumonia and Respiratory Distress Syndrome exhibited much shorter desaturated-phosphatidylcholine half-lives than term newborns with normal lungs. Larger amounts of exogenous surfactant were associated in our study with a less severe shortening of disaturated phosphatidylcholine half-life.

Key words

Surfactant • Pneumonia • Newborn • RDS • Stable isotopes

Parole chiave

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Riassunto

Il ruolo del surfactant nella polmonite neonatale è in gran parte sconosciuto e la somministrazione di surfattante esogeno è oggetto di discussione.

Obiettivo. *Valutare la quantità e la vita media della fosfatidilcolina disatura nell'aspirato tracheale di 4 gruppi di neonati: a termine con polmonite, pretermine con polmonite, a termine con polmone sano, pretermine con Respiratory Distress Syndrome.*

Metodi. *Tutti i neonati hanno ricevuto una dose tracciante endotracheale di dipalmitoil-fosfatidilcolina marcata con ^{13}C. Nei gruppi di neonati a termine con polmonite, pretermine con polmonite, e pretermine con Respiratory Distress Syndrome il tracciante è stato aggiunto al surfactant esogeno. La quantità di fosfatidilcolina disatura e l'arricchimento isotopico del surfattante sono stati misurati con gas-cromatografia e spettrometria di massa.*

Risultati. *La quantità di fosfatidilcolina disatura dagli aspirati tracheali è risultata simile nei 4 gruppi. La vita media della fosfatidilcolina disatura è risultata significativamente più corta nei neonati a termine con polmonite (19.3 ± 7.3 h), pretermine con polmonite (34.8 ± 9.4 h) e pretermine con Respiratory Distress Syndrome (28.7 ± 15.9 h) in confronto con i neonati a termine con polmone sano (61.8 ± 28.6 h), $p = 0.002$. Con l'analisi della regressione multipla la dose totale di surfattante somministrato e l'indice di ossigenazione sono risultati i predittori significativi della vita media della fosfatidilcolina disatura ($R^2 = 0.5$; $p = 0.03$).*

Conclusioni. *Neonati a termine e pretermine con polmonite e con Respiratory Distress Syndrome hanno mostrato una vita media della fosfatidilcolina disatura marcatamente più corta di neonati a termine con polmone sano. La dose di surfactant esogeno più elevata si è associata a un minor accorciamento della vita media.*

Introduction

Neonatal pneumonia remains a major medical problem in neonatal medicine for its high incidence [1] and associated mortality and morbidity. Frequent infections in term and pre term infants admitted to neonatal intensive care units, apart from the environmental risk, could be attributed to several factors: a) impaired immunologic response [2]; b) low IgG levels due to premature interruption of transplacental antibodies transfer [3]; c) decreased surfactant specific proteins SP A and/or SP D, as a consequence of lung immaturity and ventilator injuries [4].

Most newborns with pneumonia have respiratory symptoms, laboratory and radiological evidence resembling the Respiratory Distress Syndrome (RDS) [5]. Moreover there is also increasing evidence that infection during pregnancy and the perinatal period plays an important role in the pathogenesis of neonatal chronic lung disease [6].

Standard therapy for severe neonatal pneumonia consists in antibiotics and mechanical ventilation. Surfactant treatment has been advocated since alveolar inflammation leads to protein leak into the alveoli and consequent inhibition of surfactant function. Beneficial effects of surfactant therapy in pneumonia were found in animal studies and postulated in case reports [7-9].

Herting et al. studied retrospectively the effect of exogenous surfactant in a group of newborns with connatal pneumonia caused by group B streptococcal (GBS) infection, in comparison with a control population of noninfected infants treated with surfactant for RDS. The study comprised 118 infants with respiratory failure and GBS infection proven by culture results. A nonrandomized control group of 236 noninfected infants was selected from the same database as control group. Within 1 hour of surfactant treatment, median fraction of inspiratory oxygen was reduced from .84 to .50. The authors concluded that surfactant therapy improved gas exchange in the majority of patients with GBS pneumonia but the response to surfactant was slower than in infants with RDS, and repeated surfactant doses were often needed [10].

Conversely Sherman et al. speculated that in animal models surfactant treatment might serve as a nutrient for bacteria and thereby promote microbial growth [11]. This hypothesis seems to be confirmed in surfactant preparations in vitro, where bacterial growth is affected by microbial species and by surfactant composition and amount [12].

Although Rudiger et al. showed that infants with pneumonia had a sufficient amount of surfactant [13], higher doses were suggested to overcome surfactant inhibition in pneumonia [14] or in meconium aspiration syndrome [15]. Surfactant use in congenital pneumonia still remains controversial.

Studies on surfactant kinetics in newborns with pneumonia could address some of these issues. Most importantly tracer studies could be used for measuring the half life (HL) of the treatment dose. Surfactant HL provides an in vivo estimation of disappearance of surfactant disaturated phosphatidylcholine (DSPC) from the lungs and therefore could give insights into the timing and dosage of exogenous surfactant.

We recently described a new method to measure surfactant DSPC-HL in pre term infants with RDS [16]. In the present study we applied this methodology to term and pre-term newborns with severe pneumonia.

Materials and methods

Patients

We enrolled preterm and term newborns with severe pneumonia, requiring mechanical ventilation and treated with exogenous surfactant. A group of pre-term infants with RDS without signs of infection and a group of term infants with no lung disease acted as controls. All study infants were admitted to the Neonatal Intensive Care Unit (NICU) of the Department of Pediatrics, University of Padova and to the Pediatric Intensive Care Unit, Institute of Anaesthesia and Critical Care, University of Padova from January 1997 to December 2001. We compared these infants with preterm infants with RDS but no signs of pneumonia, and with term newborns with normal lungs but requiring mechanical ventilation for major abdominal surgery, airway malformations or for neurological impairment leading to respiratory failure.

The study protocol was approved by the Ethics Committee of the General Hospital of Padova, and written informed consent was obtained from at least one parent for each child.

Diagnosis of severe pneumonia was based on the following criteria: 1) respiratory distress, 2) radiographic findings and 3) at least one of the following laboratory signs of infection: (a) presence of a positive blood culture, (b) increased C reactive protein plasma levels (CRP), (c) leukocytosis (d) immature to total ratio of neutrophilic granulocytes (I:T ratio) > 0.2 [13].

Newborns with congenital malformations, sepsis, or renal or liver failure were excluded from the study. All pre-term and term infants with pneumonia received one or more doses (100 mg/kg) of porcine surfactant (Curosurf; Chiesi Farmaceutici S.P.A., Parma, Italy). The indication for surfactant treatment was a fraction of inspired oxygen (FiO_2) above 0.40 or a mean airway pressure (MAP) higher than 7.5 cmH_2O in pre-term infants with pneumonia or RDS and a $FiO_2 > 0.50$ or a MAP > than 9.5 cmH_2O in term infants with pneumonia. Term infants with normal lungs did not receive exogenous surfactant treatment.

Study design

A tracer dose (2.5 mg/kg) of dipalmitoylphosphatidylcholine (DPPC) with both palmitic acids (PA) uniformly labeled with the stable isotope ^{13}C (U-^{13}C-PA) was added to the exogenous surfactant and administered endotracheally to all study infants as previously described [16].

Term newborns with normal lungs received only the tracer dose. Briefly, a bolus of surfactant and/or tracer was administered via a small catheter inserted through the endotracheal tube. After the procedure, the neonates were hand-ventilated for 1 min and then reconnected to the mechanical ventilator at pretreatment settings. The mode of ventilation during the study period was standardized and adjusted so that the PaO_2 ranged from 50 to 70 mmHg, oxygen saturation was between 88 and 96% and PCO_2 between 45 and 55 mmHg.

Tracheal aspirates of all infants were collected before administration of the tracer (time = 0), then every 6 hour up to 72 h, and thereafter every 12 h until extubation. The airways were routinely suctioned with 0.5 ml/kg of 0.9% saline injected through the endotracheal tube. The tracheal aspirate was kept at 4 °C for no longer than 3 hours, and was brought to a final volume of 2 ml with 0.9% saline. The sample was gently vortexed for 1 min and centrifuged at 400 x g for 10 min. The supernatant was recovered and kept at -20 °C until analysis. Fifty microliters of each labeled exogenous surfactant dosing, or tracing dose in case of the term controls who did not receive exogenous surfactant treatment, were stored at -20 °C in order to determine the ^{13}C enrichment of DSPC.

Clinical data, including ventilator support, blood gas analysis, surgical and pharmacological interventions, and complications, were recorded throughout the whole study period. Oxygenation index (OI), calculated as (MAP x FiO_2)/paO_2, was computed twice a day in infants with pneumonia or RDS and at least three times in term newborns with normal lungs during the study period.

Analytical methods

Lipids from tracheal aspirates and from exogenous surfactant were extracted according to the method of Bligh and Dyer [17] after addition of the internal standard. One third of the extract was oxidized with osmium tetroxide [18]. DSPC was isolated from the lipid extract by thin layer chromatography (TLC) [19]. DSPC fatty acids were methylated, and the amount of DSPC measured by gas chromatography as previously described [20]. For each tracheal aspirate DSPC amount was corrected for the volume of normal saline added during tracheal suctioning and sample processing.

The enrichment of U-^{13}C-PA- DSPC in the tracheal aspirates was measured by gas chromatography-isotope ratio mass spectrometry (GC-IRMS) as previously described [21-23]. The isotopic enrichment was expressed in mole percent excess (MPE), calculated from the increase in percentage of ^{13}C atoms in the total carbon dioxide obtained from the combusted compounds above baseline enrichment (before tracer administration). Enrichments were corrected for the contribution of unlabeled carbon atoms added during derivatization.

Calculations

Tissue-bound and alveolar surfactant were regarded as one pool. The half-life (HL) of U-^{13}C-PA-DSPC was calculated by exponential curve-fitting at the final, monoexponential part of the downslope of the enrichment value curve over time after administration of the surfactant dose.

Each study variable was expressed as mean ± SD.

All statistical analysis was performed using SPSS 9.0 and Microsoft Excel 97. To test differences among groups we used ANOVA analysis of variance with the post-hoc Bonferroni test for differences between individual study groups. Stepwise multiple regression analysis (entry 0.05, removal 0.10) was performed to assess predictors of DSPC-HL. Ventilatory index ($MAPxFiO_2/PaO_2$), oxygenation index, cumulative surfactant dose, days of ventilation, postnatal age at study start, birth weight, gestational age, plasma CRP were tested as the independent variables and surfactant DSPC-HL as dependent variable. Significance was set at 0.05.

Results

We studied 26 infants: 6 term infants with pneumonia (TP), 7 term newborns with normal lungs (TC), 7 preterm newborns with pneumonia (PP) and 6 preterm newborns with RDS (P-RDS). Clinical characteristics of the 4 groups are reported in Table I.

Ventilator and oxygenation parameters of the 4 study groups are reported in Table II. There was no difference in ventilator support or degree of respiratory insufficiency, as expressed by the mean FiO_2, MAP and OI over the study period, in the three study groups with lung disease. Significant differences were found in the TC group as expected by the study design. (Tab. II).

Total exogenous surfactant dose tended to be higher, (but not to a statistically significant extent, p = 0.06), in the PP group than in the P-RDS and the TP groups.

Mean amount of DSPC obtained from tracheal aspirate *during the study period* was not significantly different between the four study groups, being 0.53 ± 0.51 mg/dl in TP, 0.45 ± 0.25 mg/dl in TC, 0.52 ± 0.22 mg/dl in PP and 0.58 ± 0.40 mg/dl in P-RDS (Tab. II).

Mean DSPC-HL was 19.3 ± 7.3 h in TP, 34.8 ± 9.4 h in PP, 28.7 ± 15.9 P-RDS and 61.8 ± 28.6 h TC respectively (p = 0.002) (Fig. 1). Term infants with pneumonia had the shortest HL, but no significant differences were found between term and pre term infants with pneumonia and pre-term infants with RDS (Fig. 1).

Since the degree of ventilator support, expressed by mean FiO_2, MAP and OI were similar in all infants with lung diseases (Tab. II), we pooled these infants together and applied a multiple regression analysis to assess which clinical parameters (*see materials and methods*) were significant predictors of surfactant DSPC HL.

We found that the cumulative dose of exogenous surfactant and mean OI were best predictors of HL (R^2 0,5 and p = 0.03). Cumulative surfactant dose was positively correlated with HL (coefficient β 0.53, p = 0.01) and OI was negatively correlated with HL (coefficient β -0.41 p = 0.03).

Tab. I. Patient characteristics.

	Term pneumonia (6)	Term controls (7)	Preterm pneumonia (7)	RDS (6)	p value
GA (weeks)	37.1 (1.1) [a]	38.8 (2.1) [a]	30.0 (1.7) [b]	29.8 (1.9) [b]	< 0.001
Weight (g)	2920 (657) [a]	2590 (464) [a]	1167 (358) [b]	1134 (261) [b]	< 0.001
I. A. at 5 min.	7.3 (2.4)	8.0 (1.7)	7.4 (0.9)	8.8 (1.1)	0.38
Prenatal steroids	0 [a]	0 a	7 (100%) [b]	5 (83%) [b]	< 0.001
Exogenous Surfactant (mg/kg)	133 (51)	-	242 (78)	216 (98)	0.06
Mechanical ventilation (days)	5.5 (1.5)	14.3 (17.6)	9.0 (6.5)	9.0 (3.7)	0.4
CRP (mg/L)	45.6 (48.3) [a]	1.6 (0.5) [b]	18.7 (0.63) [a]	3.5 (0.06) [b]	0.049
Start of study (days)	2.7 (2.0)	4.7 (6.6)	1.1 (0.4)	1.3 (0.6)	0.25
Survival	5 (86%)	7 (100%)	6 (85%)	6 (85%)	0.4

Data are expressed as means (standard deviation) or numbers (%). Values with different superscripts (a or b) are significantly different with the Bonferroni test ($p \leq 0.05$).

Tab. II. Patients' ventilator and oxygenation parameters.

	Full term with pneumonia (6)	Healthy controls (7)	Pre-terms with pneumonia (7)	RDS (6)	P value
Mode of ventilation	3 (HFOV) 3 (SIMV)	7 (SIMV)	1 (HFOV) 6 (SIMV)	1 (HFOV) 5 (SIMV)	
OI	4.43 (3.6)	1.42 (0,4)	2.23 (1.2)	3.31 (1.9)	0.09
FiO2	0.45 (0.16) [a]	0.25 (0.04) [b]	0.30 (0.05) [a]	0.40 (0.08) [a]	0.004
MAP (cm H_2O)	10.2 (3.6) [a]	5.5 (1.1) [b]	7.1 (2.2) [a]	8.3 (2.3) [a]	0.019
DSPC (mg/ml)	0.53 (0.5)	0.45 (0.2)	0.52 (0.2)	0.58 (0.4)	0.92

Data are expressed as means (standard deviation) computed *during the study period*. Values with different superscripts (*a or b*) are significantly different with the Bonferroni test ($p \leq 0.05$). OI = oxygenation index = MAP x FiO_2/paO_2.

Discussion

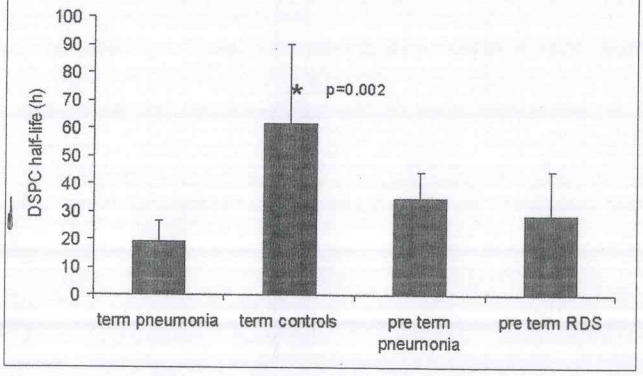
In this study we measured surfactant kinetics in preterm and term newborns with pneumonia using a safe method based on stable isotopes, as recently reported by our group [16]. This method is applicable to patients who require endotracheal intubation, thus in patients in whom sampling of airways is feasible via endotrachel tube.

The study of surfactant kinetics with isotopic tracers is based on several assumptions: 1) the distribution of exogenous surfactant is similar to the distribution of endogenous surfactant; 2) the phospholipid composition of surfactant in various pulmonary compartments is uniform; 3) the surfactant system is pulse labeled and there is no endogenous synthesis of the marker. It is reasonable to assume that most of the assumptions have been fulfilled. Hallman et al. [24] and Torresin et al. [16] have extensively discussed surfactant kinetics assumptions and we refer the reader to their work.

The major finding of our study was that DSPC-HL was significantly shorter in infants with pneumonia and RDS than in with infants with normal lungs. A faster DSPC-HL is the consequence of a faster U-^{13}C-palmitic acid-DPPC disappearance from the DSPC pool. Disappearance rate of U-^{13}C-palmitic acid-DPPC reflects the DSPC disappearance rate from lung DSPC. Tracer disappearance can be caused either by increased DSPC catabolism (loss of stable isotope DPPC molecules to the upper airways or to the blood-stream), or by an increased deacylation/reacylation pathway [25]. In the latter case the tracer palmitic acid produced by breakdown of DSPC is reincorporated into newly synthesized non-DSPC molecules, and it is thus lost from the DSPC pool.

A larger number of infants will have to be evaluated to understand whether the disease itself, beyond the severity of the respiratory failure/support, is responsible for DSPC-HL shortening. In a recent study [23] we compared surfactant kinetics in pre term infants with bronchopulmonary dysplasia (BPD) but no lung disease. We found that DSPC HL was 19.4 ± 2.8 h which was significantly shorter than in age matched controls. In the BPD study [23] as well as in the present study a shorter HL suggests a faster tracer breakdown. Elevated levels of pro-inflammatory cytokines (TNF-, IL 1, IL6 and IL8) in bronchoalveolar lavage have been found in term and pre term newborns with RDS, pneumonia and BPD (26-29). We speculate that the inflam-

Fig. 1. Mean DSPC HL (h) in the four groups (term pneumonia, term normal lungs, preterm pneumonia and preterm RDS). DSPC HL was significantly shorter in term and pre-term infants with lung disease, compared with term infants with normal lungs. Data are expressed as mean ± SD.



matory process leading to increased cytokines release could be responsible for the accelerated surfactant turnover and surfactant alterations, which in turns could exacerbate lung injury.

In the present study the cumulative surfactant dose and mean OI were significant predictors of DSPC-HL (R^2 0,5 and p = 0.03). Total surfactant dose correlated positively with HL (R 0.533, p = 0.008) and negatively with mean OI (R -.408 p = 0.035). The positive correlation between dose of surfactant administered and HL might be explained by an increased amount of surfactant in the lungs after exogenous surfactant administration. However term control patients, who did not receive exogenous surfactant, had the longest HL in the four groups. Therefore the main conclusion holds that HL is much shorter in sick than in healthy lungs.

In our study infants birth weight, gestational age, postnatal age and duration of mechanical ventilation did not significantly predict DSPC-HL. This suggests that the degree of mechanical ventilation plays a much bigger role on surfactant kinetics than the above-mentioned clinical variables. Mechanical ventilation and oxygen exposure may act as promoter and propagator of injury especially in the lungs of pre-term infants [30]. In adult animal lungs, ventilation with gas volumes that approached or exceeded total lung capacity resulted in pulmonary edema and pro-inflammatory cytokine release [31].

In the present study we did not measure de novo DSPC synthesis and/or pool size and we used the amount of DSPC recovered from tracheal aspirates as an indicator of the alveolar surfactant status. We found that DSPC amount in infants with lung disease was similar to that of term infants with normal lungs, possibly suggesting an adequate amount of exogenous surfactant delivered to our infants with lung disease. Whether the lack of surfactant in pneumonia was related to surfactant deficiency or inactivation is still matter of debate. Rudiger et al. suggested that respiratory insufficiency in pneumonia is most likely caused by altered properties of the surfactant [13]. In our infants with lung disease DSPC HL significantly correlated with the total amount of exogenous surfactant administered during the study period, suggesting a protective function of exogenous surfactant on DSPC disappearance from the lungs.

In conclusion we report here for the first time the measurements of surfactant kinetics in term and pre-term newborns with pneumonia. DSPC-HL was markedly reduced in both pre-terms and term newborns. DSPC-HL was associated with OI, which is a proxy for severity of lung disease. Exogenous surfactant dosing was associated with a slower disappearance of DSPC from lungs.

Larger studies will be needed to demonstrate differences in clinical outcomes and to confirm that prolongation of DSPC-HL by exogenous surfactant administration is associated with a better clinical outcome in neonatal pneumonia.

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