Precursor preference in surfactant synthesis of human preterm infants

Pulmonary surfactant, a mixture of phospholipid and protein, stabilizes lungs by lowering surface tension and preventing alveolar collapse at end expiration. Respiratory distress syndrome (RDS) in infants most commonly results from a quantitative deficiency of pulmonary surfactant after premature birth (1,2). Nutritional composition may affect the composition and function of pulmonary surfactant (3). It is unclear whether preterm human infants prefer de novo synthesis of fatty acids versus preformed fatty acids in the metabolism of surfactant. Recently developed methods utilizing naturally occurring, stable, non-radioactive isotope labeled metabolic precursors of phospholipid synthesis provide the opportunity to understand possible influences on surfactant metabolism in infants (4-8). We will use intravenous infusions of surfactant phospholipid precursors (administered [1,2,3,4-$^{13}C_4$] palmitate, and [1-$^{13}C_1$] acetate) and gas chromatography/mass spectrometry (GC/MS) to test **the hypothesis that: palmitate is the preferred substrate for surfactant synthesis in preterm infants less than 28 weeks gestational age and compare the results to term infants with normal lungs.** Acetate's and palmitate's respective rates of incorporation into the surfactant of preterm infants will be compared. The use of labeled metabolic precursors of surfactant phospholipid provides a unique and powerful approach to evaluate disruption of surfactant metabolism and will lead to specific and clinically useful interventions to restore pulmonary function in infants with RDS.

Specific Aim: 1) To determine the rate of surfactant synthesis using de novo synthesized fatty acids (acetate).

Specific Aim: 2) To determine the rate of surfactant synthesis using preformed fatty acids (palmitate).

Specific Aim: 3) To compare the rates of incorporation in preterm infants versus term infants with normal lungs.

We will measure the rate of incorporation of ^{13}C into surfactant obtained from tracheal aspirate samples after simultaneous 24 hour infusions of [1-$^{13}C_1$] acetate and [1,2,3,4-$^{13}C_4$] palmitate. **Eligible infants for the control group include intubated infants with normal lungs including 1) full-term infants (greater than 37 weeks EGA), 2) < 6 weeks of age, 3) clear lung parenchyma on chest radiograph, 4) requiring less than 30% fractional inspired oxygen. These infants will be intubated for reasons other than lung disease and will be eligible if continued intubation is likely to be longer than 5 days. The inclusion criteria for premature infants include: gestational age is 28 weeks or less and require mechanical ventilation as part of the routine management of their illness. In the preterm infant study group infants will be excluded if death appears imminent and those with known infection, congenital anomalies and pulmonary hemorrhage. Exclusion criteria for the control or normals group will include, 1) imminent death, 2) clinical lung or blood infection (positive blood cultures or**

tracheal aspirates in clinical context), 3) Development of respiratory insufficiency as evidenced by oxygen requirement >30% and abnormal chest x-ray. After consent is obtained from the patient's medical care team and parents, baseline blood and tracheal effluent samples will be obtained. Infants will then receive concurrent 24 hour intravenous infusions of [1,2,3,4-$^{13}C_4$] palmitate and [1-$^{13}C_1$] acetate. Blood samples (0.5 cc) will be obtained approximately every 8 hours for 24 hours, then tracheal aspirate samples will be obtained every 3-6 hours for 1 week, then every 12 hours for the second week or until the infant is extubated. In general, tracheal aspirates and blood specimens will be drawn at clinically indicated times if at all possible to reduce unnecessary handling of the infant. After 2 weeks, if the infant still requires mechanical ventilation and has indwelling vascular access, the infusion and sample acquisition will be repeated with assent of medical attending and consent of family.

Based on previous studies using these techniques, we estimate that 15-20 preterm infants will permit detecting a 25% difference between fractional synthetic rates of acetate and palmitate with a significance level of 0.05 with 90% power (4,5). However, for the term infants with normal lungs we estimate we will only need 10 infants.

Demographic data, including pregnancy history, birthweight, gestational age, race and sex will be obtained from each medical record. Clinical data to be obtained during the course of the study will include type, degree and duration of mechanical ventilation, blood gas analyses, electrolyte determinations, medications, specifically corticosteroids and nutritional support, specifically glucose, protein and lipid infusion rates and pathology reports. These clinical data will be linked with the surfactant metabolic data to determine the relationship between the kinetics of surfactant metabolism and the clinical expression of pulmonary dysfunction.

The samples will be prepared for measurement with GC/MS and GC/C/IRMS. We will measure indices of precursor incorporation into surfactant-derived palmitate: the lag time from the start of the tracer infusion until additional enrichment is first detected in surfactant phospholipid, the time from the start of the tracer infusion until maximum enrichment is reached, and the half life of surfactant clearance from the airspaces, and the fractional synthetic rate of surfactant palmitate from both plasma and de novo synthesized palmitate.

The Tapp, FSR and half-lives of the [^{13}C-1] acetate and [1,2,3,4-$^{13}C_4$] -palmitate will then be compared using the paired t-test. All data will be entered into a database and analyzed with the SAS system for personal computers (SAS Systems, Cary, NC).

The application of labeled metabolic precursors of fatty acid and protein synthesis provides a unique and powerful approach to evaluate differences in surfactant metabolism and the ability to test hypotheses about the effect of clinical interventions on surfactant metabolism.

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