9084

A Recombinant Fragment of Human Surfactant Protein D (rfhSP-D) Decreases Inflammation in Ventilated Pre-term Lambs

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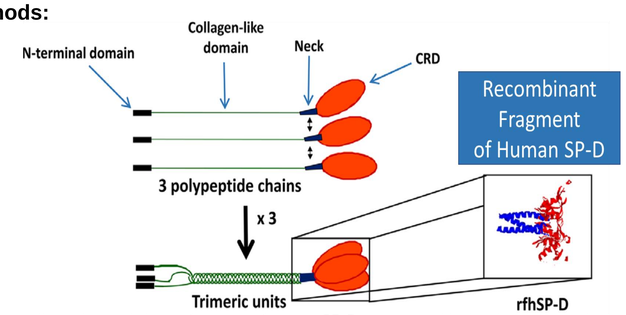
Rationale:

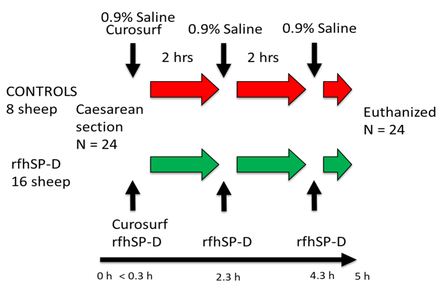
Premature babies have immature lungs and develop neonatal respiratory distress syndrome (RDS). Their immature lungs are deficient in lung surfactant, and current treatment combines respiratory support with exogenous surfactant therapy. Currently available animal-derived surfactants provide phospholipid components and two of the four surfactant proteins (SP-B and SP-C). SP-A and SP-D are not present. Low levels of SP-D in preterm infants with RDS have been linked to an increased risk of developing neonatal chronic lung disease and it has previously been shown that adjunctive surfactant therapy including whole length native SP-D decreases inflammation in a preterm ventilated lamb model of neonatal RDS (Sato et al., 2010)

Hypothesis: *A recombinant fragment of human surfactant protein D (rfhSP-D) has previously been shown to have anti-inflammatory effects in murine models of infectious and allergic inflammation (Knudsen et al., 2007). We hypothesized that surfactant treatment including this truncated rfhSP-D would decrease ventilator induced inflammation similarly to full length SP-D.*

Methods:

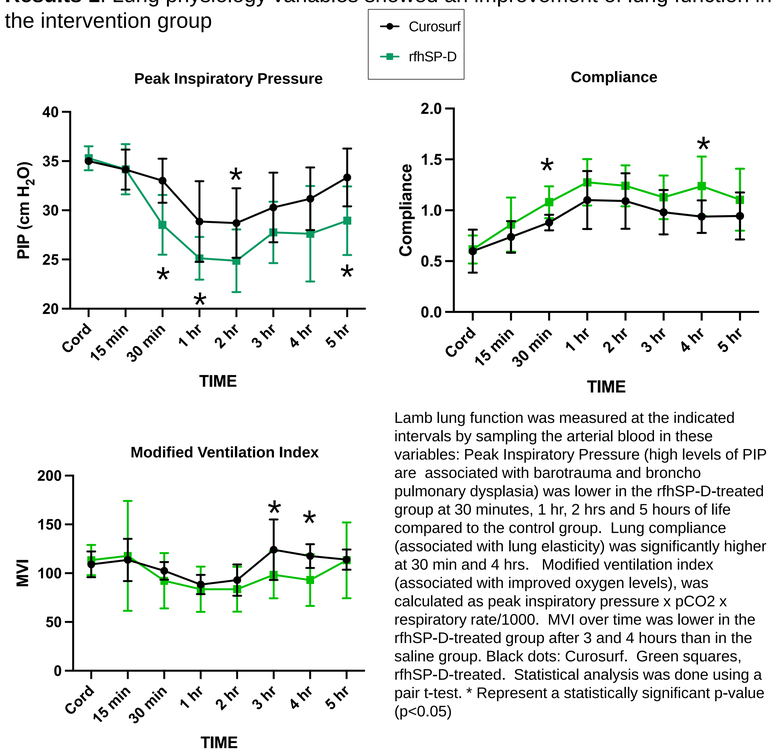


A genetically engineered shorter fragment of human SP-D was created by leaving the carbohydrate recognition domain intact and isolated to purity using HPLC.



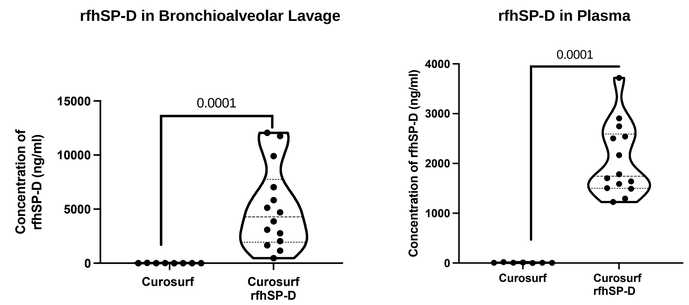
Preterm lambs of 127±1 days gestational age were delivered by caesarean section and injected with ketamine as a sedative (upper left photo). Lambs were either treated intratracheally (IT) with Curosurf (Poractant alpha) only or Curosurf plus rfhSP-D **Control**: **Intervention** was approximately 1:2 (8 lamb: 16 lamb). All lamb received a single bolus instillation of Curosurf and either three instillations of 0.9% saline (control group) or 1.5 mg/kg rfhSP-D in 0.9% saline (intervention) with two hours between each instillation. Arterial blood gas measurements and blood samples collection were done at 15 minutes, 30 minutes, and then every hour for 5 hours. After 300 min (5 hrs) of post-treatment, lamb were sacrificed to obtain tissue samples and analyzed by qRT-PCR or ELISA.

Results 1: Lung physiology variables showed an improvement of lung function in the intervention group



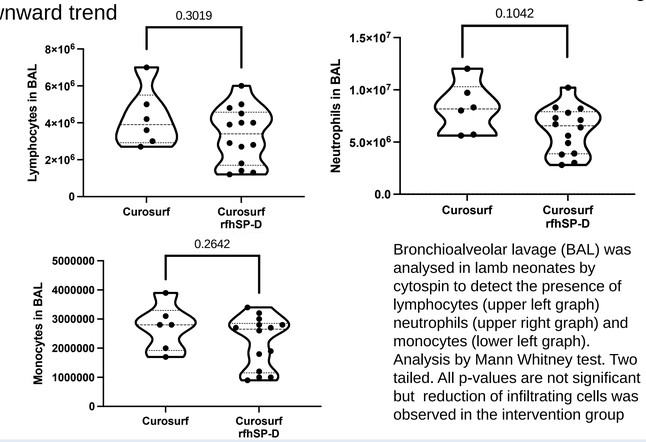
Lamb lung function was measured at the indicated intervals by sampling the arterial blood in these variables: Peak Inspiratory Pressure (high levels of PIP are associated with barotrauma and broncho pulmonary dysplasia) was lower in the rfhSP-D-treated group at 30 minutes, 1 hr, 2 hrs and 5 hours of life compared to the control group. Lung compliance (associated with lung elasticity) was significantly higher at 30 min and 4 hrs. Modified ventilation index (associated with improved oxygen levels), was calculated as peak inspiratory pressure x pCO2 x respiratory rate/1000. MVI over time was lower in the rfhSP-D-treated group after 3 and 4 hours than in the saline group. Black dots: Curosurf. Green squares, rfhSP-D-treated. Statistical analysis was done using a pair t-test. * Represent a statistically significant p-value ($p<0.05$)

Results 2: rfhSP-D was confirmed in the BAL and plasma of preterm lambs



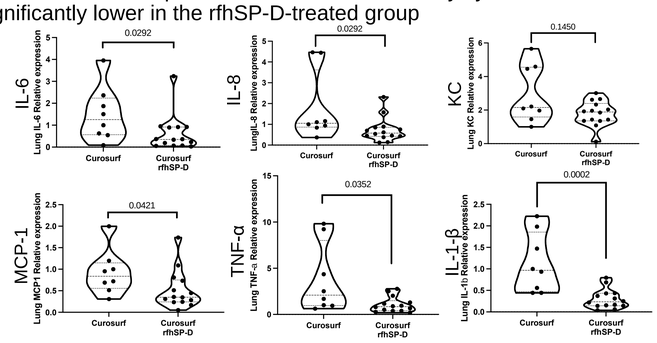
Enzyme-Linked Immuno Assay (ELISA) determination of rfhSP-D in Bronchioalveolar lavage (BAL) and in plasma confirmed rfhSP-D was delivered efficiently to the lamb lungs. Statistical analysis was performed with a Mann Whitney test. Two tailed. $p<0.05$ represent a statistically significant p-value

Results 3: Reduced cell infiltration was observed in the rfhSP-D-treated group as a downward trend



Bronchioalveolar lavage (BAL) was analysed in lamb neonates by cytospin to detect the presence of lymphocytes (upper left graph) neutrophils (upper right graph) and monocytes (lower left graph). Analysis by Mann Whitney test. Two tailed. All p-values are not significant but reduction of infiltrating cells was observed in the intervention group

Results 4: Gene expression levels of inflammatory cytokines in lamb lungs were significantly lower in the rfhSP-D-treated group



mRNA levels encoding lamb interleukins IL-6, IL-8, keratinocyte chemokine (KC), monocyte chemoattractant protein-1 (MCP1), tumour necrosis factor alpha (TNF-α) and interleukin-1 beta (IL-1β) in the lung were measured in lung homogenates by quantitative reverse transcription RNA using $2^{-\Delta\Delta Ct}$ method. L32 was used as an internal control. Horizontal bar(s) (long) is the median or standard deviation of the population (short). All the p-values were obtained with a Mann Whitney test. Two tailed.

Conclusions:

1. rfhSP-D was effective in reducing the levels of ventilation-induced inflammation in the lungs of preterm lambs
2. The above levels of decreased inflammation and improved lung function were similar to the levels previously observed iin preterm lambs treated with full-length nSP-D (Sato et al 2010)
3. These results suggest rfhSP-D may be a potentially useful therapy for neonatal respiratory distress syndrome in combination with currently available surfactants

Acknowledgements:

We gratefully acknowledge Chiesi Farmaceutici for providing Curosurf for these studies

Attendance for this conference was made possible by funding from MRC and from the American Thoracic Society International Trainee Scholarship Award

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