Surface Activity and Therapeutic Efficacy of Synthetic Surfactant with Recombinant Combo Peptide Combining Properties of SP-B and SP-C in Experimental Respiratory Distress Syndrome

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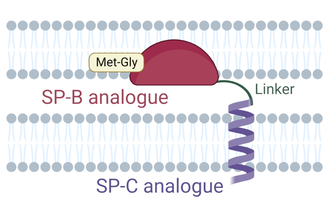
BACKGROUND

Exogenous surfactants restore surface activity and effective lung ventilation of premature infants with respiratory distress syndrome (RDS). The presence of surfactant protein (SP)-B and SP-C is essential for the proper functioning of any surfactant phospholipid complex. Recently, a new Combo analog has been developed that combines SP-B and SP-C into a single polypeptide chain. In acute RDS (ARDS) as a result of damage to the alveolocapillary membrane, plasma components penetrate into the alveolar spaces and inactivate the endogenous surfactant. Surfactant therapy in ARDS is still controversial, probably because the exogenous surfactants studied so far have been susceptible to inhibition by plasma proteins. The aim of the study is to verify the resistance of a new synthetic surfactant with a modified recombinant Combo peptide to the inhibition of plasma components in vitro and its therapeutic potential on lung functions in a rabbit model of RDS in vivo.

METHODS

Synthetic surfactant was prepared by mixing 3% (w/w) recombinant (E. coli BL21, vector pT7) purified Combo peptide with a 50:50 (w/w) DPPC/POPG phospholipid mixture. The combo peptide was designed as a fusion of the SP-B analogue Mini-BLeu and the SP-C analogue SP-C33Leu into a single polypeptide using a short GSG linker. Surfactant surface activity in the presence of plasma inhibitors was assessed by a pulsed bubble surfactometer (PBS). The RDS model involved ventilated preterm New Zealand White rabbit fetuses (caesarean section at gestational age 27 days of 32) treated with bolus intratracheal applications of Combo surfactant or Poractant alfa (Curosurf) 2.5 ml/kg, 80 mg phospholipids/ml. Respiratory volumes were regularly recorded during 30 min after surfactant administration.



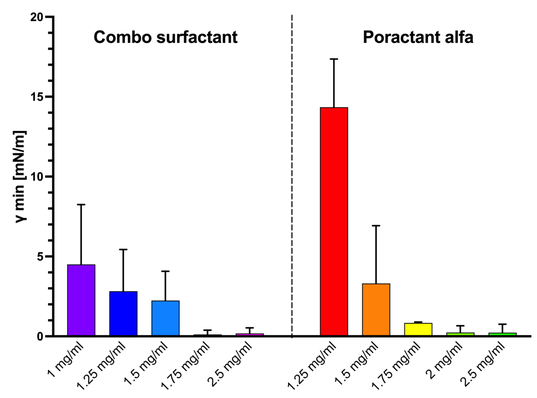
New combo peptide sequence

Initial seq	MG
SP-B analogue	LWLLRALIKRIQALIPKGGRLLPQLVLRLVLRLS
Linker	GSG
SP-C analogue	IPSSPVHLKRLKLLLLLLLILLLILGALLLGL

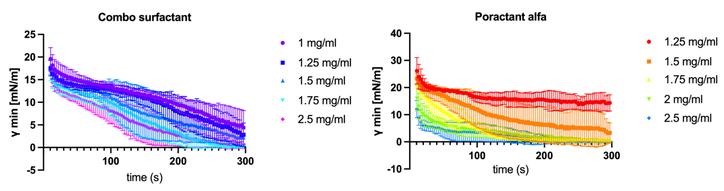
*Animal model of pulmonary surfactant deficacy mimic neonatal respiratory distress syndrome. Animals were ventilated in parallel with pressure-constant ventilator system (*Servo Ventilator 900 B; Siemens-Elema*); FiO_2 0.21, ZEEP, RR 40/min and I:E 1:1.*

RESULTS

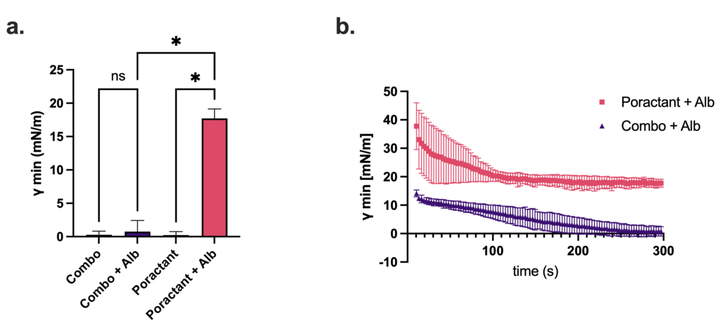
Albumin or fibrinogen at a concentration of 4 mg/ml significantly increased the minimum surface tension (γmin) of Poractant alfa in contrast to no effect on Combo surfactant at a concentration of 2.5 mg/ml after 5 min of pulsing in PBS. Moreover, the γmin of Combo surfactant was significantly lower compared to Poractant alfa at a concentration of 1.25 mg/ml. In RDS, Surfactant and Poractant alfa Combo therapy significantly improved tidal volumes during ventilation and resulted in significant alveolar opening and increased lung volume compared to untreated controls.

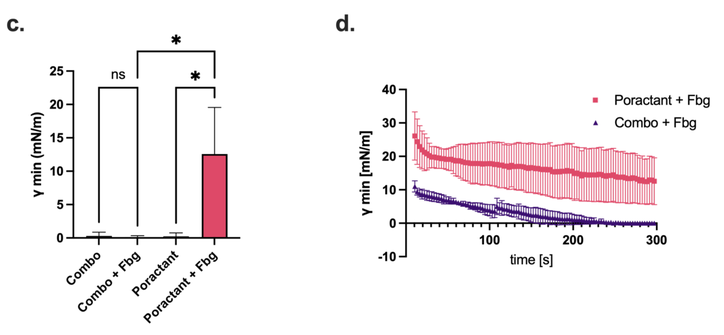


Biophysical activity of the Combo surfactant. Minimum surface tension (γmin, mN/m) after 5 min of pulsation in PBS. γmin ± SD of Combo surfactant compared to Poracant alfa at a concentration of 1.25 mg/ml, 2.82±2.61 mN/m vs. 14.34±3.02 mN/m, p=0.008.



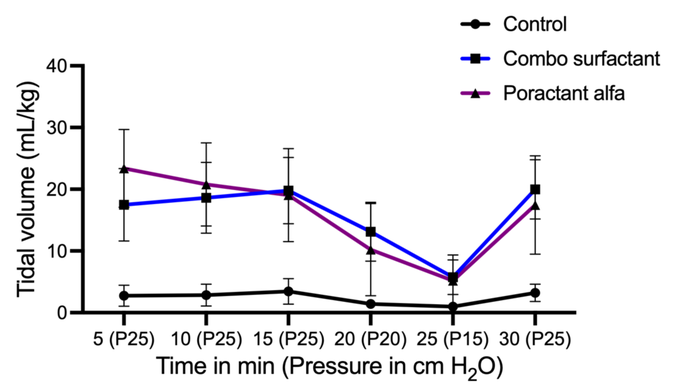
Minimum surface tension (γmin) of Combo surfactant and Poractant alfa at different concentration throughout the analyzed period during 5 min of cycling in PBS. Data are presented as mean ± SD.

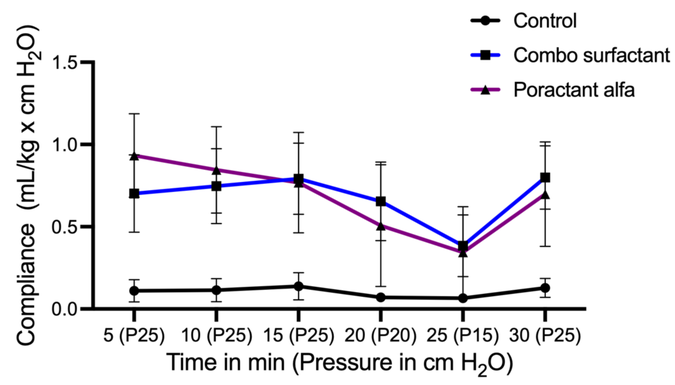




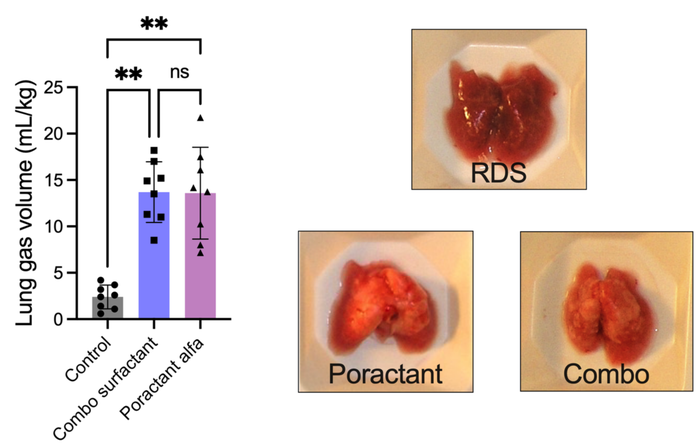
*Minimum surface tension (γmin) of the Combo surfactant and Poractant alfa at concentration 2.5 mg/mL mixed with (a) albumin (Alb) and (c) fibrinogen (Fbg) both at concentration 4 mg/mL after 5 min in PBS. γmin during the whole period of cycling in PBS mixed with (b) albumin and (d) fibrinogen. Data are presented as mean ± SD. *p < 0.05, **p < 0.01*

RESULTS





Combo surfactant is highly efficient in rabbit fetus model of neonatal RDS. Tidal volumes and compliance values during during the 30 min ventilation period. Data in line graphs are presented as means ± SD.



*Lung gas volumes at the end of animal experiment and representative macroscopic appearances of lungs from untreated controls, Poractant alfa treated and Combo surfactant treated animals. Data are presented in dot plot as median and interquartile ranges. **p < 0.01*

CONCLUSION

An absence of adequate levels of pulmonary surfactant results in malfunctions of the lungs. Surfactant replacement therapy is the primary option in the management of neonatal RDS. The synthetic surfactant with the new Combo peptide was therapeutically effective in the RDS model and highly resistant to inactivation by plasma proteins, and even at low concentrations it showed its functionality with minimal surface tension on PBS, indicating its potential for the treatment of ARDS.

REFERENCES

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