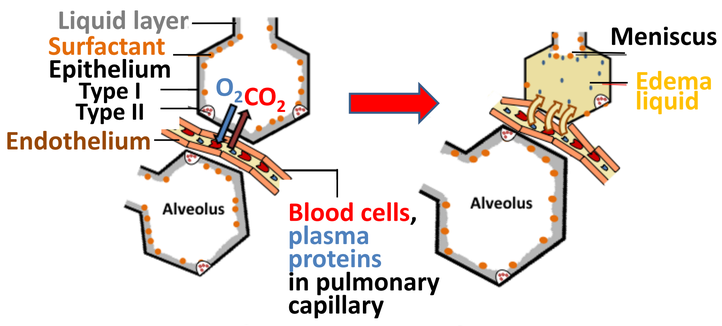
Ventilation Induced Surfactant Spreading In Pulmonary Edema

Angana B. Kharge and Carrie E. Perlman

Stevens Innovation Expo

Introduction

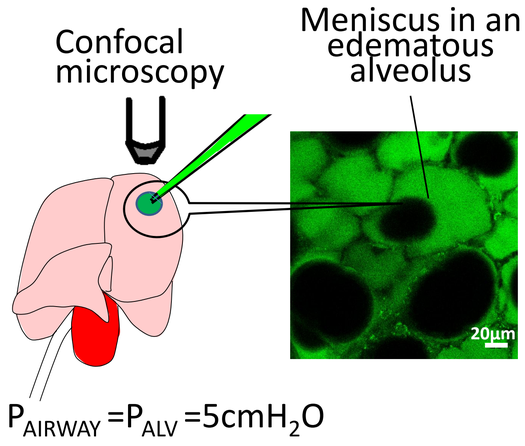


- Surfactant, secreted by alveolar type II cells, reduces surface tension, *T*, at the alveolar air-liquid interface and prevents the lung from collapsing.
- From experiments *in vitro,* plasma proteins in edema liquid are thought to inactivate surfactant activity and increase T[1-3]. We have developed a novel method for determining *T in situ* in edematous alveoli of the isolated rat lung.
- Our results indicate that protein inclusion does not alter *T.* To explain the *in vitro-in situ* difference, *in situ* we replicate the excessive surface area compression (*ΔA*) that was applied *in vitro*[4-6] while determining *T.*

Methods

I. Isolated rat lung preparation
- Anesthetize rat, cannulate trachea
- Excise heart and lungs

II. Alveolar edema model
- To flood alveoli, microinject 5% albumin or 5% dextran solution in surface alveoli
- For liquid visualization, include fluorescent BCECF in injected solution
- Observe interspersal of air and liquid filled alveoli
- To visualize lamellar bodies (LBs) or surfactant, include either 100 nm LTR or 500 nm FM4-64 in the injected solution



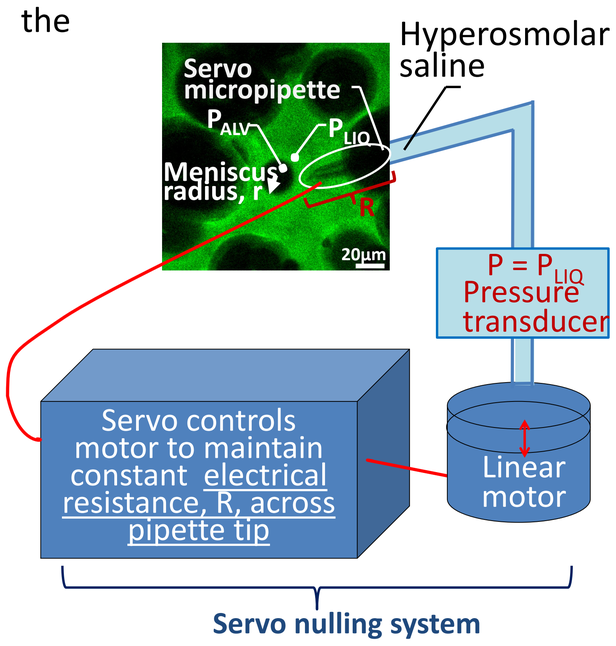
iii. Ventilation Protocol

Ventilate 60x
- 5-15 cm H_20, physiologic *ΔA*
- *- or -*
- 1-30 cm H_20, excessive *ΔA*

iv. Determine *T* at 15 cmH_20 on deflation using Laplace Law

$$T = ½\ r\ (P_{ALV}-P_{LIQ})$$

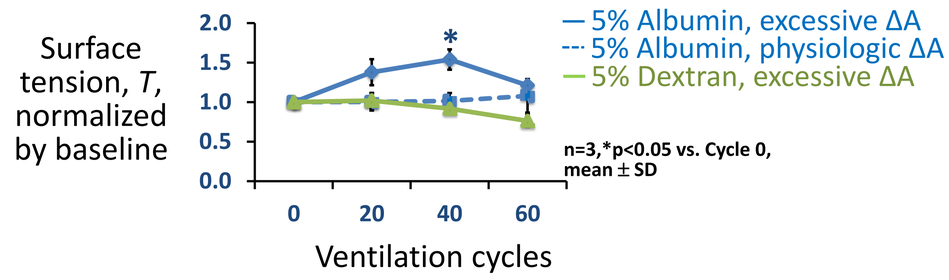
r = radius of curvature at air liquid interface determined in 3D from a Z stack of confocal images
P_{ALV} *= Inflation pressure* at trachea
P_{LIQ} *= Alveolar liquid pressure* - measured using servo system



Results

1. *In vitro- In situ* comparison

We flood alveoli with albumin or dextran solution, ventilate the lung with physiologic or excessive *ΔA* and determine *T* in edematous alveoli at cycles 0, 20, 40 and 60.

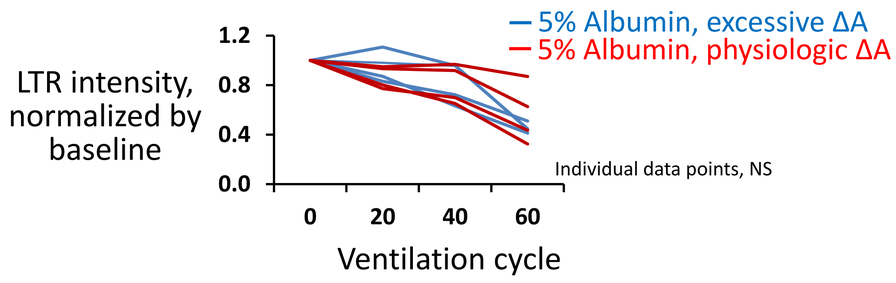


Results: (a) Proteins and excessive *ΔA* are both required to increase *T*
(b) Regardless of protein content, excessive *ΔA* decreases *T* after 40 cycles

2. *Investigate decrease in T with excess ΔA ventilation*

2A. Ventilation effects on surfactant release

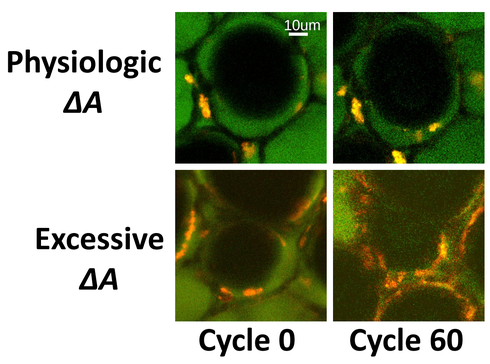
We ventilate the lung with physiologic or excessive *ΔA* and image tracked LB's at cycles 0, 20, 40 and 60.

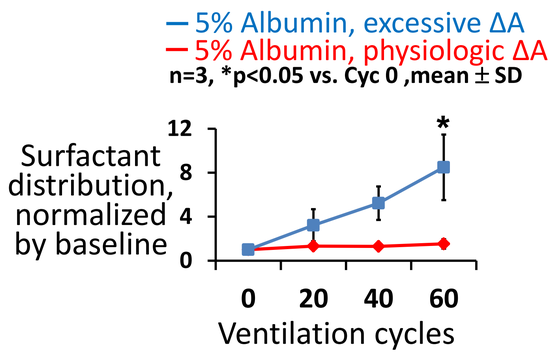


Results: Over 60 ventilation cycles, LTR fluorescence decreases progressively in both physiologic and excessive *ΔA* groups (n=4;N.S.)

2B. Ventilation effects on surfactant spreading

We ventilate the lung with physiologic and excessive *ΔA* and image surfactant at the edematous alveolar interface at cycles 0, 20, 40 and 60. We quantify surfactant distribution(%) at the interface as the fraction of ***total meniscus circumference*** over which ***FM4-64 fluorescence*** is evident





Results: At cycle 0 (baseline), FM4-64 distribution comprises 11±2.0% of the interface (n=4/group, N.S.). Ventilation with physiologic *ΔA* does not alter FM4-64 distribution (N.S.). Ventilation with excessive *ΔA* increases FM4-64 distribution to 53±8.5% (p<0.05) over 60 cycles.

Discussion

Our *in-situ T* measurements indicate that both albumin inclusion and excessive *ΔA* were required for cycling to increase *T*. In the absence of either albumin or excessive *ΔA*, *T* remained constant to cycle 40. Surprisingly, after 40 cycles and regardless of the presence of protein in the edema liquid, *T* decreased significantly with excess *ΔA*. We visualized surfactant secretion by labeling surfactant-storing acidic LB's with pH sensitive LTR. A decrease in LTR fluorescence indicates LTR loss due to LB fusion with the plasma membrane, thus surfactant secretion. LTR fluorescence decreased to the same degree in both physiologic and excessive *ΔA* groups suggesting no correlation between ventilation pattern and surfactant secretion. In contrast, excessive ΔA ventilation was particularly effective at promoting surfactant spreading (>50% compared to 11% with physiologic ΔA) at the alveolar air-liquid interface.

Conclusion

Protein inclusion does not increase *T* in the lung during physiologic ventilation. But regardless of protein content, excessive *ΔA* ventilation decreases *T* after 40 cycles by promoting surfactant spreading at the interface. In patients with cardiogenic lung edema (non-proteinaceous edema liquid), transient ventilation with low end expiratory pressure might reduce *T* and increase compliance.

References

1. Warriner, *Biophys J* 82:835, 2002.
2. Hills, *J Appl Physiol 87:1567, 1999.*
3. Zasadzinski, *Biochim Biophys Acta* 1798:801, 2010.
4. Tierney, *J Appl Physiol* 20:1253-1260, 1965.
5. Seeger, *J Appl Physiol* 58:326-338, 1985.
6. Hallman *et al*., *J Clin Invest* 70:673, 1982.