MECHANICAL STRETCH INCREASES ALVEOLAR EPITHELIAL PARACELLULAR PERMEABILITY AND EQUIVALENT PORE RADIUS

Kenneth J. Cavanaugh, Jr. and Susan S. Margulies

Department of Bioengineering
University of Pennsylvania
Philadelphia, PA

INTRODUCTION

Patients who require mechanical lung ventilation are at risk for ventilator-induced lung injury (VILI), which has an associated mortality rate of 34 – 60% [1]. VILI can result from ventilating with excessively large lung volumes and is characterized by an increase in alveolar fluid and macromolecule content. The alveolar epithelium provides the main barrier to unrestricted paracellular transport in the alveolus due to the extremely non-permeable tight junctions between adjacent type I cells. Previously we have shown that large physiological magnitudes of mechanical stretch can disrupt tight junction structure and function *in vitro* [2,3]. In this series of experiments we hypothesized that high magnitudes of stretch increase the paracellular permeability of the cultured alveolar epithelium to tracers of various sizes, and that this permeability increase can be represented as an increase in the radius and number of hypothetical paracellular pores. We found that high cyclic stretch magnitudes increased the paracellular permeability of the cells, altering the distribution and size of the equivalent pores. Our results support *in vivo* and *ex vivo* data gathered by other researchers, and indicate that low physiological stretch magnitudes may be safer for clinical mechanical ventilation.

METHODS

Cell Culture and Stretch Application

Primary rat alveolar type II cells were isolated according to a previously described method [4]. All cells were cultured onto flexible co-polyester membranes mounted in custom-made wells. Cells were cultured for five days, by which time the cells formed a confluent monolayer and adopted a type I phenotype, representative of the majority of the alveolar surface area *in vivo*. At least one well from at least four isolations was used for each observation.

Uniform equibiaxial strain was applied to the wells at 37ºC using a previously characterized device [4]. Cyclic stretch was performed for 1 hr at 0.25 Hz using peak magnitudes of 12%, 25%, and 37% change in surface area (ΔSA). These changes in surface area approximately correspond to strains experienced by the alveolar epithelium *in vivo* at 70%, 90%, and 100% total lung capacity, respectively [5]. Additional sets of wells were stretched cyclically at 0.25 Hz between 12% and 25% ΔSA, stretched statically (0 Hz) for 1 hr at 25% ΔSA, or used as unstretched controls.

Permeability Measurement

After stretch, each well was mounted into an Ussing chamber. Both sides of the chamber were filled with Ringer's solution. The apical reservoir was spiked with 10 mM of one of six biologically inactive tracers. These tracers were methylamine, alanine, valine, alanine-alanine, alanine-alanine-alanine, and leucine-leucine, with molecular radii of 1.5, 2.2, 2.8, 3.6, 4.4, and 5.5 Å, respectively. Basal reservoir samples were drawn at time points of 0, 30, 60, 90, and 120 minutes. The basal fluid volume was replenished with fresh solution after each sample was taken. The tracer concentration in the samples was determined by mixing each sample with 0.1% fluorescamine and measuring the fluorescence using a microplate assay.

A first-principles model of paracellular transport was derived for this system. Assuming that tracer mass in this system is conserved and that tracer transport across the epithelium follows first-order Fickian diffusion, then the following equation describes transport for each tracer:

$$\ln\left(\frac{C_{A0}-\left(1-\frac{V_B}{V_A}\right)C_B(t)}{C_{A0}-\left(1-\frac{V_B}{V_A}\right)C_{B0}}\right)=-\frac{\left(1-\frac{V_B}{V_A}\right)PS}{V_B}\left(t-t_0\right) \qquad (1)$$

Here P is the tracer permeability and all other parameters are constants or experimental data. Fitting the tracer data to this model and correcting for sample removal resulted in P values for each type of stretch and tracer tested. For each tracer, Dunnett's test for multiple comparisons to a control group was used to detect significant differences in permeability between the stretched groups and the unstretched group. Statistical significance was defined as $p \leq 0.05$.

Equivalent Pore Radius Calculation

The epithelial equivalent pore radius, a surrogate measure of the overall epithelial permeability [6], was calculated as a function of applied stretch by comparing the permeability of each tracer relative to a reference solute (alanine-alanine) and considering steric restrictions imposed on tracer transport by the pore walls. We found that the data were best represented by two populations of equivalent pores, one large and one small. We also calculated the number of pores of each type present before and after application of each type stretch.

RESULTS

One hour of cyclic 37% ΔSA produced a significant increase (100% – 2200%) in paracellular permeability (Figure 1). This increase was significant for all tracers studied and was more pronounced for larger tracers (those with a radius greater than 3 Å). Cyclic stretch at 12% ΔSA and both cyclic and static stretch at 25% ΔSA resulted in increased permeability for all tracers examined (1% – 42%), but these increases were not statistically significant compared to unstretched values. Stretch between 12% and 25% ΔSA slightly increased the permeability of the monolayer to smaller tracers (1% - 11%) and slightly decreased the permeability to the larger tracers (1% - 7%), although none of these changes was significant.

In each of the experimental groups, the total equivalent pore population was vastly dominated by the smaller pores, which represented over 99% of all equivalent pores in the monolayer. The radius of the small and large equivalent pores changed little (less than 15%) after application of moderate stretch magnitudes (less than 37% ΔSA). The radii of both large and small pores increased dramatically after 1 hr of 37% ΔSA (Table 1), indicating that this stretch magnitude facilitates paracellular transport of larger molecules. After 37% ΔSA, the total number of small pores decreased from 3.7×10^{10} to 2.6×10^{9} pores/cm^2, while the amount of large pores increased from 5.4×10^{5} to 5.0×10^{6} pores/cm^2. Although the total number of equivalent pores and the total pore area both decreased at this stretch magnitude by 93% and 61%, respectively, tracer permeability increased because the enlarged pore radii presented less steric hindrance to paracellular tracer diffusion.

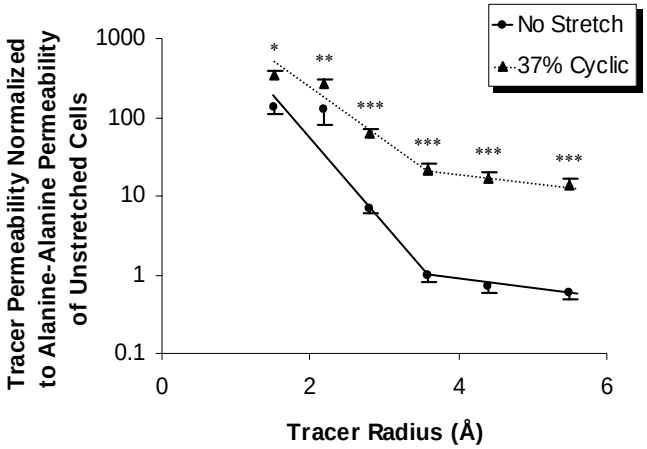

Figure 1. High physiological magnitudes of applied alveolar epithelial strain increase paracellular permeability. Data are given as mean ± standard error. Regression lines represent correlations calculated using best-fit values for large and small equivalent pore radii. Asterisks indicate a statistically significant increase in permeability after stretch (* = p < 0.05, ** = p < 0.005, *** = p < 0.0001).

	Small Pore Radius (Å)	Small Pore Number Compared to Controls	Large Pore Radius (Å)	Large Pore Number Compared to Controls	Pore Area Occupied by Large Pores
No Stretch	4.28	100%	43	100%	0.15%
12% Cyclic	4.34	92%	44	112%	0.18%
12-25% Cyclic	4.20	117%	42	105%	0.13%
25% Cyclic	4.22	128%	49	92%	0.14%
25% Static	4.21	127%	41	132%	0.14%
37% Cyclic	9.14	7%	63	924%	8.19%

Table 1. High applied strain magnitudes alter the equivalent pore characteristics of the alveolar epithelium.

DISCUSSION

Mechanical stretch only perturbs epithelial barrier function at 37% ΔSA. This is the same stretch magnitude which resulted in altered tight junction structure and function in similarly cultured cells [2,3]. These results also agree with the results of *ex vivo* animal experimentation, in which high physiological inflation volumes caused an increase in lung permeability [6,7].

The effects of tidal volume on patient health during ventilation have been studied clinically as well. In one study, low tidal volume ventilation decreased patient mortality compared with the use of comparatively high lung volumes [1]. In a separate study, use of high inflation pressures resulted in an increased risk of pneumothorax [1]. Our *in vitro* results are consistent with both of these findings.

Our data provide quantitative evidence that large cyclic strains increase the paracellular permeability of the alveolar epithelium. This information can be used to develop treatment regimens capable of making mechanical ventilation safer and more effective.

ACKNOWLEDGEMENTS

This work was supported by the National Heart, Lung, and Blood Institute Grant HL-57204 and the Whitaker Foundation.

REFERENCES

1. Ware, L. B., and Matthay, M. A., 2000, "The Acute Respiratory Distress Syndrome," New England Journal of Medicine, 342, pp. 1334-1349.
2. Cavanaugh, K. J., Oswari, J., and Margulies, S. S., 2001, "Role of Stretch on Tight Junction Structure in Alveolar Epithelial Cells," American Journal of Respiratory Cell and Molecular Biology, 25, pp. 584-591.
3. Cavanaugh, K. J., and Margulies, S. S., 2002, "Measurement of Stretch-Induced Loss of Alveolar Epithelial Barrier Integrity with a Novel in Vitro Method," American Journal of Physiology, 283, pp. C1801-C1808.
4. Tschumperlin, D. J., and Margulies, S. S., 1998, "Equibiaxial Deformation-Induced Injury of Alveolar Epithelial Cells in Vitro," American Journal of Physiology, 275, pp. L1173-L1183.
5. Tschumperlin, D. J., and Margulies, S. S., 1999, "Alveolar Epithelial Surface Area-Volume Relationship in Isolated Rat Lungs," Journal of Applied Physiology, 86, pp. 2026-2033.
6. Kim, K-J., and Crandall, E.D., 1982, "Effects of Lung Inflation on Alveolar Epithelial Solute and Water Transport Properties," Journal of Applied Physiology, 52, pp. 1498-1505.
7. Egan, E.A., 1980, "Response of Alveolar Epithelial Solute Permeability to Changes in Lung Inflation," Journal of Applied Physiology, 49, pp. 1032-1036.