



Validation Study: Postnatal Hyperoxia-induced Lung Injury in Infant Rats: Qin Zhang, Alireza Ebrahimnejad, Felisha Paniagua, , Robert Sukhu, Carol Meschter

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Introduction

Exposure of neonatal infant rats to 2 weeks of hyper-oxygenation followed by return to room air leads to pulmonary alveolar wall fibrosis, enlargement of the alveolar space and inflammation. This is analogous to pulmonary fibrosis in premature human neonates due to bronchopulmonary dysplasia, the treatment of which represents a significant unmet medical need. Similar lesions are also found in smoke inhalation injury and other pulmonary conditions leading to alveolar wall fibrosis. Endpoint determinations include lung function, histopathology, immunohistochemistry and alveolar measurements including the mean lineal intercept (MLI) method. Currently, a new method of assessment using digital image analysis provides accurate, robust and reproducible data on alveolar wall thickness and alveolar size that is comparable or superior to MLI measurements.

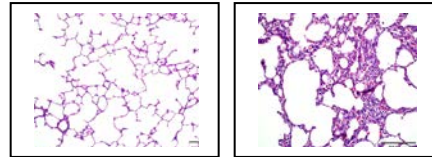
Materials and Methods

Newborn rats are exposed to hyperoxic air (~95% oxygen) from postnatal Day 4 to Day 14 and then returned to normal air. Pups are sacrificed with lung perfusion, and the left lung histopathology, IHC and digital image analysis conducted.

Results

In hyperoxygenated lungs, histopathologic findings were characterized by a multifocal to diffuse distribution of the following lesions throughout the lung, including, mild to severe (Grade 2-4, average of approximately 2.5),

fibrotic thickening of the alveolar walls (10-60 μm , normal 5-10 μm) and expansion of the alveolar diameter. Increased alveolar type II macrophages and mixed inflammation with hyperemia and congestion in the alveolar walls occurred. Normal and hyper-oxygenated lung photomicrographs are presented.



Hyperoxygenation was associated with increases in cellular expression of Collagen type I, vWF, CD68, PCNA and PDGFr.

MLI measurements

MLI data from validation studies at CBI indicate that normoxic and hyperoxic lungs from CBI are comparable to and consistent with literature reports indicating that the model is working appropriately at CBI. MLI is a count of the number of times a septal wall crosses an intercept line. There is a reduction in MLI in this model in comparison to normal lung alveoli. Neonatal lungs respond to hyperoxygenation by collagen deposition in the alveolar walls leading to thickening of the walls, and expansion of the alveolar space which leads to a reduction in the number of alveoli in a given area. This change can be measured by MLI method in which the number of times an alveolar wall, or septa, intersects with a grid line may be counted. Literature references indicate an approximate decrease of about 22% in hyperoxygenated lungs. Further, this change is also clearly visible by standard qualitative light microscopy.



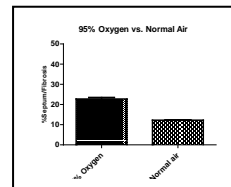
| Summary of MLI measurements | | |
|---|--------------|------------------|
| | Normoxia | Hyperoxygenation |
| CBI MLI | 35.5 ± 0.06* | 44.3±5.3* |
| Reference MLI Van Haaften, 2009 | ~35.5 ± 3** | ~50.3±2** |
| *Statistically significant, two tailed T test. p ≤ 0.001. N=10/group. (µm) | | |
| **Statistically significant, Student's two tailed T test. p ≤ 0.001. | | |

Phase Contrast Analysis. CBI has developed a validated method initially based upon Phase Contrast Analysis as presented by Jacob, et al, 2009.

Phase contract analysis is not directly correlative to MLI measurements. In this assessment, two parameters are measured: the alveolar wall thickness and the size of the alveolar spaces as a percentage of an area of tissue (um²) . It is clearly visible histopathologically that in hyperoxygenated lungs, there is a marked increase in the thickness of the alveolar walls, due primarily to an increase in collagen in the

alveolar walls. This thickness is measured and the differences between normoxic and hyperoxic alveolar wall clearly and consistently visible using the CBI digital image analysis technique. Digital image analysis clearly demonstrated statistically significant differences in alveolar wall thickness and alveolar space size in hyperoxygenated versus normal air lungs.

Conclusion

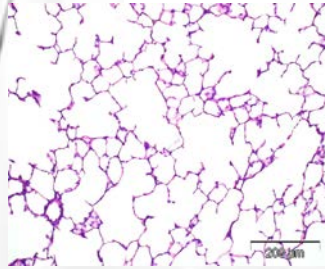


The pulmonary hyperoxygenation model as validated at CBI is characterized by statistically significant increases in alveolar size, increased alveolar wall fibrosis, upregulation of Collagen type I, vWF, CD68, PCNA and PDGFr. Results from image analysis method developed at CBI are consistent and superior to reports of increases as demonstrated by the antiquated MLI technique.

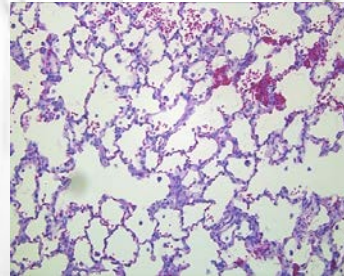


Histologic Findings

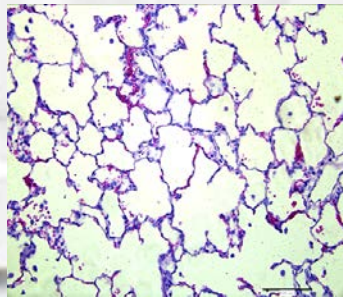
Normal lung



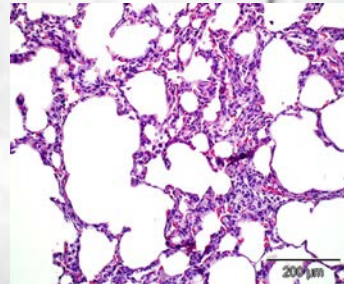
Hyperox-lung



Hyperox-lung 805



Hyperox-lung 904



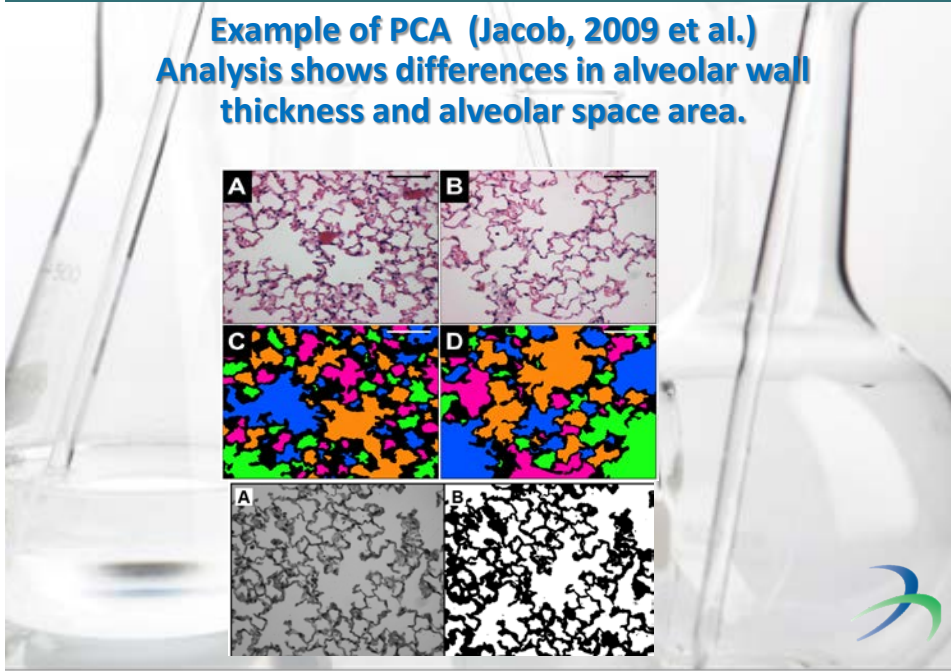
Hyperox-lungs: There is thickening of the alveolar walls, with inflammation, edema, and hemorrhage and consequently reduced alveolar area





A TRANSLATIONAL APPROACH TO PRECLINICAL RESEARCH

**Example of PCA (Jacob, 2009 et al.)
Analysis shows differences in alveolar wall
thickness and alveolar space area.**



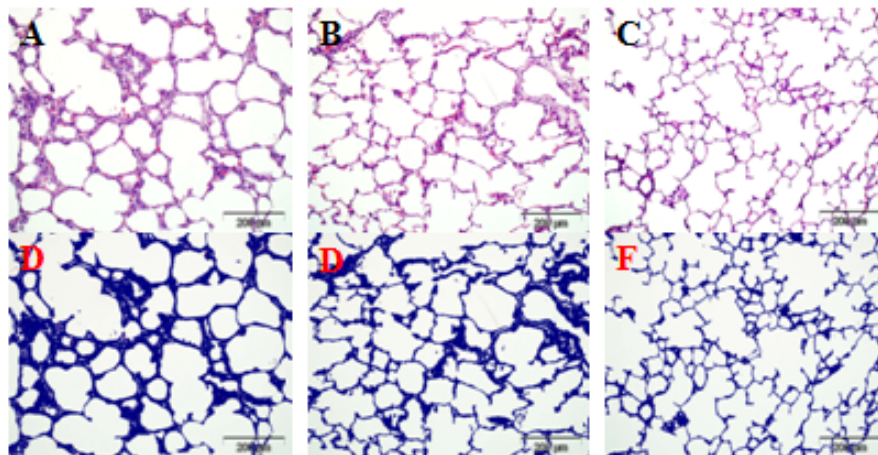
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**PCA at CBI: 95% oxygen vs normal air. There are clear
differences in alveolar wall thickness and alveolar
space size**

A&B 95% O₂

C Normal Air

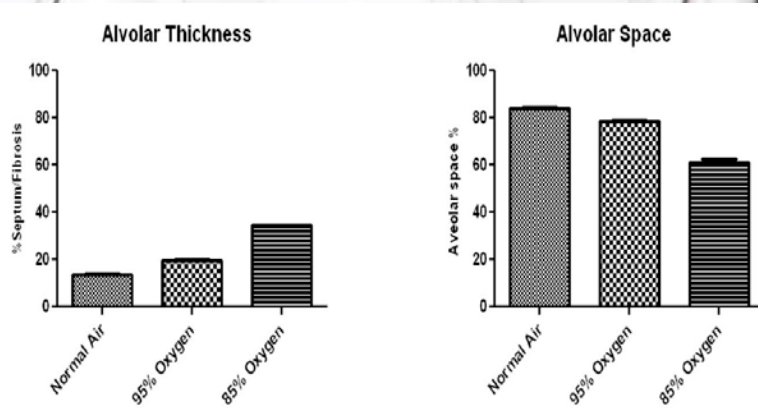


Top: H&E Images of lungs in a hyperoxic chamber at 95 % Oxygen (A&B) and normal air (C).
Bottom: The corresponding pixel image analysis was shown in (D, E and F)



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CBI PCA data showing clear significant increases in alveolar wall thickness and differences in the area of the alveolar spaces



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CBI PCA data showing clear significant increases in alveolar wall thickness

