COMPARATIVE BIOSCIENCES, INC
A TRANSLATIONAL APPROACH TO PRECLINICAL RESEARCH
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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

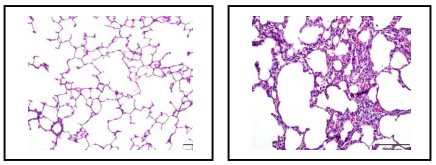
Exposure of neonatal infant rats to 2 weeks of hyper-oxygenation followed by return to room air leads to pulmonary alveolar wall fibrosis, enlargement of the alveolar space and inflammation. This is analogous to pulmonary fibrosis in premature human neonates due to bronchopulmonary dysplasia, the treatment 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). 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 published MLI measurements.

Materials and Methods

Newborn rat puts are exposed to hyperoxic air for 10 days postnatally and then returned to normal air for a defined period of time. Pups are sacrificed, lungs are perfused, and the left lung is evaluated by histopathology, immuno-histochemistry (IHC), and digital image analysis.

Results

In hyper-oxygenated 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 (Figure 1). 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.



IHC assessments

A host of IHC stains are available to understand mechanistic responses in the hyperoxia model. Hyper-oxygenation was associated with increases in presence of Collagen type I (fibrosis), vWF (Von Willebrand factor), CD68 (pan-macrophage), and PCNA (proliferating cell nuclear antigen).

MLI measurements

MLI data from validation studies at CBI indicate that normoxic and hyperoxic lungs are comparable to and consistent with literature reports indicating that the model is performing appropriately at CBI. MLI is a count, assessed histologically, of the number of times a septal wall crosses an intercept line on a structured grid. There is an MLI increase in this model in comparison to normal lung alveoli. Neonatal lungs respond to hyper-oxygenation by collagen deposition in the alveolar walls leading to thickening of the walls, and expansion of the alveolar space, literature references indicate an approximate increase of about 22 – 41% in lungs exposed to 90 – 95% hyperoxia. At CBI we showed a 25% increase in MLI with exposure to

95% oxygen, confirming performance of the hyperoxia model and MLI measures. Further, the alveolar changes are also clearly visible by standard qualitative light microscopy.

Phase Contrast Analysis (PCA)

CBI has developed a validated method initially based upon Phase Contrast Analysis as presented by Jacob, et al, 2009 (Figure 2).

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 (um^2). It is clearly visible histopathologically that in hyper-oxygenated lungs, there is a marked increase in the thickness of the alveolar walls, due primarily to an increase in collagen. This thickness is measured and the differences between normoxic and hyperoxic alveolar wall is clearly and consistently demonstrated using the CBI digital image analysis technique (Figure 3). Similarly, image analysis clearly demonstrated significant differences in alveolar space in hyper-oxygenated versus normal air lungs (Figure 4). And such changes were reproducible across groups exposed to 95% oxygen (Figure 5). Histomorphometric phase contrast analysis via digital imagery represents an improvement over MLI in assessment of distal lung responses by being more robust, reproducible, and descriptive of the complexity of change.

Conclusion

The pulmonary hyper-oxygenation model as validated at CBI is characterized by significant increases in alveolar space, increased alveolar wall fibrosis, upregulation of Collagen type I, vWF, CD68, and PCNA. Results from the image analysis method developed at CBI are consistent with and superior to reports of increases as demonstrated by published MLI techniques.

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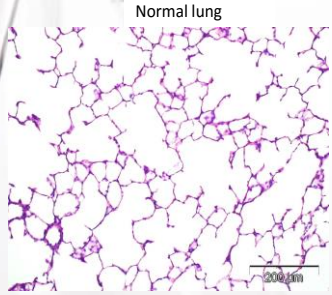
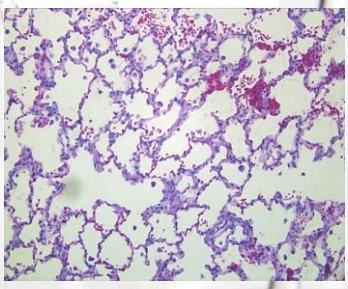
Figure 1

Histologic Findings

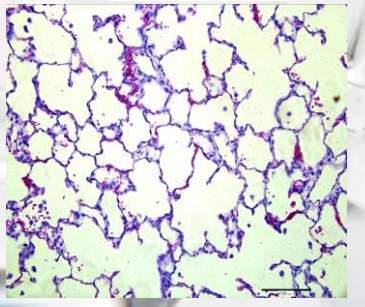
Normal lung



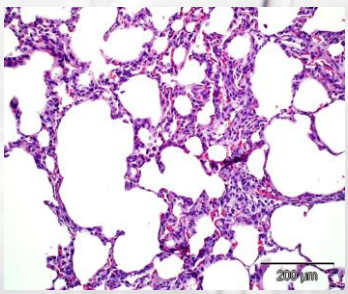
Hyperox-lung



Hyperox-lung



Hyperox-lung



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

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Figure 2

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

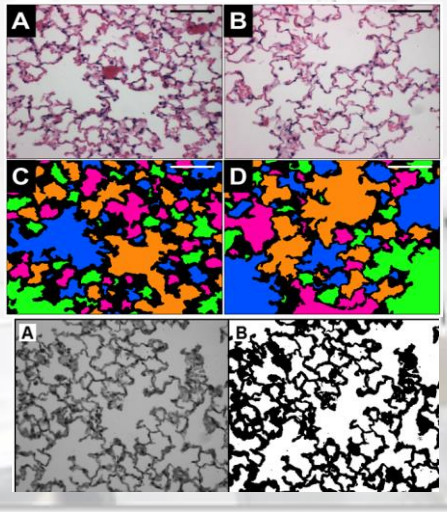

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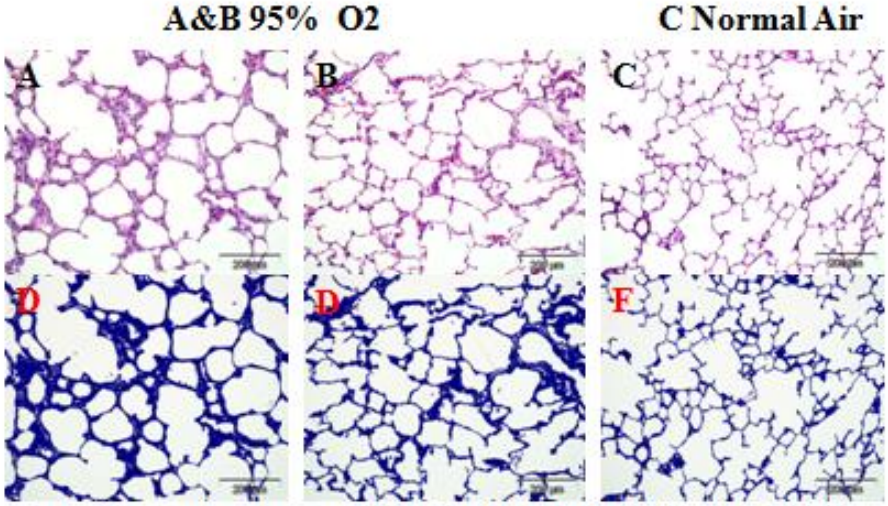
Figure 3

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



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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Figure 4

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

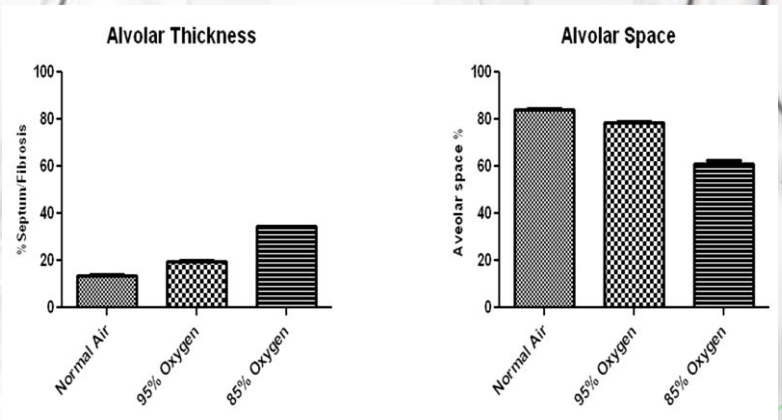

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Figure 5

CBI PCA data showing clear, significant, and repeatable increases in alveolar wall thickness