Respiratory Function and Quantitative Measurement of Fibrosis in a Rat Model of Bleomycin-Induced Lung Fibrosis

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1 Introduction

Idiopathic Pulmonary Fibrosis (IPF) is a chronic progressive lung disease with limited treatments. IPF is believed to be driven by repetitive damage to the alveolar barrier with abnormal healing leading eventually to lung fibrosis. Administration of bleomycin to rats causes lung fibrosis and is used widely to model IPF. The objective of this study was to determine whether impaired lung function in conscious rats could be correlated with the extent of lung fibrosis as assessed by quantitative image analysis.

2 Methods

Rats were administered bleomycin directly to the lung on one or seven occasions.

- Group 1: Vehicle Control/Single Dose (Saline, n = 3)
- Group 2: Bleomycin/Single Dose (1.5 mg/kg, 2.5 U/kg), n = 5)
- Group 3: Bleomycin/Single Dose (3.0 mg/kg, 5.0 U/kg), n = 5)
- Group 4: Vehicle Control/Multi-Dose (Saline, n = 5)
- Group 5: Bleomycin/Multi-Dose (0.5 mg/kg, 0.8 U/kg), n = 5)
- Group 6: Bleomycin/Multi-Dose (1.0 mg/kg, 1.7 U/kg), n = 5)

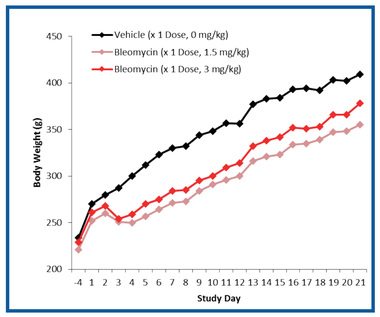
Respiratory measurements were determined using head-out plethysmography and the extent of lung fibrosis was determined on picrosirius red (PSR) stained formalin-fixed lung sections utilising a customised digital image analysis algorithm (OracleBio). The study was conducted for 22 days and respiratory measurements were determined on Day 18. Body weights were measured daily and lung weights were determined at necropsy.

3 Statistical Analysis

Data presented as mean ± S.D. Statistical comparisons were performed against Vehicle Saline Control using ANOVA with Dunnett's post test analysis.
*p≤0.05, **p≤0.01, ***p≤0.001, ****p≤0.0001

4 Results

Figure 1: Body weight gain reduced in bleomycin-dosed animals; the extent of weight reduction greatest in the multi-dosed animals at 1 mg/kg.



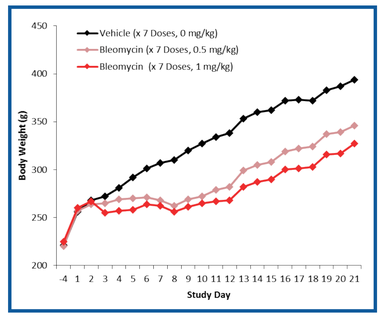


Figure 2: Group mean lung weights were increased in bleomycin-dosed animals.

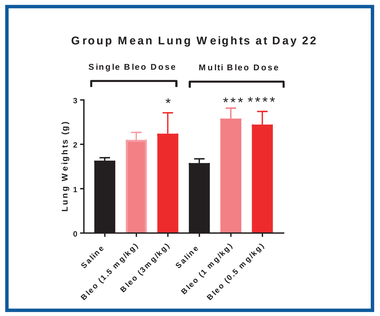


Figure 3: Group mean respiratory rates were significantly increased in the bleomycin-dosed animals in the multi-dose bleomycin model (Day 18).

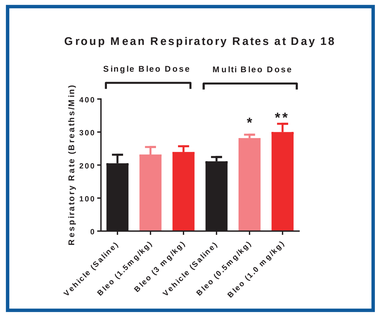
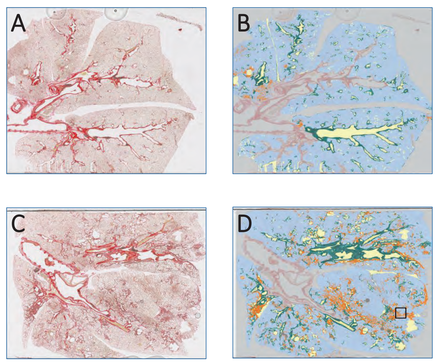


Figure 4: Examples of image analysis on control and bleomycin-treated lungs



A: Control Lung PSR stained

B: Control Lung – Image overlay to shown collagen (green) and lung parenchyma (blue)

C: Bleomycin Lung PSR stained

D: Bleomycin – Image overlay showing basal collagen (green), fibrotic lesion (orange) and lung parenchyma (blue)

Figure 5: Higher magnification view of image analysis on bleomycin-treated lungs

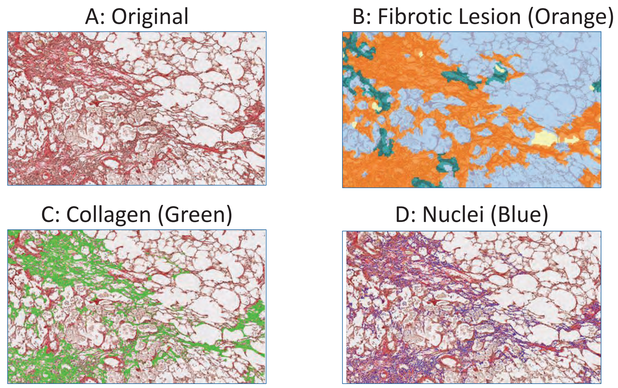


Figure 6: Group Mean Values for the percentage lung for the fibrotic lesional area. The extent of lesional area is increased in all bleomycin-dosed groups.

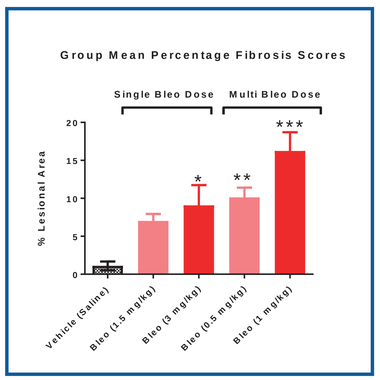
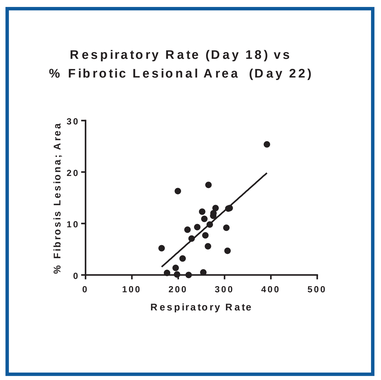


Figure 7: Individual respiratory rates vs % fibrotic lesion area significantly correlated (P<0.001 Pearson's Coefficient, $R^2 = 0.43$)



5 Conclusion

These data demonstrate that lung function measurements, and specifically respiratory rate, are significantly correlated with the extent of lung fibrosis as assessed by quantitative morphology on fixed lung sections. These data suggest that in-life respiratory rate measurements may be a useful method to help evaluate efficacy in studies to evaluate new treatments for fibrosis.