

Old Drug, New Target: inhaled Lithium attenuates changes in Lung Mechanics in Bleomycin induced animal model of Lung Fibrosis

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Abstract

Background: Idiopathic pulmonary fibrosis remains an incurable disease with poor prognosis. Recently the focus in research on the possible pathogenesis of this debilitating illness has shifted, at least in part, to premature accelerated aging of lung tissue as a driving force leading to fibrosis. Telomere shortening as a marker of premature aging, may play an important role in an approach to understand idiopathic pulmonary fibrosis pathophysiology. The goal of this study was to test the effect of inhaled lithium on pulmonary fibrosis, as a potential new treatment modality. The reason for such an approach was based upon observational results published in several articles demonstrating the ability of lithium to elongate telomeres of patients on long-term lithium treatment for bipolar disorder.

Results: We conducted an animal study in a Bleomycin induced model of pulmonary fibrosis to investigate this hypothesis. Results of this study have shown a beneficial effect of inhaled lithium. Mice were protected from changes in gas exchange (hypoxia) as well as from changes in lung mechanics, experiencing reversion of these changes that are characteristic of pulmonary fibrosis in the Bleomycin induced model of pulmonary fibrosis.

Conclusion: Taking in account the multiple cellular and physiological effects of lithium we do not want to be bound by one theory of the beneficial action of inhaled lithium to Bleomycin induced lung fibrosis. Given these positive preliminary results we suggest that further studies are indicated to expound upon the therapeutic implications of this approach to idiopathic pulmonary fibrosis.

Introduction

IPF is a rare, chronic, age-related, progressive, irreversible, and fatal fibrotic lung disease of unknown cause and characterized by the underlying histopathologic and/or radiologic pattern of Usual Interstitial Pneumonia (UIP), as further defined below. The pathogenesis and etiology of IPF are not fully understood, but it is believed to be a result of a combination of chronic micro-injury and the pathological repair process in genetically predisposed individuals. Premature and accelerated cellular senescence is hypothesized as a major driving force in IPF. Genetic and epigenetic changes, disturbance in protein synthesis, profibrotic growth factors, and epithelial to mesenchymal transition are thought to play a role in the pathophysiology of IPF. Mutations in the genes TERT and TERC, which code the most important telomerase components (specialized polymerase responsible for telomere elongation), are associated with up to 15% of familial pulmonary fibrosis and rare sporadic IPF cases. Alder et al. (2008) Cronkhite et al. (2008) Interestingly, nearly all patients with IPF have short telomeres even in the absence of TERT or TERC mutations. Armanios (2012) MUC5B promoter region rs35705950, a common gain-of-function variant with low penetrance, has been confirmed as the risk factor for development of both familial and sporadic IPF. Seibold et al. (2011) Epigenetic changes are likely to be involved in the control of gene expression in IPF. Yang and Schwartz (2015) Smoking and aging have a major effect on epigenetics. Issa (2014) Liu et al. (2010)

Disbalance between cellular demand for protein synthesis and the endoplasmic reticulum's capacity to meet this demand activate a cellular response termed the Unfolded Protein Response (UPR). If the UPR cannot match the demand, the cell sacrifices itself through apoptotic pathways. There is emerging evidence of the UPR in the pathogenesis of IPF. Tanjore et al. (2012)

The level of active transforming growth factor beta (TGF-beta) is increased in lungs of patients with IPF. Possible profibrotic processes associated with TGF-beta activation include inhibition of aortic endothelial cell (AEC) proliferation, differentiation of fibroblast to myofibroblasts, Scotton and Chambers (2007) and activation of programming that promotes mesenchymal transition of epithelial cells. Kim et al. (2006) Normal tissue homeostasis does not require epithelial-mesenchymal transition (EMT). However, EMT is activated during conditions of tissue injury and remodeling and plays an important role in fibrogenesis. Kim et al. (2006) In the damaged lung, fibrocytes contribute to IPF through differentiating into fibroblasts and myofibroblasts, and secreting profibrotic cytokines. Maharaj et al. (2013) Cellular senescence markers are abundant in IPF lungs, with p16 expression increasing with disease severity, and senescent cell depletion rejuvenates pulmonary health in aged mice. Schafer et al. (2017)

IPF may present at any time during middle-to-late adulthood, but most commonly arises in the sixth and seventh decades. To this end, IPF is practically absent in patients younger than 50. Raghu et al. (2016); Raghu et al. (2006)

The classic presentation of IPF primarily occurs in late adulthood with onset of unexplained chronic exertional dyspnea, and commonly presents with cough, bibasilar inspiratory crackles, and finger clubbing. Gastroesophageal reflux disease (GERD) is a frequent comorbidity in IPF patients. Costabel et al. (2018) Median survival for IPF is 3 years, Vancheri et al. (2010) and death typically occurs from respiratory failure. Tobin et al. (1998)

Definitive diagnosis of IPF is associated with the histological and radiological pattern of UIP, and requires: (a) exclusion of other known causes of interstitial lung disease (ILD) (e.g., domestic and occupational environmental exposures, connective tissue disease, and drug toxicity); (b) the presence of a UIP pattern on high-resolution computed tomography (HRCT) in patients not subjected to surgical lung biopsy; and (c) specific combinations of HRCT and surgical lung biopsy pattern in patients subjected to surgical lung biopsy. Raghu et al. (2011) Disease progression monitoring in IPF includes pulmonary function tests such as forced vital capacity (FVC). Raghu et al. (2011)

Disease progression and symptomatology is variable and unpredictable. Most patients with IPF demonstrate a gradual worsening of lung function over years, with a minority of patients remaining stable or declining rapidly. Some patients may experience episodes of acute respiratory worsening despite previous stability. Disease progression is manifested by increasing respiratory symptoms, worsening pulmonary function test results, decrease of oxygen saturation, progressive fibrosis on HRCT, acute respiratory decline, or death. Overall, prognosis for IPF is poor, with a mean survival of about 2.5-5 years. du Bois (2012)

Current therapies of IPF are limited in their effectiveness.

Pirfenidone, an orally administered pyridine with anti-inflammatory, antioxidant and anti-fibrotic properties, has shown some promise in its ability to reduce the rate of functional decline in IPF patients. Fujimoto et al. (2016); Sgalla et al. (2018) The precise mechanism of action of pirfenidone remains unknown, but it is believed to play a role in the regulation of TGF-beta expression.

Similarly, the use of nintedanib, a multiple inhibitor of tyrosine kinase receptors, including PDGF and VEGF receptors, Hilberg et al. (2008) has shown efficacy in reducing the rate of functional loss in IPF patients. While both pirfenidone and nintedanib have shown efficacy in reducing the functional decline in IPF, neither were able to significantly improve IPF patient survival. Sgalla et al. (2018)

Results of anti-acid treatment on the natural course of disease in IPF patients are inconclusive. Despite earlier published data suggesting a positive effect of baseline antacid treatment to slow significantly decreases in forced vital capacity (FVC) Lee et al. (2013), more recent studies did not find that baseline anti-acid treatment was associated with more favorable outcome in patients with IPF. Costabel et al. (2018) Kreuter et al. (2016)

For selected IPF patients, lung transplantation may be a treatment option, but mortality related to this procedure remains high and mean post-transplant survival is only six years. Laporta Hernandez et al. (2018) Chambers et al. (2017) Oxygen therapy is commonly prescribed for IPF patients. Despite its frequent use, there is a lack of evidence supporting the effectiveness of oxygen therapy for prolonging survival of IPF patients. Bell et al. (2017)

Palliative care is commonly used for IPF patients for physical, psychosocial support, and advanced care planning. Lindell et al. (2017)

We performed an animal study to evaluate the effect of inhaled Lithium on the extent of fibrosis development in a Bleomycin Mouse Model of IPF. The Bleomycin model of IPF is the most widely used and best-characterized model of IPF, due to its ability to reproduce many aspects of IPF and evaluate anti-fibrotic strategies. Bleomycin produces epithelial cell injury which leads to an early inflammatory phase which transitions into fibrosis after 5–7 days. Within this model, administering experimental therapies are classified as either preventative (starting < 7 days after bleomycin installation) or therapeutic (> 7 days). Kolb et al. (2020) Further, this model induces many of the cellular and molecular mechanisms directly relevant in the pathogenesis of IPF. Peng et al. (2013) Finally, the Bleomycin animal model has been used for development of both Pirfenidone and Nintedanib, which are approved by FDA for IPF. Wollin et al. (2019) Liu et al. (2017)

Methods

An animal study as an outsourcing work model was done with the UNC Lung Disease Model Center with full accordance with their internal policy for approval and performing mice lung research to evaluate the

effect of Lithium Carbonate on the extent of fibrosis development in a Mouse Model of Bleomycin-Induced Fibrosis.

The study was performed to determine the preventative (“early treatment”) (< 7 day after Bleomycin challenge) and therapeutic (“late treatment”) (> 7 days after Bleomycin challenge) effect of Lithium Carbonate by oropharyngeal (OP) aspiration route of administration in mice with Bleomycin-induced pulmonary fibrosis. This study measured the effect of the drug on pulmonary function: lung resistance (Rrs), tissue elastance (H), dynamic compliance (Crs), and static compliance (Cstat).

Animals and administration:

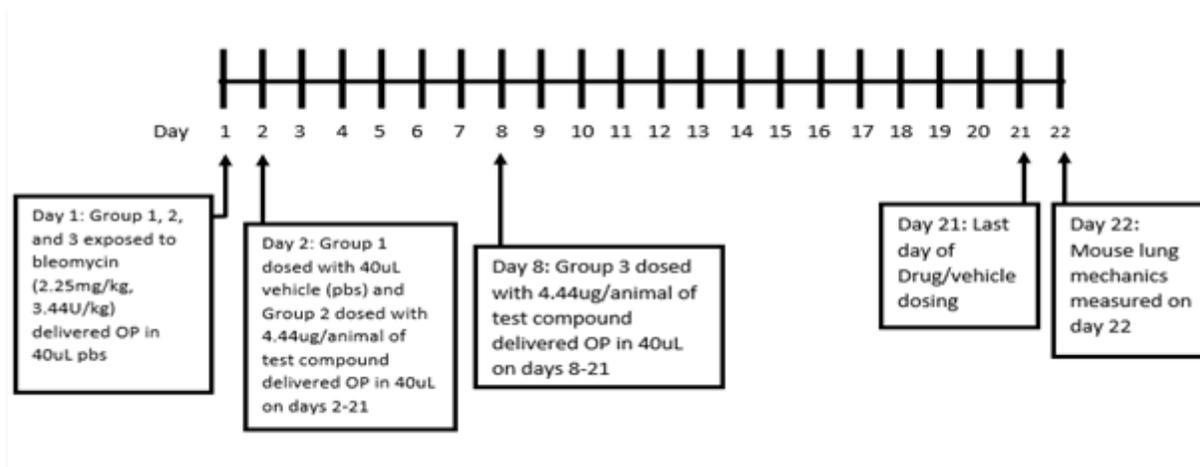
Female C57BL/6 mice 8–12 weeks old from Jackson Laboratories.

Study design (Table 1) On day 1, three randomized groups of mice were given Bleomycin (2.25 mg/kg/3.44 U/kg) by oropharyngeal aspiration as a single daily dose.

On day 2 through day 21, group# 1 (no-treatment control) was given vehicle by oropharyngeal aspiration and group# 2 (preventative “early” treatment group) was treated with Lithium Carbonate (4.44 µg/animal) by oropharyngeal aspiration daily through day 21.

On day 8, group# 3 (therapeutic “late” treatment group) was treated by oropharyngeal aspiration with Lithium Carbonate (4.44 µg/animal) through day 21.

Table 1 Study design



Preventative effects of the treatment were determined by comparing group #1 (no-treatment control) vs group #2 (preventative treatment). Additional therapeutic effects of Lithium Carbonate were analyzed by comparing group #1 (no-treatment control) vs group #3 (therapeutic treatment group).

Establishment of IPF-like phenotype was confirmed by decreased oxygen saturation in all three groups of animals by Day-8 (Fig. 1)

In addition to oxygen saturation, lung function was evaluated using the FlexiVent protocol. FlexiVent is regarded as the “gold standard” for in vivo lung function measurements. Bleomycin induced model of IPF is characterized in the FlexiVent protocol by increased respiratory system resistance, increased tissue elastance, reduced compliance, and shifting pressure-volume (PV) loop downward. Devos et al. (2017)

Statistical analysis:

Comparison among groups was analyzed by 2-way ANOVA. P-values were determined using an unpaired t-test.

Results

Therapeutic effects of Lithium carbonate on Bleomycin model of IPF in this study suggested efficacy of such treatment:

a) Oxygen saturation levels improvement in treatment groups:

Oxygen saturation levels were measured for each treatment group, which revealed: (1) a statistically significant difference between the “early” drug treated group (group# 2) and the vehicle treated group (group# 1) on day 15 ($p = 0.0235$); and (2) a statistically significant difference between both “early” treated group (group# 2) ($p = 0.004$) and “late” drug treated group (group# 3) ($p = 0.0014$) compared to the vehicle treated group (group 1) on day 22. (Fig. 2)

b) Various measures of mouse lung mechanics were evaluated during and after the treatment period.

Rrs (Respiratory System Resistance)

Lung resistance was significantly reduced in both the “early” ($p = 0.0141$) and “late” ($p = 0.0118$) drug treated groups compared to the vehicle treated group. (Fig. 3)

Crs (Respiratory System Compliance)

Respiratory system compliance (the change in lung volume per unit change in pressure) was observed to be statistically significantly increased in “early” and “late” drug-treated mice compared to vehicle treated mice. ($p = 0.033$ and $p = 0.0255$, respectively) (Fig. 4)

H (Tissue Elastance)

Tissue elastance, which is increased in mice with Bleomycin induced model of pulmonary fibrosis compared to normal (naïve) mice, Devos et al. (2017) was observed to be lower in the lithium-treated groups. (Fig. 5)

Cst (Static Compliance)

To further determine the impact of “early” and “late” Lithium Carbonate treatment, static compliance (Cst) was calculated. A) Cst was calculated prior to deep inflation in PV loop 1. Both early and late drug-treated mice demonstrated a significant increase in static compliance compared to vehicle-treated mice ($p =$

0.0379 and $p = 0.0243$, respectively). B) In PV loop 2 both early and late drug-treated mice experienced a significant increase in static compliance ($p = 0.0434$, $p = 0.0283$) after deep inflation compared to animals that received vehicle. (Fig. 6)

PV: Pressure-Volume

PV Loop

Shifting of the PV loop up and to the left indicates that a lower pressure is needed to achieve the same lung volume. Such a shift was observed in Lithium carbonate treated animals (groups #2 and #3) vs no-treated control (group #1) prior to deep inflation, (Fig. 7A) proving that the lungs of treated animals are more compliant. The fact that the PV loop remaining shifted up and to the left, even after deep inflation, (Fig. 7B) which opens closed airways and reduces atelectasis, confirms that lungs of animals in Lithium carbonate treated groups are more compliant than in non-treated control animals.

In this study, mice treated with lithium carbonate as a preventative treatment (group #2), and most importantly as a “late” therapeutic treatment group (group #3), showed significant benefit with lithium carbonate. They were protected from changes in gas exchange (hypoxia) and protected from changes in lung mechanics, experiencing reversion of these changes that are characteristic of pulmonary fibrosis in the Bleomycin induced model of IPF.

Discussion

In spite of recent advances in the management of Idiopathic Pulmonary Fibrosis, this affliction remains an incurable disease with a worse prognosis than many types of cancer. Vancheri et al. (2010) As a result, clinical manifestations are lung dysfunction and ultimately decline secondary to respiratory failure. IPF is the most common and most deleterious type of the over 150 recognized types of interstitial lung disease. Michaelson et al. (2000)

There is still much unknown regarding etiology and the mechanisms of disease progression in IPF, but there are growing lines of supporting scientific discoveries that cellular senescence is a major driving force in the pathophysiology of IPF. Schafer et al. (2017) This hypothesis is supported by changes in age-related disease biomarker(s); telomere shortening observed in IPF patients, (Duckworth et al.) 2020, van Batenburg et al. (2020) and findings that telomere length in lungs of IPF patients is shorter than in their other organs. van Batenburg et al. (2020) As such, Lithium Carbonate is approved for the treatment of bipolar disorder and has demonstrated to elongate telomere length. Martinsson et al. (2013) Squassina et al. (2016) Coutts et al. (2019) Lundberg, Millischer, et al. (2020)

Lithium has multiple cellular effects including inhibition of inositol monophosphatase (IMPA) and glycogen synthase kinase 3-beta (GSK 3-beta), as well as effects on mitochondrial respiration and apoptosis. Malhi and Outhred (2016) Lithium inhibits TGF beta induced epithelial mesenchymal transition. Stump et al. (2006) Lithium, as recently shown by Chen and colleagues reduces migration and collagen synthesis activity in human cardiac fibroblasts by inhibiting Calcium entry to the cells. Chen et

al. (2021) Lithium causes its innumerable physiological and biochemical effects by competing for macromolecular sites that are relatively specific for other cations, most especially for sodium and magnesium. Jakobsson et al. (2017)

Effect of lithium on telomere length is not completely understood, but observed elongation in long-term users could be explained, at least partially, by the fact that expression of telomerase reverse transcriptase positively correlates with duration of lithium treatment in bipolar disorder. Lundberg, Biernacka, et al. (2020)

Our choice of inhalation route of administration was dictated by our intention to deliver our drug directly to affected organ, and decrease the dose and systemic drug level, Rau (2005) taking in account known adverse effects and the narrow therapeutic index of oral lithium treatment. Geddes and Miklowitz (2013)

Is a shorter telomere a risk factor for IPF like smoking, or a major driving force of IPF pathogenesis? Does lithium inhalation produce its positive effects in our animal experiments of Bleomycin induced pulmonary fibrosis through telomere elongation or by a modifying effect of TGF-beta of epithelial to mesenchymal transition, or by inhibiting Calcium entry or other additional mechanisms involved? These and many other questions will need to be addressed in future studies, to better delineate the mechanism behind the observed therapeutic benefits of Lithium in our study.

Conclusion

Our observations suggest that lithium carbonate inhalation may provide effective therapy for IPF patients. Given the lack of effective drugs available to treat IPF, a great need for novel therapeutics exists. Therefore, inhalation of Lithium Carbonate may potentially provide a new therapeutic modality for treatment of IPF patients.

Abbreviations

IPF	Idiopathic Pulmonary Fibrosis
UIP	Usual Interstitial Pneumonia
GERD	Gastroesophageal Reflux Disease
HRCT	High-resolution Computed Tomography
ER	Endoplasmic Reticulum
UPR	Unfolded Protein Response
TGF	Transforming Growth factor
TERT	Telomerase Reverse Transcriptase
TERC	Telomerase RNA Component
AEC	Aortic Endothelial Cell
EMT	Epithelial-to-Mesenchymal Transition
PDGF	Platelet-derived growth Factor
VEGF	Vascular Endothelial Growth Factor
FVC	Forced Vital Capacity
cAMP	Cyclic Adenosine Monophosphate
OP	Oropharyngeal

Declarations

Ethics approval

The animal study was approved by the UNC IACUC ethical commission. UNC is an AAALAC accredited institution. UNC (University of North Carolina) IACUC (Institutional Animal Care & Use Committee) approval protocol number: 17-113. All animal studies were carried out in full accordance with relevant guidelines and regulations. The study was carried out in compliance with the ARRIVE guidelines.

Consent for publication

Not applicable

Availability of data and materials

The datasets generated by this study and analyzed during the current study are available from the corresponding author on reasonable request.

Competing interests

The authors declare the following financial interests which may be considered as potential competing interests: Lispiro LLC is a pharmaceutical company and owns a patent for using inhaled lithium for treatment of idiopathic pulmonary fibrosis.

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Author's contribution

PI conceived of the presented idea. PI and KR designed and planned the experiments. PI and KR analyzed the results of the experiments. PI has drafted the manuscript. KR substantively revised it.

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Figures

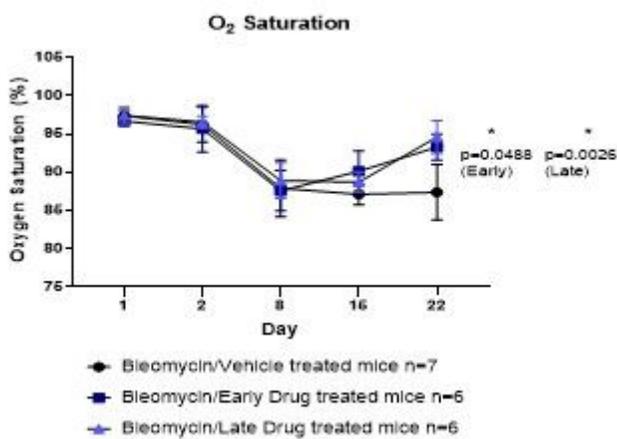


Figure 1

Oxygen Saturation

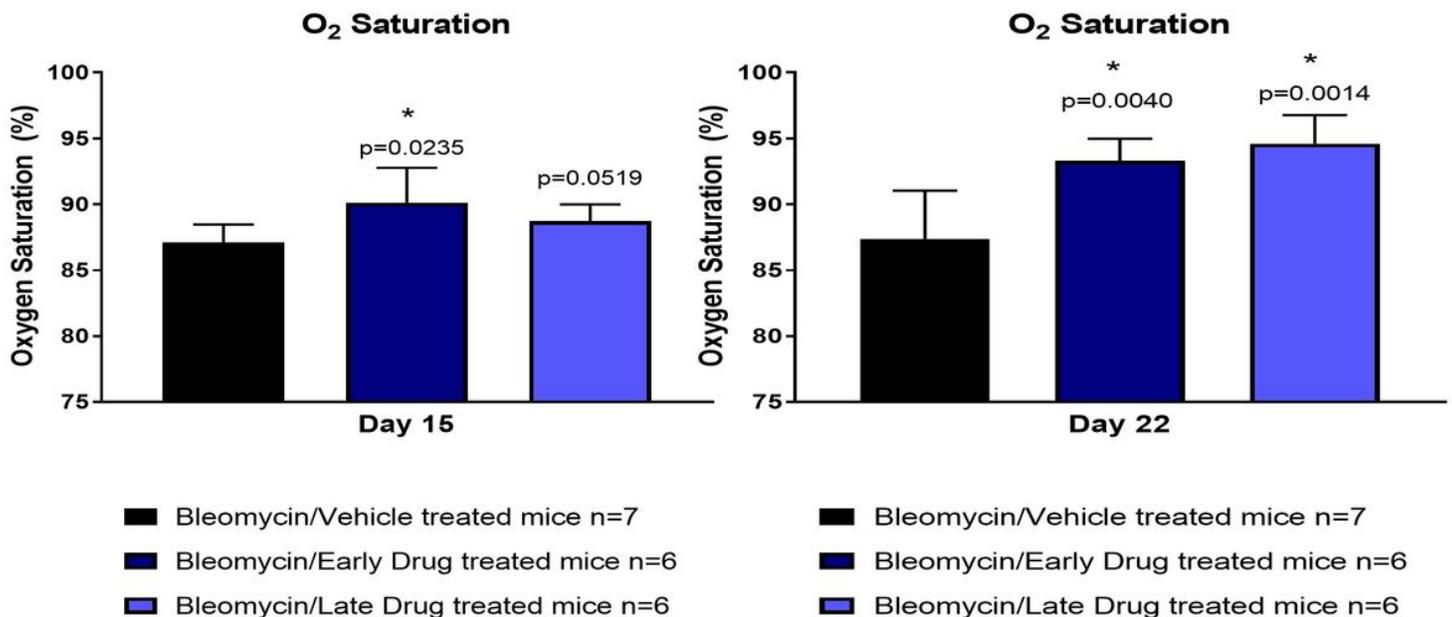


Figure 2

Oxygen Saturation b) Various measures of mouse lung mechanics were evaluated during and after the treatment period.

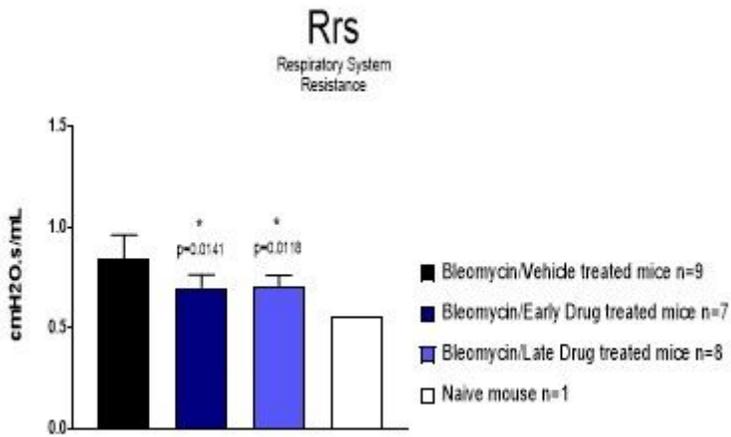


Figure 3

Respiratory System Resistance Following Drug-Treatment

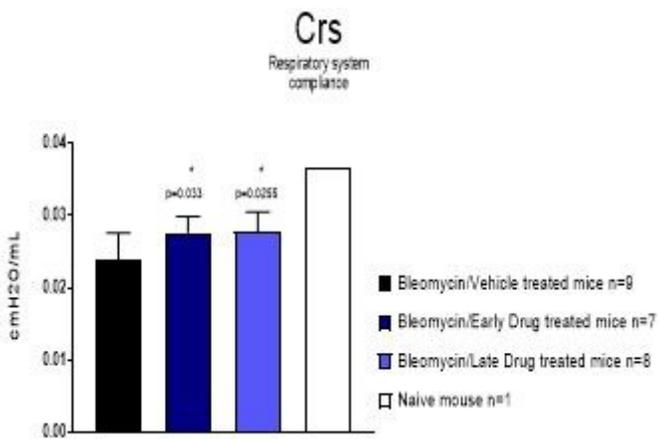


Figure 4

Respiratory System Compliance Following Drug-Treatment

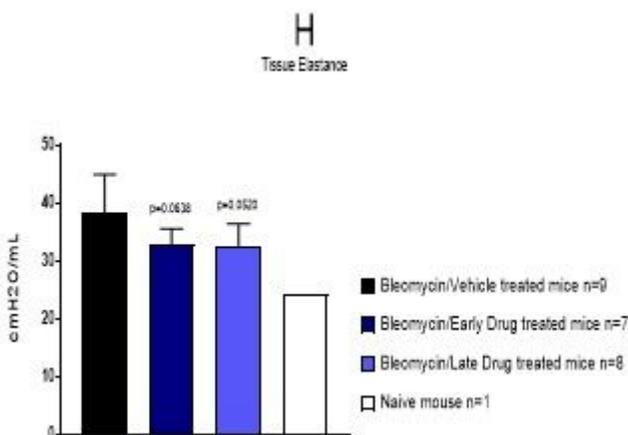


Figure 5

Tissue Elastance Following Drug-Treatment

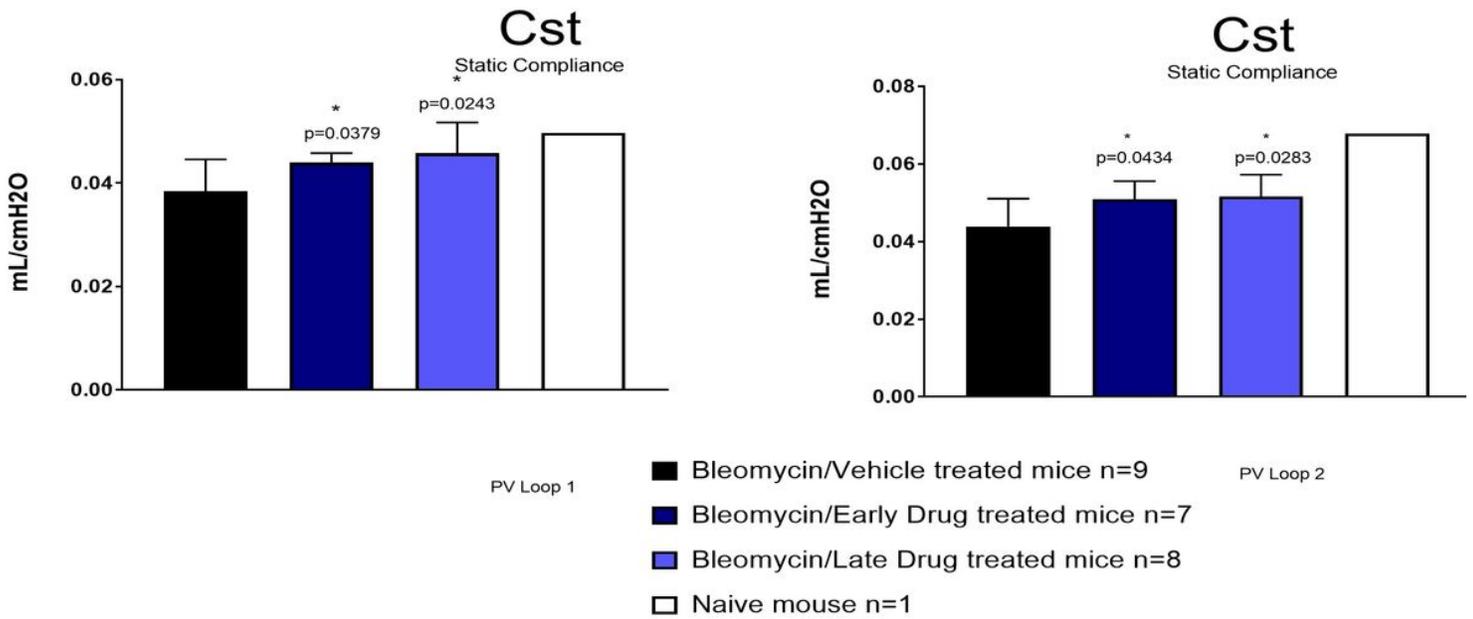


Figure 6

Static Compliance Following Drug-Treatment prior and after deep inflation

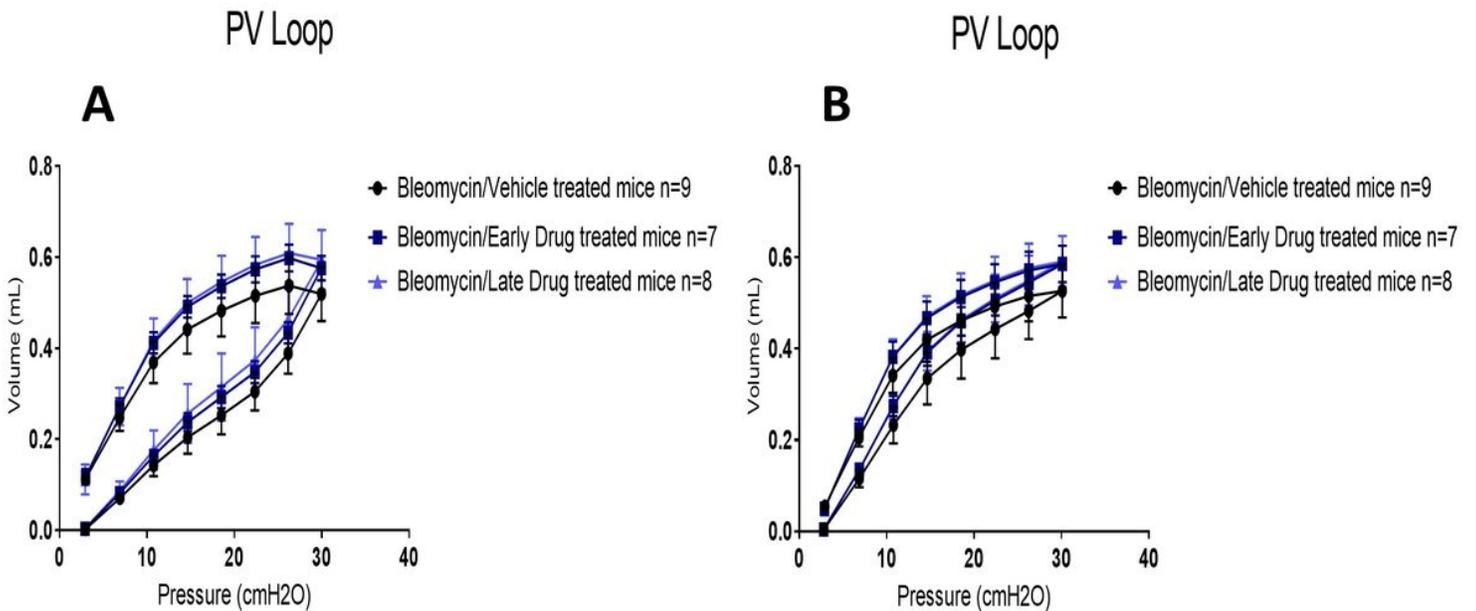


Figure 7

Static Compliance Following Drug-Treatment prior and after deep inflation