Evaluation of lung function and lung fibrosis in mouse models of interstitial lung disease

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Abstract

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INTRODUCTION: Development of mouse models of lung fibrosis requires methods that assess the severity of lung fibrosis and the accompanying decline in lung function. These methods should be quantifiable and clinically-relevant.

In the clinic, lung fibrosis and other pathological changes in the lung are normally assessed using high-resolution computed tomography (HRCT). The decline in lung function that accompanies fibrosis and destruction of the lung architecture is routinely assessed using spirometry techniques. Typically, spirometry measures parameters such as forced vital capacity (FVC) that reflect fibrosis-induced changes in lung compliance and elastic recoil.

METHODS: Using the mouse model of bleomycin-induced lung fibrosis, we are optimizing methods that allow us to quantify lung fibrosis by measuring tissue X-ray CT density using micro-CT. In addition, we assess respiratory mechanics and lung function in our models using the flexiVent (SCIREQ Inc., Montreal, Canada) respiratory mechanics system. These methods allow us to measure respiratory mechanics and lung function parameters that include correlates for the spirometry parameters used in the clinic.

RESULTS: We demonstrate that there is a significant increase in high-density lung tissue in fibrotic lungs in our mouse models of lung fibrosis, including the bleomycin model, as assessed by 3D volumetric visualization and analysis of the micro-CT images. This parallels the development of lung fibrosis as assessed by expression of profibrotic and extracellular matrix-associated genes, histopathological analysis (e.g. increase in Ashcroft score), and an assay for tissue collagen (hydroxyproline).

We also show a reduction in forced vital capacity (FVC) in mouse models of bleomycin- and transforming growth factor β (TGFβ)-induced lung fibrosis, accompanied by changes in lung mechanics that reflect changes in the resistive properties of lung tissue.

CONCLUSIONS: Together, these micro-CT and flexiVent approaches meet the technical challenges posed by the small size of mice, and allow us to recapitulate methods used to monitor interstitial lung diseases and treatment efficacy in the clinic.

Introduction

- **Idiopathic pulmonary fibrosis (IPF) is a restrictive lung disease (i.e. lungs are restricted from fully expanding).**
- **Clinical monitoring of IPF patients, and clinical trials of new therapies, usually rely on morphometric analysis by high resolution computed tomography (HRCT) and on changes in physiological parameters (e.g. restrictive changes in lung function).**
- **Pulmonary function tests of IPF patients typically show:**
  - Reduced lung capacity (e.g. reduced forced vital capacity, FVC)
  - Reduced airflow (forced expiratory volume in 1 second, FEV1), or increased FEV1/FVC ratio

    (because of increased elastic recoil of fibrotic lungs)
  - Reduced lung compliance (i.e. increased lung elastance)
- **We are establishing methods that will also allow us to monitor efficacy of test compounds in mouse models of lung fibrosis using these clinically-relevant techniques.**
- **Our goal is to translate additional clinically-relevant assessment tools into our mouse models.**

Assessment of lung function in mice

Methods

- **Lung fibrosis was induced in mice using two methods:**
  - Intrapulmonary bleomycin.
  - Transgenic overexpression of TGFβ1 in the lung. CC10-tTS-rtTA-TGFβ1 transgenic mice received oral doxycycline to induce the TGFβ1 gene.
- **Twenty one days after bleomycin administration, or TGFβ1 gene induction, mice were anesthetized and lung function was measured using the flexiVent system (SciReq Inc.).**

The flexiVent system (SciReq Inc.)

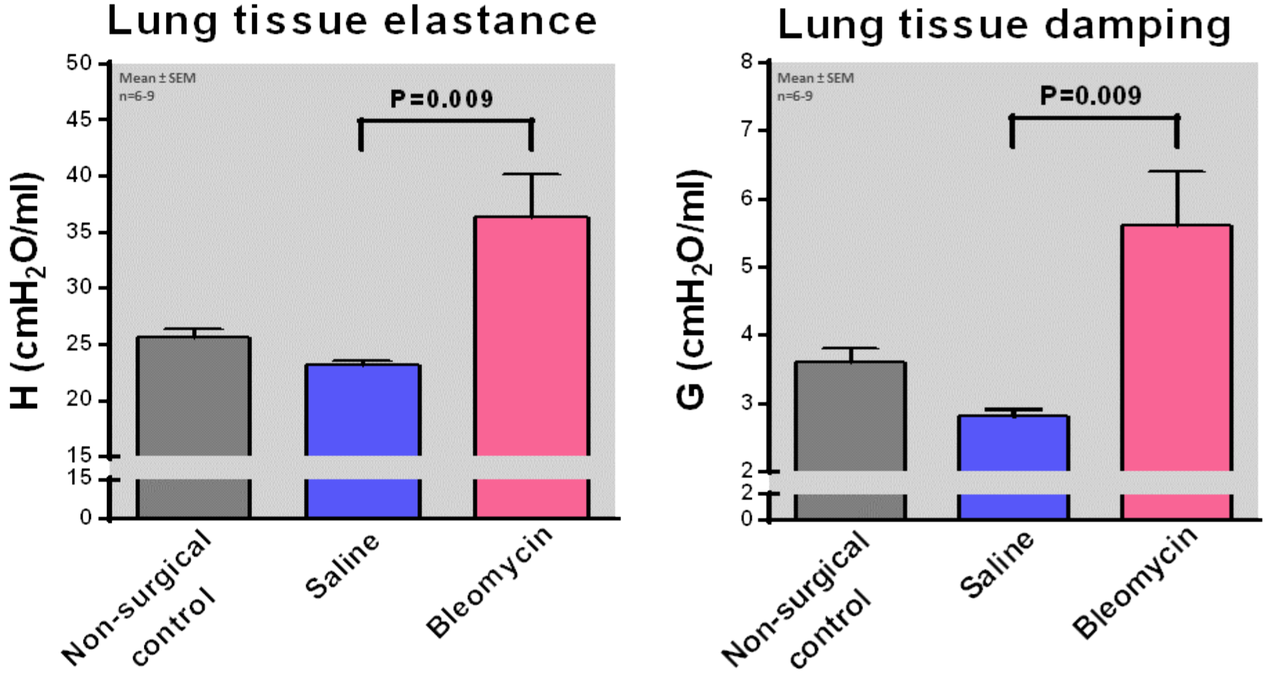
- **The flexiVent system uses a series of preprogramed ventilator maneuvers to measure multiple respiratory mechanics. These maneuvers include:**
  - **Forced oscillations.** Detailed measurements of respiratory mechanics, such as lung tissue elastance and damping.
  - **Pressure-volume loops.** Measurements of clinically-relevant parameters such as respiratory system compliance.
  - **Forced expirations.** Mimics clinical spirometry. Measurement of clinically-relevant parameters such as forced vital capacity (FVC) and forced expiratory volume (FEV).

Lung fibrosis in mice is accompanied by increased lung elastance

Results from forced oscillation maneuvers

An oscillatory volume waveform, containing a wide range of low and high frequencies, is forced on the mouse lungs by the ventilation pump.

The respiratory system input impedance is then analyzed to produce highly-detailed measurements of respiratory mechanics, such as lung tissue elastance and damping.



- **Lung tissue elastance and damping (measures of energy conservation and dissipation in the tissue) are both increased in bleomycin-exposed mice.**

Lung fibrosis in mice is accompanied by pressure-volume changes

Results from pressure-volume maneuvers

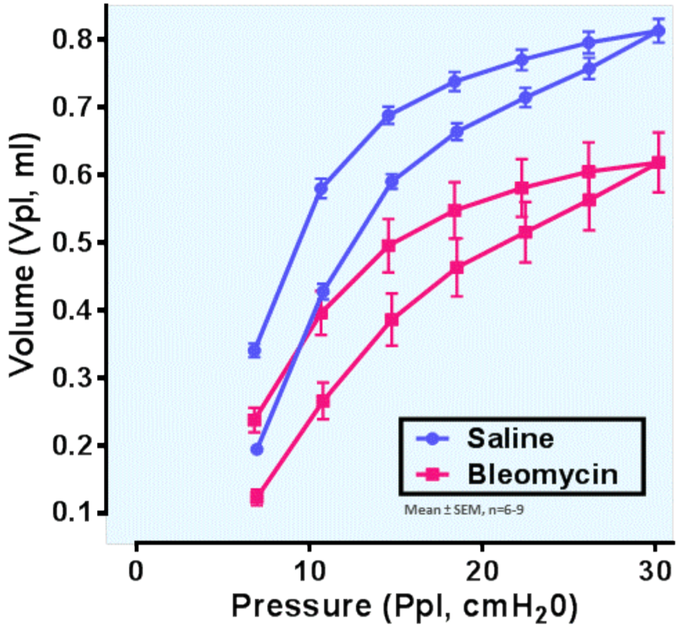
The mouse lungs are inflated in a step-wise manner to total lung capacity, and then deflated.

A pressure-volume loop is constructed that captures the quasi-static mechanical properties of the respiratory system.

This allows calculation of clinically-relevant parameters such as compliance of the respiratory system.

Quasi-static pressure-volume loops from mice

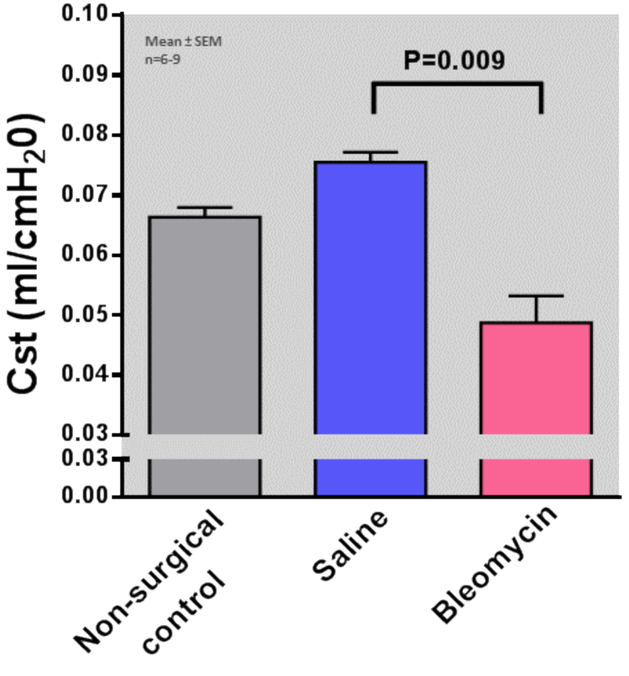
Curves from our bleomycin-exposed mice with lung fibrosis show a shift down and to the right, indicating decreases in lung capacity and compliance.



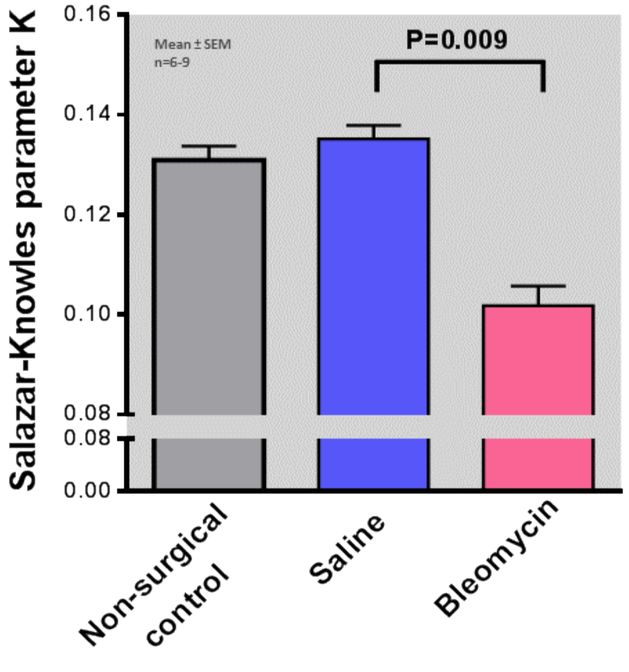
Lung fibrosis in mice is accompanied by pressure-volume changes

Results from pressure-volume maneuvers

Quasi-static lung compliance



Deformation of deflation curve



- Parameters of lung compliance, derived from pressure-volume loops, are reduced in bleomycin-exposed mice.
- The same changes in pressure-volume curves are reported in studies of IPF patients.

Lung fibrosis in mice is accompanied by a fall in FVC

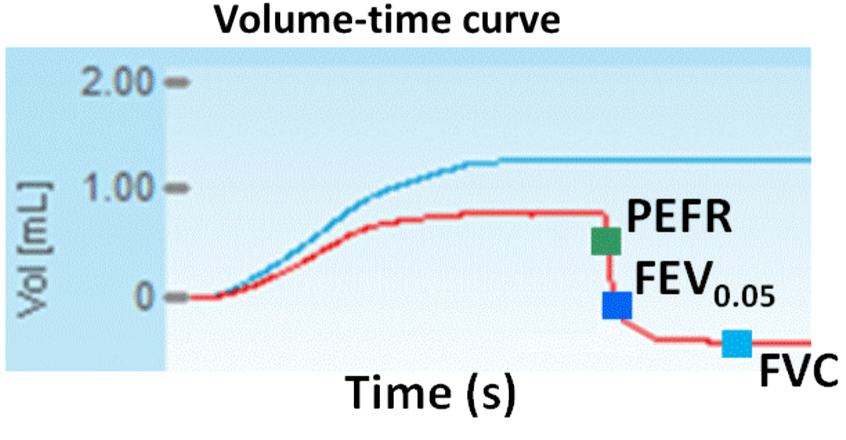
Results from forced expiration maneuvers

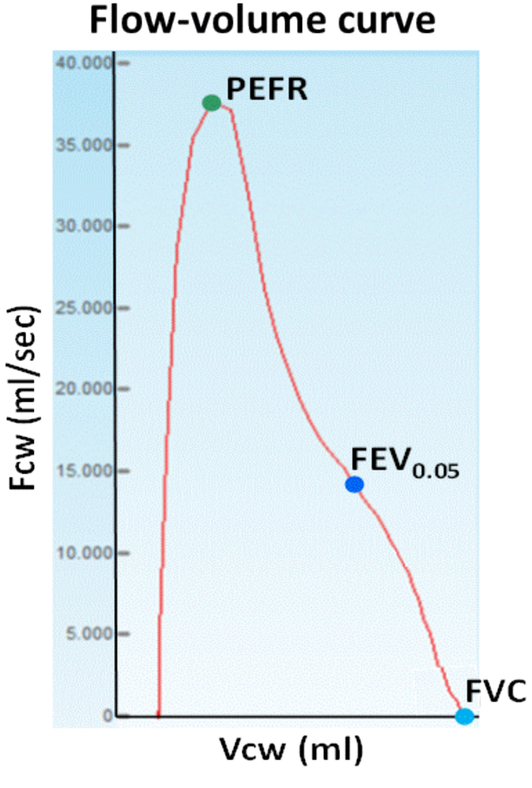
The mouse lungs are inflated and then rapidly exposed to a negative pressure that causes a forced expiration.

This attempts to mimic clinical spirometry, and allows measurement of clinically-relevant parameters such as forced vital capacity (FVC) and forced expiratory volume (FEV).

Typical forced expiration curves from a mouse

Curves from our bleomycin-exposed mice with lung fibrosis show reduced forced vital capacity (FVC) and reduced forced expiratory volume (FEV)

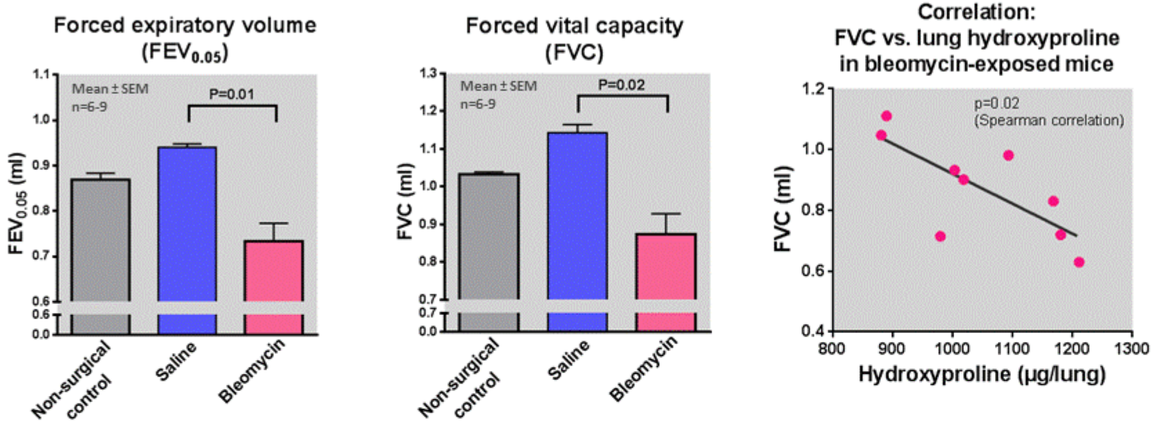




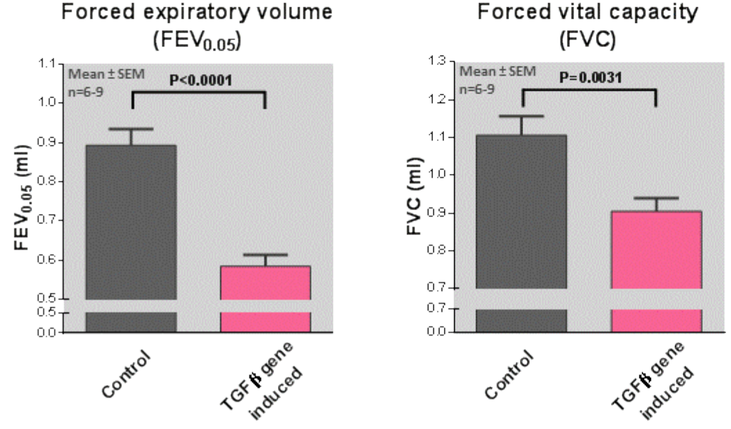
Lung fibrosis in mice is accompanied by a fall in FVC

Results from forced expiration maneuvers

Bleomycin-exposed



TGFβ-overexpressing



- Forced vital capacity and forced expiratory volume are both reduced in bleomycin-exposed, or TGFβ over-expressing mice.
- Reduced forced vital capacity is a commonly used marker of IPF disease progression and prognosis.

Conclusions

- Readouts from mouse models of lung fibrosis should be clinically-translatable.
- The flexiVent respiratory mechanics system now allows us to measure correlates for the spirometry parameters used in the clinic, together with changes in lung mechanics. We have demonstrated that lung fibrosis is accompanied by a significant fall in FVC, together with changes in lung mechanics that reflect increased restrictive properties of the lung tissue.
- We are optimizing methods that allow us to quantify lung fibrosis using micro-CT. We demonstrate that bleomycin causes a significant increase in high-density lung tissue, as assessed by 3D volumetric visualization.
- Together, these flexiVent and micro-CT systems meet the technical challenges posed by the small size of mice, and allow us to recapitulate methods used to monitor interstitial lung diseases and treatment efficacy in the clinic.