Repeat low doses of bleomycin induces progressive changes in murine lung mechanics associated with the development of pulmonary fibrosis

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Introduction

Pulmonary fibrosis is a progressive interstitial lung disease characterised by increased deposition of extra cellular matrix and scarring of lung tissue over an extended period of time. A frequently used animal model of pulmonary fibrosis is a single intratracheal administration of bleomycin; however, this model has been subject to some criticism as certain features of pulmonary fibrosis such as the progressive nature of the disease are not well recreated. The aim of this study was to develop a model to mimic progressive functional aspects of fibrosis observed in patients with IPF by using repeated low doses of systemic bleomycin.

Methods

Male C57BL/6J mice were administered intraperitoneal (I.P.) injections of either saline or bleomycin hydrochloride twice a week for up to four weeks (up to a total of eight injections). Cohorts of mice were euthanized on Days 7, 14, 21, 28 and 35 following the first I.P. injection. Lung function measurements were performed using a forced oscillation system to measure airway resistance, compliance, elastance, Newtonian resistance, tissue damping and tissue elastance. Total and differential cell counts and TGF-β levels were assessed in Bronchoalveolar lavage (BAL) and lungs were fixed in 10% neutral buffered formalin for histopathological analysis.

Results

Bleomycin administration resulted in a progressive weight loss which reached an average of 18% on Day 35 post first injection. No animals had to be euthanized prior to their scheduled day of termination (Figure 1).

There was a progressive decline in lung function observed from Day 14 post first bleomycin injection which peaked on Day 35 (Table 1). Significant changes in lung function included increases in whole lung elastance and corresponding significant decreases in whole lung compliance (Figure 2).

A similar progressive increase was recorded in BAL TGF-β1 concentration, peaking on Day 28 post first injection (Figure 3).

Histopathologically increases in both incidence and severity of interstitial fibrosis was observed peaking on Day 35 post first injection (Figure 4).

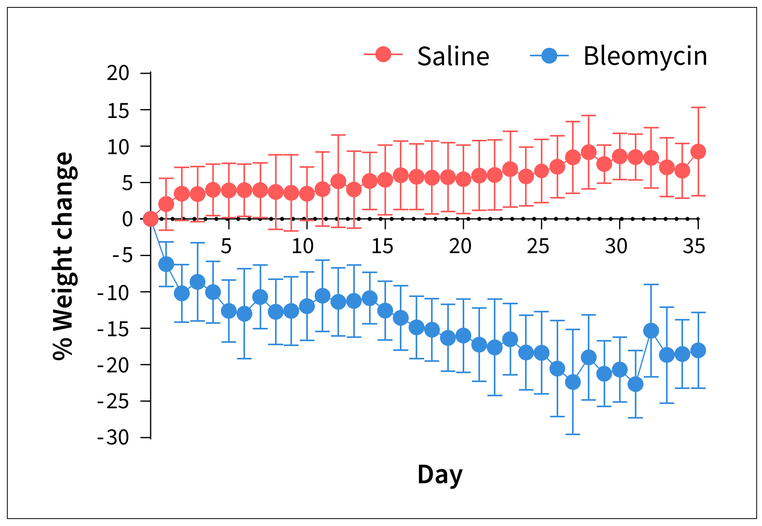

Figure 1. Repeat I.P. administration of bleomycin induces a progressive weight loss.

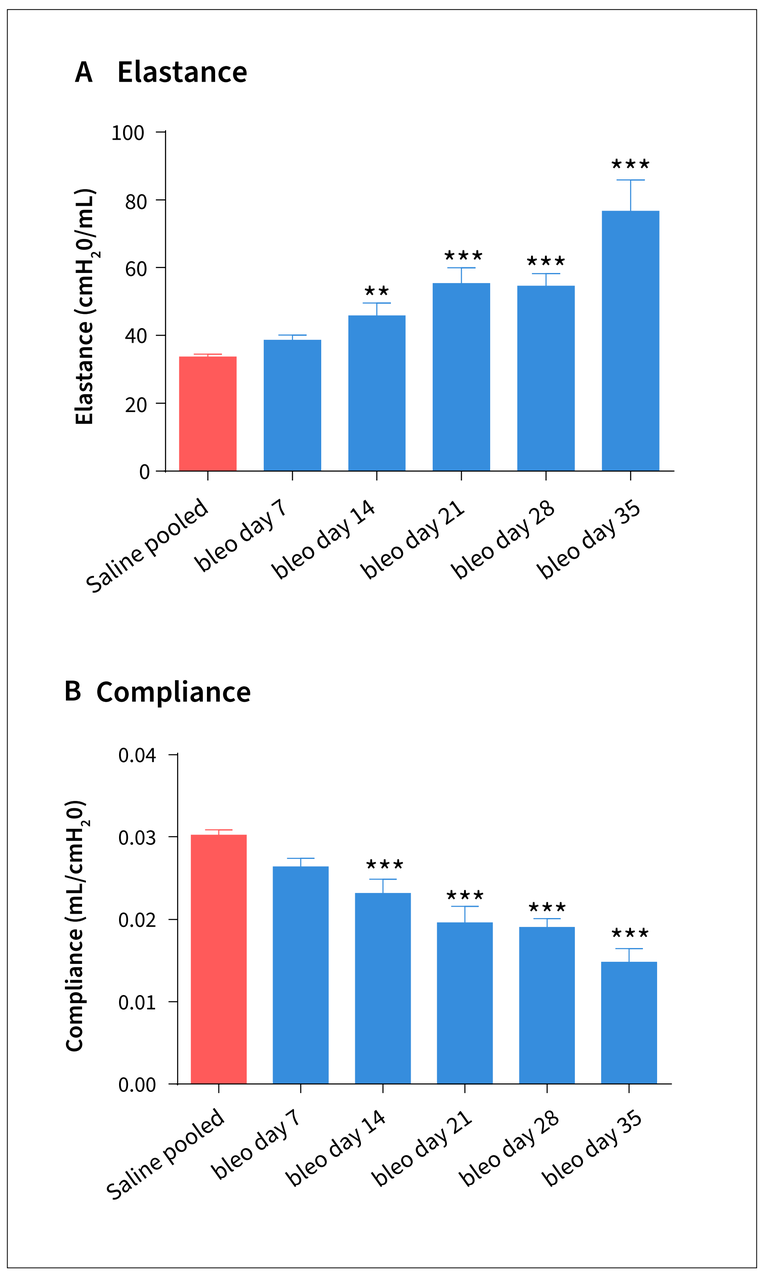

Figure 2. Repeat bleomycin administration induces a progressive decline in lung function. Increases in elastance A and decreases in compliance B suggest a stiffening of the lung tissue.

Figure 3. Repeat I.P. bleomycin administration induces a progressive increase in BAL TGF-β1, peaking at Day 28.

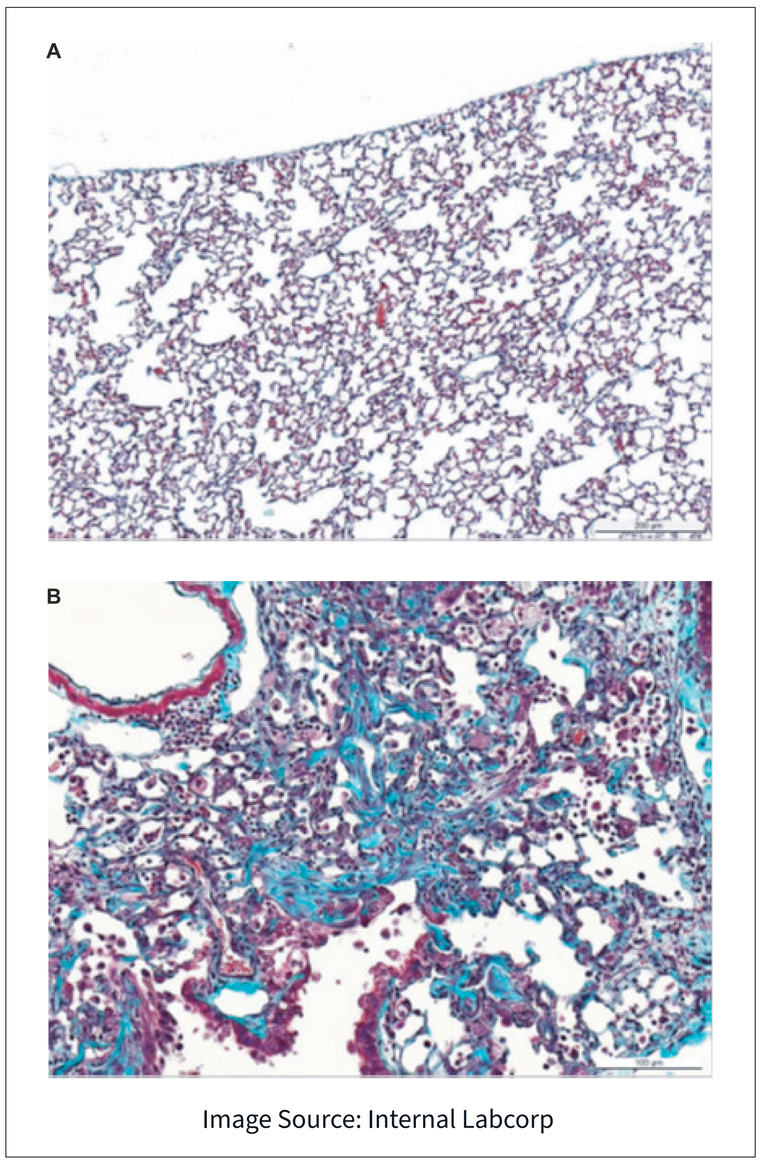

Image Source: Internal Labcorp

Figure 4. Massons trichrome stained lung sections from a saline control animal (A) and a bleomycin treated animal euthanized on Day 35 (B).

	% Change relative to saline control				
	Day 7	Day 14	Day 21	Day 28	Day 35
Airway resistance	+3	+28	+22	+14	+44 **
Elastance	+14	+36 ***	+64 ***	+62 ***	+128 ***
Compliance	-13	-23	-35 ***	-37 ***	-51 ***
Tissue damping	+11	+46 *	+52 **	+33	+62 ***
Tissue elastance	+18	+34 **	+69 ***	+56 ***	+163 ***

Table 1. Repeated bleomycin systemic administration induced a progressive decline in lung function

*** p<0.001, ** p<0.01, * p<0.05 when compared to the saline treated group

Discussion

These results show that repeated low systemic doses of bleomycin led to a progressive pulmonary fibrosis in mice. This repeated injury model may be more akin to how pulmonary fibrosis is thought to develop in humans compared to the single bleomycin dose model that is commonly utilised for the investigation of pulmonary fibrosis. This model may be able to be used to investigate novel anti-fibrotic compounds in a more clinically relevant model.

Animal experiments were conducted according to the Animals (Scientific Procedures) Act, 1986, and 2012 amendments following local ethical approval. Work was conducted in an AAALAC-accredited facility.