Reviewer's report

Title: Structural and functional changes that persist in a segmental challenge model of bleomycin-induced pulmonary fibrosis

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Reviewer: ying sun

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Reviewer comments:

Idiopathic interstitial pneumonias (IPF) is one the Fibrotic Lung diseases with poor prognosis, which shows heterogeneous patterns of both usual and nonspecific interstitial pneumonia (UIP and NSIP respectively). To better understand and define its disease pathogenesis, extensive attempts have focused on the animal models of lung fibrosis and great breakthroughs have been made although almost none has been reflective of the disease process in human. In this manuscript, the authors set up a segmental challenge model of bleomycine-induced pulmonary fibrosis which showed patchy fibrosis predominantly in the lung parenchyma, mimicking the pattern of fibrosis reported in IPF patients. This is a new addition to the animal model pools of lung fibrosis diseases. However, there are several major comments that need to be addressed:

1. Due to the fact that the single-dose model is reported to resolve over time, controls should be set up to compare 1 single dose with 2 doses and 2 weeks with 7 weeks after the last dose, convincing the audience that the segmental challenge model in this manuscript is better mimicking the pathology of human fibrotic lung disease.

2. Other than traditionally systematical or lung specific delivery of Bleomycin, the authors here administered it to the appropriate lung segment via the biopsy port of a fibre-optic bronchoscope, inducing a lobe-restricted fibrosis, which is pathologically different from the defused fibrosis found in IPF patients. It should be clarified what advantages of this delivery system has compared with the traditional ones.

3. In the results part, on page 16, the authors mentioned that “there was no difference in fibrosis scores between these two time points ” and also stated that “inflammation reduced in the tissue at seven-week post-injury time point compared to the two-week time point”. Does it mean that the fibrosis process slows down or stops or maybe begins to resolve? Since some studies reported a self-limiting response in Bleomycin models, combined with these findings here, longer time window should be used to monitor the fibrosis process.

4. Also in the results part, on page 18, Why were TGF- # levels examined at two and six weeks points but not two and seven weeks time points while other parameters were test at seven weeks time point?
5. According to Figure 11, TGF-β level of 4 sheep was elevated while decreased in the other 5 at six-week time point, compared with the two-week time point. Since TGF-β is a potent profibrotic cytokine, does the decreased level mean that the fibrosis process would stop and the body would recover? This point should be explained in the discussion part.

6. Figure 5 showed inflammatory score, fibrosis score and overall score. It’s confusing to mention “overall score” without explaining what it stands for nor where this data came from.

7. In this manuscript, the authors mentioned that no other Bleomycin animal models of pulmonary fibrosis have been reported extended period time, However, Dr. Amber L. Degryse from Vanderbilt University School of Medicine, Nashville, TN set up a Bleomycin model up to 30 weeks.

8. It is confusing about the part of Necropsy and tissue sampling” that if Individual segment was inflated with OCT, how can “each treated segment be fixed in 10% neutral-buffered formalin”? If you want to do paraffin-embedded tissue you must inflate the lung segment with formalin. It doesn’t make sense that the lung sample inflated with OTC was then fixed with formalin for paraffin-embedded tissue sections.

9. In the result part on page 16, Figure 6 should be Figure 7 and Figure 7 should be Figure 6.

Minor points:

10. There are grammer and word usage errors in the main text with some examples outlined below:

i. In page 15 line 336, “than saline” should be “than that of saline.”

ii. In page 16 line 357, “C8” should be “CD8.”

iii. In page 16 line 36, “was still present” should be “still presented.”

iv. In page 19 line 436, “were” should be corrected to “was.”

v. In page 22 line 513, “bleomycine administration in” should be “bleomycin in”

Level of interest: An article whose findings are important to those with closely related research interests

Quality of written English: Needs some language corrections before being published

Statistical review: No, the manuscript does not need to be seen by a statistician.