Reviewer’s report

Title: Increased expression of lung TRPV1/TRPA1 in a cough model of bleomycin-induced pulmonary fibrosis in guinea pigs

Version: 1 Date: 04 Oct 2018

Reviewer: Mariana Brozmanova

Reviewer's report:

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The authors have submitted a well-conceived and conducted study to evaluate expression of lung TRPV1/TRPA1 in a cough model of bleomycin-induced pulmonary fibrosis in guinea pigs. The authors made great efforts in response to their concern using more relevant methods including immunohistochemistry, RT-PCR, histology, and micro-CT scans, which increase the worth of this manuscript. Mostly, the studies are well designed; the techniques appropriate to address the question at hand. The data are interesting, appropriately illustrated and accurately referred. Mostly, the experiments are appropriately controlled. Unfortunately, I have reservations about designing and evaluating cough reflex with some inaccuracies that requires a major adjustment.

There are a few issues to address and some additional details which would benefit the reader. The following points I think the authors might take into account.

Major

1) The model of pulmonary fibrosis is needed to describe in detail, to mention of only single intratracheal administration is insufficient. In which part of trachea was positioned BLM administration?

2) I suppose that cough study design with tussive challenge model is not entirely complete, the authors did not mention using pneumotachograph or it was not used? How did they evaluate the cough response? For objectivity of data did they use only a sound? In that case the results may not be valid. How did authors distinguish cough reflex from sneezing and other cough like artefacts. In addition, there is missing control data in tussive challenge model. From presented manuscript it is not clear at what time point PBS-group was exposed to capsaicin. With respect to true protocol a control group should be tested at the same time as experimental group, it means at days 13 and 27, too. In that case the results are not accurately controlled. Additionally, the authors focused on TRPV1/TRPA1 in cough model of BLM-induced pulmonary fibrosis, therefore why didn’t they test TRPA1-induced cough? It would be more valuable for this manuscript corresponding to TRPA1 expression study.
Because the authors were expected the increased cough response to capsaicin with hypersensitivity. I wonder why they decided to use relatively high concentration of CAPS. I have own experiences with cough studies and 50-70 coughs are extreme response, usually associated with bronchoconstriction, hypersecretion, dyspnea, hypoxia with signs of cyanosis and this is out of welfare of animals. Finally, what was the clinical status of these animals? Results of cough response should be stated in separate section out from body weight. The proportion to cough study is too low. It is not clear when PBS cough was studied. I suggest reporting cough response to capsaicin more in details using numerical values and compare to control values.

3) Discussion. I recommend studying papers referred to subtypes of cough-triggering nerves (Canning, Mazzone, Undem). Besides, it’s needed to recognize jugular C-fibres and nodose C-fibres with different function and so to complete discussion. The previous findings by Coleridge JC and Coleridge HM from 1984 are widespread by a new concept of cough.

4) Fig. 2, mainly 2B is needed to approve, the font size is too small and not unreadable. Fig. 3C and 3D - again the font size should be enlarged. Fig. 4 should be described more more accurately (Aa,b,c,d,e,f) and (Ba,b,c,d,e,f), figures Ag and Bg are small, hardly readable. Fig. 5 I suggest to add a description of the units on y-axis.

5) The language of manuscript needs editing required on some places.

Minor
Grammar - Using articles in front of a noun require a revision on several places.
Page 3, line 15-16: The sentence requires a revision.
Page 4, line 8: correct "vivo" on "in vivo"
Page 5, line 1: What does mean specific pathogen-free mouse facility?
Page 5, line 3: correct "24°C" on "24°C"
Page 5, line 12: correct "to assessed" on "to assess"
Page 6, line 15: correct "Simens" on "Siemens"
Page 7, line 22/ Page 9, line 15: correct "felids" on "fields"
Page 10, line 9: The title of this section requires a revision.
Page 13, line 12: correct "becomes" on "become"
Page 13, line 12: correct "needs" on "need"
Page 14, line 5-7: the sentence requires a revision.

Are the methods appropriate and well described?
If not, please specify what is required in your comments to the authors.

Yes
Does the work include the necessary controls?
If not, please specify which controls are required in your comments to the authors.

Yes

Are the conclusions drawn adequately supported by the data shown?
If not, please explain in your comments to the authors.

Yes

Are you able to assess any statistics in the manuscript or would you recommend an additional statistical review?
If an additional statistical review is recommended, please specify what aspects require further assessment in your comments to the editors.

I am able to assess the statistics

Quality of written English
Please indicate the quality of language in the manuscript:

Needs some language corrections before being published

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