Reviewer’s report

Title: Regulating Autonomic Nervous System Homeostasis improves Pulmonary Function in rabbits with Acute Lung Injury

Version: 0 Date: 27 Dec 2016

Reviewer: Tobias Piegeler

Reviewer’s report:

In their manuscript, entitled "Regulating Autonomic Nervous System Homeostasis improves Pulmonary Function in rabbits with Acute Lung Injury", the authors are evaluating the effect of vagus nerve stimulation, intravenous tetrahydroaminocridine or stellate ganglion blockade on measures of lung injury after tracheal instillation of HCl in rabbits.

The overall idea of the study is interesting. There are some linguistic issues, which can be resolved easily. However, I have several concerns that I'd like the authors to address:

Major:

1. Methods p4/5, "Surgical procedure": Here the description is rather short and therefore also incomplete. Please add the following descriptions and parameters:

   a. Where has venous access been established? What catheter? Were the animals awake during this procedure or anesthetized, e.g. by an inhalational agent?

   b. What was the FiO2? This information is crucial in any study regarding lung injury.

   c. Did the animals receive any form of pain treatment?

2. Methods p5, L33: The authors state that bupivacaine "was continuously administered […] as a bolus injection". This is confusing. Has there been a bolus administration or a continuous administration or a repeated bolus? Please clarify.

3. Methods p5, L41: How was the discharge frequency in the specific nerve trunks measured?

4. Methods p6, L4: How were the leukocytes and PMNs stained and analyzed? Please add this information.

5. Methods p6, "Histological assessment": Besides several language issues, this paragraph contains several points that will need clarification:

   a. L29: The lung W/D ratio is a measure to estimate the amount of pulmonary edema, not necessarily "lung injury" as stated by the authors.
b. L32: after "4 μm" the word "slices" or something similar should be added.

c. There is no detailed description about the assessment of either the H&E sections or the EM sections. How were these slices evaluated? A scoring system? If there was no scoring system applied, this might be something the authors could consider adding, as it might help with quantification and subsequent analysis.

6. Methods p6, "Statistical analysis":

a. Reporting of a mean +/- SEM is inappropriate. Please always report mean +/- SD.

b. There is no information whether the values were assessed for normal distribution. Did the authors do such an analysis prior to statistical testing? If yes, what test was used?

c. If there should have been any non-normally distributed data, further assessment by ANOVA might be inappropriate. In that case non-parametric testing should be used. Please clarify and adjust your statistical assessment if necessary.

7. Results p7: The description of the results is not detailed enough. To me it is not clear, which group has been tested against which (at least regarding Figures 1-3). Additionally, the description of the statistical methods suggests that the groups have been tested against each other via ANOVA at a certain time point. However, no information regarding this issue is provided in the results section and figures 1-3 also do not have any marks, which might help the reader understand the conducted statistical analysis. Also, the authors should consider adding more numbers and values to the text, to enhance the reader's ability to follow the analysis.

8. Results, general: Please consider reporting the exact p-values for ALL reported results, also the non-significant ones. The description of p-values just as > or < 0.05 is inappropriate.

Minor:

1. Abstract p2, L15: The sentence "Then animals randomly received different treatments [...]" should also mention the control groups (saline and HCL only) in order to improve understanding of the experimental setup even in the abstract.

2. Abstract p2, L29: "activity was more active", should be changed to "activity was higher".

3. Abstract p2, L38: "alleviated" should be "alleviate" instead.

4. Background p3, L15: "play" should be "plays" or "may play"

5. Background, p3, paragraph starting in L41: Is acid aspiration really a common clinical feature? The paragraph might be misleading, as I assume the authors just wanted to use HCl instillation as a model (as explained well in the following paragraph on page 4). I would consider omitting this paragraph.
6. Methods p5, "ALI model": To me it is not clear, when exactly the treatment in the different groups was started after HCl instillation (immediately?). Please clarify.

7. Methods, general: For the most part, the description of the methods is lacking detailed information regarding manufacturers or specific description of the equipment and agents used. This should be added.

8. Methods, p6, L24: "killed" should be changed to "sacrificed".

Overall:

The idea of the study is interesting. However, due to the lack of a detailed description of the procedures and the subsequent analysis, interpretation of the results is rather difficult at this point but might be improved after the addition of a more detailed description as outlined above.

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

No

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.

No

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

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