Reviewer’s report

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

Version: 0 Date: 21 Dec 2016

Reviewer: Daniela Sevecova- Mokra

Reviewer’s report:

Aim of the study was to investigate whether the inhibition of sympathetic hyperactivity and/or enhancing parasympathetic activity can alleviate lung inflammation and deterioration of the lung functions in HCl-induced acute lung injury in rabbits.

Design of the study is rather well performed with complex measurement of cardiovascular, pulmonary, and inflammatory parameters. However, I have several comments and objections to the manuscript:

Major comments:

1. Data on the changes of the mean arterial blood pressure are missing. Could you please add these data into the manuscript if they are available.

2. Data and Methods (Par. 4 Measurements): It is not clear where the leukocyte count and examination of PMNs were done (in the blood? in the BAL fluid?). What was the methods of these investigations?

3. HRV methodology is not sufficiently described. Specifically, the part related to methodological processing for ECG recording is missing. Furthermore, it is not clear which method for linear HRV analysis was used - autoregressive analysis or Fast Fourier transformation?

4. Major criticism is related to frequency band in HF-HRV ranged from 0.15-0.4 Hz that is appropriate for analysis of RR intervals in humans. The HF-HRV strongly depends on respiratory rate, therefore, the frequency range used in this study is not appropriate to respiratory rate in rabbits. In this context, the interpretation of HF-HRV is vague. I suggest to add publications where the heart rate variability has been evaluated in rabbits, e.g., J Physiol Pharmacol. 2013;64(6):751-759, Can J Physiol Pharmacol. 2008;86(11):804-814, Acta Vet. Brno 2006, 75: 3-12. Moreover, the authors should describe procedure for removal of potential artefacts from ECG recording.

5. The length of RR intervals used in HRV analysis (short-term or long-term analysis) is crucial for correct interpretation of the results. This information is missed in the manuscript.

6. In the part Data and methods, the authors should describe calculation of LFnu and HFnu.
7. Data and methods (Par. 5 Histological assessment): For how long and at what temperature was the lung tissue dried for estimation of wet-dry lung weight ratio? What pathological changes in the lung tissue observed by light microscope and electron microscope do you mean? More details should be provided on the methods.

8. In the part Discussion, the interpretation of LF/HF as an index of sympathovagal balance is controversial. Importantly, short-term HRV is predominantly mediated by parasympathetic activity, therefore, the description of changes in sympathetic nervous system from short-term HRV analysis is questionable.

9. How can you explain the second peak of HRV response at 6 h after the treatment?

10. The part of Discussion (Par. 6), where changes in the parameters of HRV are given into the relationship with inflammatory markers should be little enlarged. I suggest to add information from the review in Respir Physiol Neurobiol. 2013;187(1):78-81.

11. Generally, English should be improved throughout the manuscript including Abstract.

Minor comments:

1. Please use „autonomic nervous system" instead of „automatic nervous system".

2. In Data and Methods (Par. 1 Animals), I suggest to explain again the abbreviations for expression of the groups 3 (VHS), 4 (THA) and 5 (SGB) for better understanding.

3. In Data and methods (Par. 2 Surgical Procedure), I suggest to explain why the lactated Ringer's solution was infused into the trachea of rabbits at that dose (10 ml/kg/h).

4. Data and methods (Par. 3 ALI model and treatment): It is not clear from the text how bupivacaine was administered - continuously or as a bolus injection?

5. Data and Methods (Par. 4 Measurements): Please use „blood gases" instead of „blood gas".

6. Discussion, Par. 3, Sentence 3. I suggest to change it to : „However, a local anesthetic (bupivacaine) was given ... „

7. Discussion, Par. 4, Sentence 3: „Lyudmila et al. suggested... (15)" Are you sure that this is a correct reference? Lyudmila et al. is not present in the list of references.

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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