Author's response to reviews

Title: Comparison of the Effects of Moderate and Severe Hypercapnic Acidosis on Ventilation-Induced Lung Injury

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Author's response to reviews: see over
Dear reviewer:

1. I am still unclear as to whether this was a randomized study. The authors state that ‘animals were randomly assigned to one group, then randomly assigned to another group etc. Can they provide further detail on this, as I don’t understand how the animals can have been randomly assigned if they were only assigned to one group, then subsequently to a different group. The key question is: did each animal have an equal chance of being allocated to any of the 5 groups studied? If the answer is ‘no’ then this was not a random allocation method. I don’t see this as a critical issue, but it is important for the reader to understand how exactly the animal allocation was performed. Given this, please detail the randomization method in the manuscript.

Answer: We have revised in the page 6 line 107.

**Experimental groups.** Seventy two rats were randomly assigned to 8 blocks of 9 animals each, with random numbers generated by SPSS (version 13.01S; Beijing Stats Data Mining Co. Ltd, Beijing, China). Among them, two blocks were randomly assigned to the sham group (anaesthetized and non-ventilated rats) and NV group (normal ventilation with PIP=15 cmH$_2$O for 4 h) served as controls for assessing the expression of NF-kB p65 protein and the inflammatory mediators in the lung. The left 6 blocks were assigned to three groups through merging two blocks of rats randomly, and including the Normocapnia (NC) group (PaCO$_2$=35-45 mmHg, n=18), the Moderate Hypercapnic Acidosis (MHA) group (PaCO$_2$=80-100 mmHg, n=18), and the Severe Hypercapnic Acidosis (SHA) group (PaCO$_2$=130-150 mmHg, n=18).

2. I also do not understand why the authors do not have the data available for the lung injury score determination for the NV group in figure 2. Contrary to their response to my point, they have presented the physiologic data for these animals in Figure 1. Also, they have stained tissue from these lungs for ICAM-1 and P65 expression. Therefore, it should just be a matter of H+E staining these slides for determination of the LIS and presenting the data and representative figures.

Answer: There is a mistake of the problem, just we only observed the vary levels of Paco$_2$ and its impact on the development of lung injury induced by high-pressure ventilation without clouding the results with other potential variables. So we have not provided a H+E for the NV group in Figure 1. However, according to the reviewer’s suggestion, we put the H+E of the NV group into the figure 2, and provide the lung injury score determination for the NV group in figure 2E. Please see in the Figure 2.

3. It is also a good idea to put the sample size calculations into the methods, given that the authors went to the trouble of performing this calculation and using it to guide their experimental design.

Answer:
Power calculations were performed prior to the commencement of the study. A sample size of 8 in each block will be sufficient to detect a difference of 0.1 U/g in MPO between the treatment and the control groups assuming a standard deviation of 0.1 U/g as reported in this population, at 80% power and 5% level of significance. This number has been increased to 9 per block (total of 72) to allow for a predicted drop-out from treatment of around 10%.

We have revised in the Statistical analysis section.