Author's response to reviews

Title: The effect and mechanisms of tight junctions protein occludin in ventilation induced lung injury

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Author's response to reviews: see over
Cover Letter

Dr. Tim Shipley,
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Dear Dr. Tim Shipley:

Thank you for your suggestions of my manuscript, my manuscript has already been copy-edited as per recent request, now I give a point-by-point response to the concerns.

1. I corrected the spelling and grammar errors in the manuscript.
2. The reviewer considered that appreciable editing and re-writing is necessary. Therefore some of lines in the manuscript have been reformatted for readability.
3. I made some changes in “Abstract”: page 2, line 17-20.
   The original text is as follows: Rats were sacrificed after mechanical ventilation. Specimens of lung tissues were harvested. Lung pathological changes were observed with optical microscope, and lung wet/dry weight ratio was measured. The occludin protein level was assayed with Immunohistochemistry staining and Western blot.
   The original text is as follows: The upper lobe of right lung tissue was quickly frozen in liquid nitrogen for Western blot analysis, the remnant right lung tissue was fixed in 4% paraformaldehyde 48~72h for HE and immunohistochemical staining, the left lung tissue was used to measure the wet lung weight, after that, the tissues were placed in an oven and maintained at a temperature of 70°C for 72 hours to gain the final dry weight.
5. I modified the steps of “Immunohistochemical staining”, and increased the specific operation steps: from page 4, line 29 to page 5 line 13.
6. Two reviewers asked the same question—“why it was chosen 4 hours of mechanical ventilation?” I have increased some evidence in the first paragraph of “Discussion”: page 8, line 1-11.
   The original text is as follows: About the tidal volume of mechanical ventilation in rats, generally set low tidal volume was 6 ~ 10 ml/kg and high tidal volume was 35 ~ 40 ml/kg[^4]. Ventilator induced lung injury is closely related to hyperventilation of tidal volume ventilation (eg: 40ml/kg, 4h). Normal tidal volume ventilation had no distinct effects on lung tissue[^3]. Therefore this study make ventilation induced lung injury model through setting tidal volume at 40ml/kg, ventilation 4h and with low tidal volume 7 ml/kg as a control. This study show that after mechanical ventilation 4 h, if serious pathological changes occurred in the rats lung tissue such as pulmonary congestion, edema, pulmonary interstitial inflammatory cell infiltration, a non-transparent film formation and W/D ratio increases, it can be considered the model of ventilation induced lung injury is success.
7. The “Results” also have some changes. I have added some details in each result.
   Some details were added in “The ratio of Wet/Dry weight in lung”: page 6, line 15-17.
   Some details were added in “The pathological changes in rat lungs ”: page 6, line 22-27.
Some details were added in “Immunohistochemistry for Occludin”: page 7, line 4-5.

Some details were added in “Western blot”: page 7, line 9-14.

8. I made some changes in “Conclusion”: page 12, line 1-6.

The original text is as follows: Finally, we conclude that mechanical ventilation can activate the PKC signaling pathway and induced the reduction of occludin in a volume-dependant manner. Pretreatment with PKC inhibitors increased occludin expression, which can reduce and delay the mechanical ventilation induced lung injury, it illustrated that ventilation-induced lung injury may be associated with the expression of occludin. But the exact mechanism still needs to be further explored.

9. I added the “Acknowledge” at the end of the manuscript: page 12, line 16-17.

The original text is as follows: The authors thank Medical Research Center of QianfoShan Hospital of Shandong Province for equipment support and technical assistance.

Thank you very much for your attention and consideration to our paper. If you have any questions please do not hesitate to contact me. I'm looking forward to receiving your reply as soon as possible.

Best regards.

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