Reviewer's report

Title: Effect of low tidal volume ventilation on lung function and inflammation in mice

Version: 2 Date: 15 February 2010

Reviewer: Vincenzo Cannizzaro

Reviewer's report:

General comment:
Some of the reviewer's issues have satisfactorily been addressed by the authors. However, many critical issues remained unanswered heavily compromising the publication of this manuscript.

Major compulsory revisions

Comment 1: Abstract:
Background: "In contrast data on the effects of low tidal volume ventilation are sparse."
This sentence should be rephrased:
In contrast data on the very short term effects...

Objective: "To investigate the functional and structural effects of low tidal volume ventilation in a mouse model."
This study, as presented, also investigated the effects of high VT ventilation. This important fact should be mentioned, too.

Results: "Oxygen saturation did not change significantly over time of ventilation in all groups (P > 0.05). However, oxygen saturation was significantly higher in the group that was ventilated with a Vt of 30 ml/kg compared to all other groups (P < 0.05)."
This statement can be misleading since it is not evident that oxygen saturation in the high VT group was elevated=normal from the beginning whereas it was very low for all other study groups.

Results: "Mechanical ventilation significantly increased infiltration of the lungs with PMN (P < 0.05). Expression of MIP-2 was significantly induced by mechanical ventilation in all groups (P < 0.05). MIP-2 mRNA expression was lowest in the group with a Vt of 10 ml/kg + PEEP."
One major finding not reported in the abstract is the fact that high VT ventilation without PEEP produced significant elevations in MIP-2 and TNF alpha. A distinction between differences compared to spontaneously breathing animals and only ventilated groups should be made and interpreted.
Conclusions: "Our data show that mechanical ventilation with lower and higher tidal volumes than 10 ml/kg results in worse lung function and enhanced inflammation."

This statement is not in line with the reported results since only G and H significantly increased while changes in resistance were not statistically significant, except for 5 mL/kg without PEEP. Also, add "very short term" (mechanical ventilation...).

"Formation of atelectasis and inadequate oxygenation may be important factors."

What is the rationale for assuming that high VT ventilation on top of PEEP results in atelectasis – as opposed to low VT without PEEP?

"Application of PEEP attenuated inflammation and ameliorated lung function."

Comment 2:
Number of animals per group should be similar, particularly since results from the 30 mL/kg study group are consistently presented AND compared with other study groups. The reviewer thinks that n=3 in the high VT group is insufficient to draw conclusions. Alternatively, authors may consider the presentation of study results without the 30 mL/kg study group, focusing on low VT ventilation strategies as stated in the abstract and title.

When considering PMN, MIP-2, and TNF alpha, high VT seems to be injurious, these are major findings the authors do not adequately discuss in their manuscript.

Comment 3:
Quality and resolution of figures is just sufficient for reading, but insufficient in case of publication.

Comment 4: Figure 1A:
The reviewer wonders why at baseline the difference between 30 mL/kg and all other groups is statistically significant, while after 30 min 30 mL/kg is not significantly increased when compared to 5 and 7 mL/kg? Please comment.

Moreover, oxygen saturation between study groups is statistically significant at baseline. Study groups exposed to lower VT likely experienced severe hypoxia. Apart from the relevant bias when comparing study groups at different baseline condition, I question whether oxygen saturation measurements below 75% are precise. Pulse oximetry devices (MouseOx included) generally become less reliable when measuring low levels of oxygen. Please comment.

Based on Figure 1A the 5 mL/kg study group had an oxygen saturation of 40-50% after 30 min of ventilation. This is extremely low when considering that primary healthy animals were ventilated for 30 min only. What is the practical and translational value of this ventilation strategy in healthy mice? Please comment.

Comment 5: Figure 5A and B:
From Figure 5 it seems reasonable to assume that TNF alpha concentration after high VT without PEEP is significantly elevated when compared to all other study groups. This finding should be highlighted and discussed. In addition, it is not clear whether statistics were used for additional comparisons, i.e. intragroup comparison with PEEP and intragroup comparison without PEEP (in addition to comparison with spontaneously breathing animals). From Panel A it could be concluded that MIP-2 concentrations were higher in the 5 and 30 mL/kg without PEEP groups when compared with the 7 mL/kg without PEEP group. Please comment.

"However, information on the use of lower tidal volumes e. g. 6 ml/kg is sparse."
Why is there a need to investigate this research question? Please provide examples to justify and emphasize the need to study very low tidal volumes in healthy mice. Irrespective of the “second goal” the reader should know whether information gained from this investigation has practical relevance in mice and/or translational value.

Comment 7: Methods:
"Mechanical ventilation was carried out for at least 30 min until a drop in oxygen saturation of > 20% or a pulse rate below 200/min." How many animals were affected (each group)? This answer has not been provided by the reviewer's revision.

Comment 8: Methods/Histology:
Though authors answered that “Data on inflation and fixation are provided (page 5, lines 25-26)”, this is not the case. Please provide values, i.e. inflation pressure

Comment 9: Results:
How many animals survived the 30 min ventilation protocol? Please provide information for each study group. This is essential to interpret results at the end of the study. Did the authors exclude animals when presenting the 30 min results?

Comment 10: Discussion, first para, page 10:
"In the present study we found that mechanical ventilation with lower tidal volumes than 10 ml/kg and the loss of PEEP were associated with worse lung function and increased expression of inflammatory mediators in a murine model."
Though n=3 is insufficient, the important and relevant findings with high VT ventilation should also be included. Alternatively, as stated above consider omission of the high VT study group.

Comment 11, Discussion: Page 10, last line:
"These data may serve as a basis for future experiments to evaluate ventilator-induced pulmonary changes below the lower inflection point in murine models."
This sentence is not clear to me. Relevant ventilator-induced lung injury occurs after many hours of ventilation. How can data from this very short-term ventilation study serve as a basis for evaluation of pulmonary changes (what kind of?) below the lower inflection point? Particularly, since no data on PV-curves are provided.

Comment 12: Page 11, para 2, first sentence:
"In the present study low tidal volumes were associated with lower oxygen saturation during mechanical ventilation." In the reviewer’s view this sentence should be rephrased as follows: …were associated with very low oxygen saturation…

In addition, how do the authors interpret the severe hypoxia at the beginning/at baseline? Extreme atelectasis in primary healthy mice following a lung volume history standardisation? Please comment.

Comment 13: Page 11, para 2, line 8:
"With the addition of PEEP survival time increased and oxygen saturation improved."

As stated above information on survival rates AND time should be provided in this study. Changes in survival time are mentioned in this part of the manuscript, but no data are presented in the results section.

Comment 14: Page 11, para 2, line 9:
"Although we did not measure the lower inflection point it seems that the PEEP used in our study was high enough based on previous data from the literature (18). On the other hand, if the PEEP is too high it carries the risk of hyperinflating the lungs and air leaks (19, 20)."

As opposed to the mentioned references (e.g. mathematical model, ex vivo rat lungs, and rabbits), recent studies in mice have clearly shown that a PEEP of 2 cmH2O is insufficient and that higher PEEP levels are not injurious in mice with primary healthy lungs after 150 min of ventilation (Allen GB et al, 2006, J Appl Physiol; Cannizzaro V et al, 2009, Resp Physiol Neurobiol). Unless the authors can provide comparable, i.e. in vivo mice studies, references supporting their statement, the above mentioned sentences need to be adjusted/deleted/modified.

Comment 15: Page 12, para 2:
"Resistance (Rn) increased during ventilation if no PEEP was applied. In contrast it remained stable with the application of PEEP except for high tidal volume ventilation (30 ml/kg). Our data agree with a study by Wilson and co-workers (21). In that study resistance showed a significant increase with high tidal volume ventilation and a small not significant decrease with low tidal volume ventilation (9 ml/kg) (21). The significant increase in tissue damping (G) reflecting tissue resistance after 30 min of ventilation supports the findings with Rn."

Based on Figures 2A and B resistance only increased after 5 mL/kg ventilation
without PEEP. This is in contrast to the first sentence. Only statistically significant differences should be discussed here, i.e. discussion of not significant elevations may be misleading.

Comment 16: Page 14, para 1:
"In our experiments mice were ventilated for 30 min. This may be considered as a very short time frame. However, changes in lung function parameters, in neutrophil influx, and in cytokine expression could be observed compared to spontaneously breathing animals. Moreover, this time frame enabled us to apply different tidal volumes without additional oxygen to stabilize oxygen saturation. This minimizes a potential confounding effect of oxygen."

Rephrasing is warranted for the following sentence:
This has to be considered as a very short time frame.

Comment 17: Page 14, para 2:
"Oxygen saturation was significantly lower in the groups with lower tidal volumes than 30 ml/kg. Some of them have to be considered as hypoxic. However, inflammation was mostly increased with very low or high tidal volumes. Therefore hypoxia may not be the only cause for changes in inflammatory markers."

Rephrasing is warranted for the following sentence: Some of them were hypoxic OR were exposed to severe hypoxia (oxygen saturation below 75% already at baseline).

Comment 18: A limitation paragraph/subsection is warranted to highlight important study limitations such as very short term protocol, survival issues (unclear how many animals per group survived), hypoxia, no information on blood gas analyses, one respiratory rate for different VT.

**Level of interest:** An article whose findings are important to those with closely related research interests

**Quality of written English:** Acceptable

**Statistical review:** Yes, and I have assessed the statistics in my report.

**Declaration of competing interests:**
I declare that I have no competing interests