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
Reviewer: Maria JC Carmona

Reviewer's report:
The authors compare the effects of moderate and severe hypercapnic acidosis on high pressure ventilation-induced inflammatory injury and evaluate the role of the NF-KB pathway in this process. The topic is original and important, considering the potential protective effects of hypercapnic acidosis on lung injury and the uncertainty regarding appropriate PaCO2.

Throughout the text, all abbreviations should be carefully described (e.g. BALF and BAL for bronchoalveolar lavage fluid). The English should be revised.

We have revised all abbreviations throughout the text.

ABSTRACT: the sample size, summary of the statistical analysis and main numerical results could be presented in the abstract.

We have revised in the abstract.

INTRODUCTION: - I suggest the authors present evidences that PIP of 30 cm of H2O promotes VILI (stretch induced injury), as well as the other causes of VILI.
We have revised in the introduction.

METHODS: The method is appropriate and adequately described, but some points should be considered:1) The sample size should be justified.

The sample size was determined (based on preliminary experiments) to be 54 animals (n=18 per group, in the NC,MHA,SHA groups) in order to detect a difference in the lung injury score of 7.8, an estimated standard deviation of 2.2, a power of 0.80, and a value of 0.05. However, we have not put it in the methods section according to the other published articles.

2) How long the animals were ventilated in the experimental group. This information is clear in the discussion, but not in the method section.
We have revised in the method section on page 7, line 117.

3) Page 7, lines 118-122: specify that the anesthesia technique (pentobarbital and pancuronium bromide) is related only with NC, MHA and SHA groups (not all rats). We have revised.
4) Concerning to BALF, the aspiration technique alters the cellularity and could be better explained (manual or vacuum suction?).
We have revised in the method section on page 8, line 140.

5) The authors mentioned that a gas mixture of carbon dioxide with oxygen was administered to maintain the target PaCO2 in the NC, MHA and SHA groups. How the FiO2 was evaluated during this period? Was the previously mentioned FiO2 (0.7) constant?

Inhaled and exhaled CO₂ and O₂ were tested using a gas monitor (DATEX Instrumentarium, Helsinki, Finland) in the method section on page 7, line 134.

RESULTS: 1) It could be interesting the inclusion of a flowchart with the distribution of the animals between groups, as well as the exclusion data and final analysis.

Thanks for the advice by the reviewer, we have explained the experimental groups in the method section on page 6, line 107, and the exclusion data in the result section on page 11, line 204.

2) Do the authors have data concerning the respiratory mechanics in NC, MHA and SHA groups?

We have data concerning the tidal volumes (Vₜ) in the table 1 of three groups, but not the respiratory mechanics.

DISCUSSION: I suggest the authors discuss the PIP of 30 cm of H₂O as the cause of VILI (stretch-induced injury). What are the evidences that VILI is related to PIP? Could it be due to FiO2, PEEP, or a combination of these factors?

We have revised in the discussion section on page 14, line 262.
Reviewer: John Laffey

Reviewer's report:
Yang et al present data from an in vivo animal experiment in which they elucidate the dose response characteristics of acute hypercapnic acidosis in the setting of VILI in the rat. The authors find that moderate hypercapnic acidosis is more protective than severe hypercapnic acidosis, and is better tolerated in terms of haemodynamic and oxygenation indices. I have a number of comments.

Major compulsory Revisions
1. It would be helpful if the authors could clarify why they selected their ventilator settings. What made them decide on these particular ventilator settings, particularly the PIP and the FiO2? VILI studies usually quantify their inflation settings as tidal volumes in ml/kg, but there is no mention of tidal volume here. We have revised in the discussion section on page 14, line 262.

2. Please clarify whether the in vivo experiments were carried out as a single 5 group series or as 1 series of 2 groups [Sham and NV groups] and 1 series of 3 groups [NC, MHA, SHA]. The total seventy two animals were randomly assigned to five groups, First, nine rats were randomly assigned to the sham group (anaesthetized and non-ventilated rats) and 9 rats were randomly assigned to the NV group (normal ventilation with PIP=15 cmH2O for 4 h) served as controls. Second, fifty-four rats were randomly assigned to three groups including the Normocapnia (NC) group (PaCO2=35-45 mmHg, n=18), the Moderate Hypercapnic Acidosis (MHA) group (PaCO2=80-100 mmHg, n=18), and the Severe Hypercapnic Acidosis (SHA) group (PaCO2=130-150 mmHg, n=18).

3. The authors should graph some of the key physiology data, including BAL protein, BAL cell counts, and Wet:Dry weight ratios, and perhaps 1-2 exemplar cytokines. According to the reviewer’s comment, we have graphed these physiology data as figure 1.

4. Can the authors include data for the NV group in Figure 1? This would help the reader understand the extent of the protection from injury conferred by MHA. The reviewer put forward an interesting issue. As we know, the severity of lung injury was assessed by histology and several other measures, including gravimetric analysis (WW:DW) and BALF protein concentration as a surrogate indicator of altered permeability. we only observed the vary levels of Paco2 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 a data for the NV group in Figure 1.
5. The authors suggest that their anesthesia regimen for the animals was ‘inappropriate’. I am unsure what this statement means, but the authors need to provide some reassurance that the animals were adequately anesthetized throughout the experimental protocol, and that this was tested at regular intervals, such as by confirmation of the absence of a motor or hemodynamic response to paw clamp.

According to the reviewer’s comment, we have revised in the method section on page 7, line 122.