Author's response to reviews

Title: Intravenous superoxide dismutase as a protective agent to prevent impairment of lung function induced by high tidal volume ventilation

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Author’s response to reviews:

We would like to thank reviewers’ scholarly comments that greatly improve this manuscript. Please note that changes made in the manuscript were in red font and the omission would not be shown. Figures associated with the responses were attached as supplementary file

Response to Reviewer1

In this very interesting paper, Nan-Chun Wu and colleagues explore the protective effect of SOD on lung impairment due to high volume ventilation in an animal model. In general the paper is well written, the aims are properly described and the results are relevant, but the article is too long and some section (methods and discussion) should be significantly reduced.

Answer: We are grateful for the reviewer’s supporting comments. In terms of shortening the manuscript, we tried to remove paragraphs in Methods and in Discussions sections without significantly altering the integrity of the manuscript.

Major comment: Sod degrades O2 to hydrogen peroxide. Adding SOD has had variable effectiveness in reducing oxidant damage in experimental systems, in some cases SOD enhances lipid peroxidation and membrane damage and in others it limits lipid peroxidation and membrane injury; the concentration of free iron and catalase influence this opposite effect; the author could comment this point.

Answer: Many thanks for the suggestions. We have added two paragraphs concerning this issue in the Discussion section as follows:
“Fukai and Ushio-Fukai reported that SODs inhibit superoxide-induced inactivation of iron-sulfur containing enzymes, namely aconitase and fumarase, and reduce the release of iron and subsequent formation for highly toxic hydroxyl radical or related iron-associated reactive species by reacting with H2O2”.

“On the other hand, adding Cu/Zn SOD may not always reduce lipid peroxidation, and that is associated with the level of iron and catalase. H2O2 derived by Cu/Zn SOD, can cross cellular membrane and form hydroxyl radicals or metal-related reactive species when interacted with redox-active transitional metal ions, such as iron or Cu, via Fenton reaction that leads to lipid peroxidation and cellular injury. Since catalase reduce H2O2 into H2O, increased Cu/Zn SOD in combination with catalase decreases lipid peroxidation.”

2- discussion should be drastically shortened (9 pages are really too for a paper). I invite the authors to concentrate the discussion on the principal point of their work, the efficacy of SOD on reducing lung inflammation, ventilatory impairment and lung injury; also the modifications of NO level is a very interesting point.

Answer: Many thanks for the valuable suggestions.

We removed some paragraphs to shortened the discussion. We focused more on the protective effects of Cu/Zn SOD against lung inflammation, lung function impairment, NO level and lung surfactants.

3- results the difference on baseline pH in the three groups are significant? If yes this point should be specified.

Answer: The differences in baseline pH were not statistically significant between groups.

4 figure 3 . It's not clear in my opinion

Answer: We added a paragraph to elucidate the results more clearly.

MINOR COMMENT: in abstract, row 10 should be indicate high tidal volume (HTV). In methods, row 50, Peep used was 0; the authors should explain because use a 0 PEEP level.

Answer: Many thanks for the suggestions. Corrections were made accordingly.

Response to Reviewer2

The authors conducted a rigorous study evaluating the impact of intravenous SOD1 on the development of high tidal volume induced acute lung injury. They document that SOD1 treatment attenuates the injury responses that occur in this model. These findings could potentially expand our understanding of the role of oxidative stress and antioxidant responses on the lung injury that occurs during mechanical ventilation. This manuscript is well written but there are some concerns that limit my enthusiasm in its present form. These are listed below:
1. They need to do a control for SOD1 infusion. It is conceivable that infusing a protein could induce protective responses that limit the extent of HTV mediated lung injury. As an example, low dose LPS attenuates experimental acute lung injury. The ideal control would be heat inactivated SOD1.

Answer: Many thanks for the valuable comments.

By following the reviewer’s suggestion, we conducted a control study with intravenous infusion of heat denatured SOD (at 95 C for 30 min) at a rate of 1000 U/kg/hr during HTV ventilation in 4 rats. The study protocol was the same as that detailed in the Materials and Methods section in the manuscript. We compared the lung mechanics (Fig. A; open bar: HTV group, solid bar: HTV+denatured SOD group; in each group, the first and 2nd bar was the baseline and post-ventilation, respectively) and MDA content (Fig. B) with treatment of heat denatured SOD during HTV ventilation to those of the HTV-ventilated rats. We found that the trend of alteration in lung mechanics, e.g. post-ventilation versus the baseline, was not significantly different with or without treatment of heat denatured SOD during HTV ventilation, suggesting no protective effectiveness of heat denatured SOD. Results were added in the Methods section.

The outcome of treatment with heat denatured SOD may not be totally surprising. Till now, we have not come across any literature, either in animal model or in human study, supporting that infusion of denatured proteins attenuates acute lung injury. Besides, the example cited by the reviewer was perplexing. Firstly, LPS (lipopolysaccharide) is a glycolipid (not a protein), composed of a polar lipid A and a chain of repeating disaccharide O antigen, existing in the outer membrane of gram-negative bacteria (1). Secondly, as an endotoxin, serum LPS binds to a specific LPS binding protein that activates the CD14 and TLR4 receptor on monocytes, macrophages and various cells, triggering the release of inflammatory mediators (2). Matute-Bello et al. (3) reported that in animals with pulmonary intravascular macrophages, even very small doses of LPS, namely at a level of μg/kg, can induce significant sepsis and lung injury. Could the reviewer kindly provide the reference supporting “low dose LPS attenuates experimental acute lung injury”?

2. They need to restate what they say in the Introduction on page 6. It's not accurate to say that there are no recommendations for optimal ventilator management strategies. Recommendations of 5-7 cc/kg are well established and ICUs are judged by their adherence to this vent strategy.

Answer: Many thanks for the suggestion. The sentence has been rephrased as “there is still no consensus regarding the optimal ventilation strategy”.

The change was made following Malhotra’s comment published in the New England Journal of Medicine (4). Malhotra’s summarized that “There was a consensus conference of the American College of Chest Physicians recommended in 1993 that low-tidal-volume ventilation be used in patients with ARDS. However, no subsequent formal guidelines dealing with low-tidal-volume ventilation have been developed by the American College of Chest Physicians, the American Thoracic Society, or the Society of Critical Care Medicine. However, all three organizations endorsed a set of industry-funded guidelines, called the Surviving Sepsis Campaign, published in 2004. The process by which these guidelines were developed has been criticized.”
3. Does the SOD1 they infuse reach the lungs? Perhaps they could add a fluorescent tag to SOD to see if it actually reaches the lavage fluid.

Answer: Many thanks for the suggestions.

The aim of this study was to investigate whether intravenous infusion of Cu/Zn SOD could protect lung against detrimental effects of HTV ventilation. Results of histological examination, lung function testing, biochemical examination, protein analyses, serum NO and NF-kB mRNA expression all supported lung protection by intravenous infusion of Cu/Zn SOD. We proposed that the protective effectiveness may be associated with increased permeability of Cu/Zn SOD. As to whether or not Cu/Zn SOD could penetrate across pulmonary capillary endothelium and basement membrane and alveolar epithelial cell into alveolar space and being collected in the BALF is a separate issue, since SOD may not penetrate but react with superoxide in endothelium, basement membrane or epithelial cells, and the outcome of fluorescent study is unlikely to alter our conclusion. Considering the bulk volume of supporting data already comprised in the manuscript, we think that conducting a fluorescent study to investigate whether Cu/Zn SOD moved from blood to the BALF may not be at a high priority.

4. The high tidal volume they choose seems to be very high. Some of the literature suggests a normal tidal volume for a 300 g rat would be about 1.6 ml. The volume used here would be more than double that (5.4 ml/300 g mouse). This is more than would occur with humans so they should at least comment on this in the Discussion.

Answer: The high tidal volume, 18 mL/kg, used in this study was well within the range of that commonly adopted by researchers for rat model of ventilator-induced lung injury; for example, tidal volume of 15 ml/kg was adopted by Chiang et al. (5), 20 mg/kg by Syrkina et al. (6) and Quinn et al. (7), 24 ml/kg by Cannizzaro et al. (8), and 30 mL/kg by Guery et al. (9), Ko et al. (10), Liu et al. (11), Kuipers et al. (12) and Kim et al. (13). In our experience, the tidal volume smaller than 15 ml/kg does not consistently induce lung injury. A paragraph has been added in the Material and Methods section.

5. The image quality for Figure 1 should be improved. Some images are much darker than others and there is no scale bar for reference.

Answer: Many thanks. We have replaced the low quality images with better ones.

We consider that scale bar may not be necessary in Fig. 1, since we only made qualitative, not quantitative, comparison of lung tissues between groups, and the images were compared at the same magnification, e.g. X100 or X400. Qualitative comparison of lung tissues was common among researchers, and that can be found in publications by Albaceta et a. (14), Almolkii et al. (15), Bregeon et al. (16), Gonzalez-Lopez et al. (17), Corry et al. (18), Vaporidi et al. (19) and many others. We added a sentence “qualitative comparison” to illustrate our purpose of Fig. 1.

6. It is not necessary to show every immunoblot in Figure 8. They should show representative examples.
Answer: Many thanks for the valuable suggestion. We amended Fig. 8 and kept the representative blots.

Reference List


Figure A and Figure B are in the supplementary file.