Author's response to reviews

Title: Hyperbaric oxygen treatment of spinal cord injury in rat model

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Version: 1 Date: 01 Feb 2017

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NURL-D-16-00525

Hyperbaric oxygen treatment of spinal cord injury in rat model
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BMC Neurology

Dear editor in chief:

Many thanks for giving us an opportunity again to resubmit our manuscript for possible publication!

Many thanks for providing us suggestive comments!

Now, we revise our manuscript according to your suggestive comments, make point-by-point responses and detail the changes.

Thank you very much!

Best regards,

Qi-feng Tang, MD.
Reviewer reports:

Reviewer #1: Excellently written article. I have no other additions or recommendations.

Response: A very suggestive comment!

Thanks !

Reviewer #2: The manuscript has relatively improved, however I still believe that there are several concerns needed to be addressed.

1. The method of tissue harvest was still unclear and the interpretation of the H&E stain remained to be inappropriate.

(a) The authors added the statement " Proteins were prepared from spinal cord tissue obtained from the lesion epicenter" in the section of Methods, this statement is very confusing and I am not sure why protein was prepared from spinal cord epicenter since the spinal cord tissue was only processed for H&E stain.

Response: A very suggestive comment!

In this paper, the focus of the study is the serum SOD and MDA, HE staining only do a general observation and verification of morphology. Immunohistochemical sections of the spinal cord injury after hyperbaric oxygen therapy and the histological findings of apoptosis and autophagy have been highlighted in other studies.


(b) At least a semiquantitative scoring of the histopathology changes (H&E) should be performed. With only demonstrating one representative H&E stain in each group, the authors cannot made a conclusion that the cystic degeneration in SCI-HBO rats was significantly less in the SCI-control rats.

Response: A very suggestive comment!

Immunohistochemical sections of the spinal cord injury after hyperbaric oxygen therapy and the histological findings of apoptosis and autophagy have been highlighted in other studies.

2. More method details should be included. For instance, it is unclear whether the authors used only female or male SD rats, or both of them. This is important since male or female rats may have different locomotor recovery pattern after SCI. It is also unclear about the meaning of Sham-SCI, do the surgically exposed mean laminectomy? How were the treatment window and duration of HBO chosen?
Response: A very suggestive comment!

We choose the female SD rats in the experiment, and there was no difference between the female and the male rats. Sham-SCI group received laminectomy only, SCI-control group received laminectomy and SCI. In some of our pre-trials, we found that HBO treatment at the earliest possible time (within 24 hours) resulted in a better therapeutic effect.

3. Still inappropriate reference and statement noted. For example in the third paragraph of Discussion, the authors only changed the word "patients" to "rats", but did not change other part of the statement accordingly (It is unusual to say clinically in preclinical study).

Response: A very suggestive comment!

We have changed the expression.

4. Why SOD and MDA analyses were not performed at the later time points (i.e. 10 and 20 days) after SCI, the authors should specify the reason.

Response: A very suggestive comment!

This study is pre experiment of a small sample. We have observed in the later period of the observation time was extended to 20 days, a large sample of experiment we have submitted to other magazines.

5. There is some grammatical and typographical errors in the manuscript, therefore the manuscript needs modification before considering it for publication.

Response: A very suggestive comment!

We have revised our paper by a native English speaker.

Reviewer #3: This manuscript explores the potential treatment of spinal cord injury with Hyperbaric oxygen. The main findings are:

- Administration of hyperbaric oxygen 2 hours post spinal cord injury resulted in an enhanced recovery in a number of behavioural assessments.

- Serum SOD showed a significant reduction post SCI, but following hyperbaric oxygen treatment SOD serum levels remained close to control levels.

- Serum MDA was increased after SCI, but treatment with hyperbaric oxygen significantly reduced serum MDA levels.

- Furthermore, hyperbaric oxygen treatment resulted in reduced cystic degeneration of the spinal cord after injury.
This elegant and simple study provides key functional evidence for the benefits in hyperbaric oxygen treatment of spinal cord injury, and provides some important correlations which suggest how this treatment may benefit spinal cord recovery post injury. Importantly, the study design has helped to make this work relevant to potential treatment, as it is feasible that patients with a spinal contusion could be treated using hyperbaric oxygen within 2 hours of injury.

Serum SOD and MDA levels show a significant reduction and increase after SCI, respectively, and hyperbaric oxygen treatment brings these levels close to control values, suggesting the hyperbaric oxygen treatment improves spinal cord injury recovery through reduced lipid peroxidation. Finally, the evidence showing reduced cystic degeneration is consistent with the functional recovery and serum SOD & MDA levels, indicating a reduced level of damage to the spinal cord following injury.

The discussion is balanced and includes a good number of references on spinal cord injury mechanisms, and therefore I do not feel needs much editing.

Response: A very suggestive comment!

Thanks!

I think this manuscript presents a clear and consistent set of data. My only real criticisms are:

- The data presented on behavioural outcomes and SOD/MDA serum levels (tables 1, 2 & 3) would be much easier to understand if presented as graphs, and I would strongly recommend that the tables be presented as graphs instead. May I suggest making a graphs from Table 1 and putting it into Figure 1A and the graph from Table 2 into Figure 1B, to put the behaviour data into one figure, and put the graph from Table 3 into a separate Figure.

Response: A very suggestive comment!

We added figure 1 from table 1, figure 2 from table 2, figure 3 and 4 from table 3.

- I do not agree that the best statistical test to use is 2-way ANOVA with Newman-Keuls post-hoc testing, and I think a 2-way ANOVA with bonferroni post-hoc testing is more robust test (it is less likely to cause a type I error, which is a common problem with the Newman-Keuls post-hoc test).

Response: A very suggestive comment!

We have redone the post-hoc testing using Bonferroni post-hoc testing.

- Table 1 shows the BBB behavioural data, and although a two-way ANOVA is presented, no post-hoc testing is performed. Currently the figure just says there is a significant difference across the groups, but it does not say which groups, or at which timepoints. I would suggest performing a bonferroni corrected post-hoc test across this set of data. I have run this analysis
using the mean±SD, & N presented, and it shows SCI-HBO significantly improves the BBB behavioural assessment compared to SCI-control at all time points post injury except day 2.

Response: A very suggestive comment!

We re-performed the post-hoc testing and noted the group and time points. As you performed before, at day 2, SCI-HBO did not significantly improve the BBB behavioural assessment compared to SCI-control.

- I am puzzled how in table 3, SOD serum levels in SCI-HBO group are significantly different from the Sham-SCI group at 2d and 5d post injury. I have run some statistics on this data myself, using 2-way ANOVA and bonferroni corrected post-hoc tests, and I find that SCI-HBO show no significant differences from sham-SCI, that SCI-control shows significant differences from SCI-HBO at 2d and 5d timepoints, and finally that Sham-SCI SOD serum levels are significantly different from SCI-control at all timepoints. Given Bonferroni is a more robust post-hoc test, I also cannot see how the comparison between sham-SCI and SCI-control before SCI is not a significant result using the Newman-Keuls post-hoc test (I cannot test it as I only have mean±SD, N and not the raw data) - but presumably this result is a false positive.

Response: A very suggestive comment!

I have used 2-way ANOVA and bonferroni corrected post-hoc tests to analyze the data again and the comparison results were just as what you had tested. Using 2-way ANOVA and bonferroni corrected post-hoc tests, the comparison between sham-SCI and SCI-control before SCI was significant difference.

- I find a similar result with the MDA serum levels. My analysis using 2-way ANOVA and bonferroni post-hoc corrections shows: No significant differences between SCI-HBO and sham-SCI at any time point, and significant differences between SCI-control and sham-SCI at 2d and 5d time points, and significant differences between SCI-control and SCI-HBO at 2d and 5d.

Response: A very suggestive comment!

I used 2-way ANOVA and bonferroni post-hoc way to test the results again and these results were the same as what you had investigated. The differences between SCI-control and sham-SCI at 2d and 5d time points showed SCI enhanced MDA level and the significant differences between SCI-control and SCI-HBO at 2d and 5d suggested the HBO effects of lowering MDA level.

- I would like to see all the statistics to use 2-way ANOVA and bonferroni post-hoc testing, and add the significant difference between the SCI-control and sham-SCI on Before SCI SOD serum levels, with sentence noting this is a false positive result somewhere in the manuscript.

Response: A very suggestive comment!
I have noted the difference with mark and sentence both in table and the manuscript. The probable reason of this false result is the limited size of sample size.

- The image in the only presented figure has a terrible resolution, making it difficult for me to assess it. Can images with a higher resolution be presented please? It looks like it is an issue with compression with a jpeg file rather than a problem with the microscope images, as even the computer text on the figure looks pixellated. In the manuscript there is a mention that there are significantly less vacuoles in the SCI-HBO group relative to the SCI-control group, yet no quantification of this is presented. I would like to see an actual quantification of the number of vacuoles and a statistical comparison of these between all three groups for such a statement to be made (its on page 1, lines 14-22, in the Section titled: Spinal cord histopathology).

Response: A very suggestive comment!

This study was to observe the changes of MDA and SOD in spinal cord injury rats after HBO intervention. Histological examination is only the general morphology of spinal cord injury observed, not the main observation object, so only a simple HE staining. Lower image resolution may be due to the lower magnification of the microscope used.

Finally, some of the grammatical errors I have noticed:

- Page 9 line 58 (Serum SOD and MDA content): Reference needs to be to table 3, not table 2 - or edited to the correct reference to the new graph of that data.

Response: A very suggestive comment!

We have changed the reference.

- Paragraph 2 in the Discussion doesn't make any sense - grammatically it is poor. Please re-write this!

Response: A very suggestive comment!

We have rewritten this paragraph.

- In a number of places spaces are missing between full stops and commas. Can these please be corrected?

Response: A very suggestive comment!

We have corrected the wrong places spaces.

Thank you very much again for your suggestive comments and hard work!