

**Author's response to reviews**

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

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Hyperbaric oxygen treatment of spinal cord injury in rat model  
Yongming Sun; Dong Liu; Qingpeng Wang; Peng Su; Qifeng Tang  
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:

Siew-Na Lim, M.D., Ph.D (Reviewer 2): In general, the authors have responded to the questions raised. A few issues remain:

1. Since this paper only focused on serum of SOD and MDA, and there was no quantification of the histopathological results, the author could not conclude that there was less cystic degeneration of spinal cord in SCI-HBO rats, in both the abstract and results sections. At least, the authors should avoid using the term "significantly" in the result section.

Response: A very suggestive comment!

It is a pity we did not perform histopathology quantification. In the manuscript, we have changed the statements in the results.

2. If HBO treatment at the earliest time point (within 24 hours) resulted in a better therapeutic effect, why the authors did not use this time window for the subsequent study? At least, the reason should be mentioned in the discussion.

Response: A very suggestive comment!

HBO treatment was employed for 5 days including the next day after SCI, and inflammation response began within 24 hours after SCI and keep rising subsequently.

3. A minor point, there should be space between "10and20 days" in the second line of the discussion section.

Response: A very suggestive comment!

These spaces were added in the manuscript. Thanks!

Steven J. West (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.

Following the first revision, the MS does read more clearly. However, there are still some essential issues which I feel need addressing:

- In reviewers comments the authors mention only female rats were used, yet this is not stated in the MS. I feel this must be stated in the MS to make it clear to readers. If they wish to state that male and female rats show a similar phenotype, they either need to show their own data on this (with relevant edits), or at least quote a previous study in their discussion. The fact only females are used should also be addressed in the discussion.

Response:A very suggestive comment!

The fact has been stated in methods and discussion parts.

- There is no statement of the N numbers for each group. I know from the first MS that the N is 15 rats per group, but now the tables have been removed, the authors should explicitly state this in the methods section.

Response:A very suggestive comment!

This detail has been stated.

- Throughout the MS the authors have used "before SCI", when I feel it would read much clearer if they state "baseline" or "at baseline", in both the text and figures.

Response:A very suggestive comment!

These words have been replaced.

- In the results section, "Serum SOD and MDA content", the authors state: "Our results showed that there was significant difference between the SCI-control and sham-SCI on before SCI SOD serum levels indicating that the results maybe are false positive. The probable reason of this false result is the limited size of sample size. Although SOD levels of the SCI-control before SCI was higher than the SCI-control before SCI and SCI was able to enhance serum SOD as previous studies, these results were in line with the SCI effects on SOD."

There are many issues with these sentences. The majority of this statement (from "indicating that the results..." to the end) is discussion and should be moved to the discussion - only statements of results should be performed in the results section. This is particularly odd as they have kept the results and discussion separate for the actual SOD and MDA SCI data! No references have been given when they have alluded to previous literature - the authors must add these. Grammatically this whole set of sentences is poor, examples of poor grammar: "there was significant difference", "on before SCI SOD serum levels", "the results maybe are false positive", "is the limited size of sample size" - these do not read very eloquently.

My corrections: "there was a significant difference", "in baseline SOD serum levels", "this is likely to be a false positive result", "is the limited sample size"

Response:A very suggestive comment!

These statements have been corrected and removed to the discussion part.

Its also stated that "SCI was able to enhance serum SOD" - but the results show SOD is reduced with SCI? So how can the authors next state that these results were in line with the SCI effects on SOD?

Response:A very suggestive comment!I am sorry for this mistake, and this statement should be "SCI was able to reduce serum SOD".

I should also say, as a side point, that typically a false positive would not be corrected by increasing the sample size (whereas a false negative would), especially as the N number is already reasonably high for pre-clinical work. The false positive would be, in my view, more likely to be eliminated by using a technique with less variability. However, the authors are free to discuss this point as they wish, and I don't consider this false positive to severely impact on the clear result the SOD serum data points to.

- Although the authors say the histology is only for general observation, they clearly state in their results "At 20 days after SCI, there was significantly less cystic degradation in the SCI-HBO rats than in the SCI-control rats (Fig. 5)". Since no quantitative assessment has even been attempted, then no statement concerning the significance of any change in histology can be made. Indeed only a single example image is presented per group, and for the authors to make any claims of significant alterations, quantification must be performed. At present, the description of spinal cord histopathology is fine, but the statement of significantly less cystic degradation should be either removed, or the appropriate quantification performed.

Response:A very suggestive comment!

This word has been removed.

- The authors also state in their comments to reviewer concerns that the cystic degradation has been reported previously - they should therefore add these references to the discussion when discussing the histopathological observations. I note that this data is not even explicitly mentioned in the discussion and I think at least a sentence or two to discuss this result in relation to previous literature should be made, given that one of the five figures is dedicated to the presentation of this data.

Response:A very suggestive comment!

Our previous reports are published in Chinese and can not be found on pubmed, so we do not recommend adding this one.

- Figures 1-4 figure legends are inadequate. They need to describe the figure, and not just state the result of the post-hoc statistical test. For example, for Figure 1, it should state what that graph is showing - the BBB Scores of animals pre- and post SCI surgery. Adding a short

description would also be nice, so the figure can be read by itself and make sense to the reader. This should be done for all figure legends. Even the statement of the statistical tests is not sufficient to understand figures 1-4, and it should be stated more clearly what the \* and # indicate (i.e. comparisons between which groups needs to be stated more clearly).

- Figure 3 The authors have failed to put on the significant difference between SCI-sham and SCI-control groups at baseline, which they now state in the MS. This must be indicated in the figure.

Response:A very suggestive comment!

We have changed the Figure 3.

- Figures 1-4 state the SCI-sham group as "before SCI" - this needs to be corrected to "SCI-sham", as its confusing otherwise for the reader.

Response:A very suggestive comment!

All the corrections in figure legends have been made.

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