Reviewer’s report

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

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Reviewer: Steven J. West

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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.

- 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.

- 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.

- 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"

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?

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.

- 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.

- 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.

- 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.

**Are the methods appropriate and well described?**
If not, please specify what is required in your comments to the authors.

Yes

**Does the work include the necessary controls?**
If not, please specify which controls are required in your comments to the authors.

Yes

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Yes

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