

Reviewer's report

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

Version: 0 **Date:** 14 Nov 2016

Reviewer: Steven J. West

Reviewer's report:

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.

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.

- 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).

- 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 \pm SD, & N presented, and it shows SCI-HBO significantly improves the BBB behavioural assessment compared to SCI-control at all timepoints post injury except day 2.

- 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 \pm SD, N and not the raw data) - but presumably this result is a false positive.

- 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 timepoint, and significant differences between SCI-control and sham-SCI at 2d and 5d timepoints, and significant differences between SCI-control and SCI-HBO at 2d and 5d.

- 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.

- 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).

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.

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

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

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?

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