Author’s response to reviews

Title: Neuroprotective effects of hyperbaric oxygen (HBO) therapy on neuronal death induced by sciatic nerve transection in rat

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Author’s response to reviews:

Response letter

Dear Editor

The corrections are marked in red.

Editor Comments:

• Combining your photomicrographs with the relevant data graph would aid understanding (i.e. Figure 2 with 3, Figure 4 with 5, Figure 6 with 7, and Figure 8 with 9).

Response: It was corrected.

• The proteins assessed by immunohistochemistry are introduced well in the Discussion; however, the reader would benefit from an explanation for their inclusion earlier in the manuscript, i.e. in the Results section as they are first mentioned.

Response: It was corrected.
• Relating to a comment from reviewer 2 - DRG consist of various functional subclasses of sensory neuron (e.g. nociceptors, mechanoreceptors, and proprioceptors), that can correlate with cell body area. For instance, small area neurons are mostly nociceptive, while larger neurons correlate with mechanoreception and proprioception. Can the authors comment on whether the phenotypes they observe when examining the DRG are more prevalent in larger vs. small area neurons? Figure 2 indicates that HBO treatment results in reduced caspase 3 levels in DRG. Looking at the representative image, the small area neurons appear to have greater levels of caspase 3 than the larger area cells – is this a true representation of the data? The same pattern is observed for COX2 in Figure 4.

Response: It should be noted that the size of the cells changes in the process of cell degeneration (chromatolysis is characterized by swelling of the nerve cell body, apoptosis was initially described by its morphological characteristics such as cell shrinkage); therefore, measuring cell size does not seem to be a very accurate and appropriate method. Also, the total amount of protein expression is measured by densitometry.

• Your graphs are not histograms, so please reword this within the text.

Response: It was corrected.

• Check your figures for typographical errors (e.g. Spinal Cord in Figure 2).

Response: It was corrected.

• The X-axis label of “Groups” is not required in your graphs.

Response: It was corrected.

Reviewer #1:

The most “important” is on the part of the discussion on S100B from Line 22 Page 16 to the end of the discussion. The authors should state in one sentence that the increase in S100B reported in SNT and brain trauma can be viewed with little doubt as an attempt of the body to counteract apoptosis. This will help the reader who is not a specialist in this field to better that the further increase of S100B by HBO is beneficial and do not oppose the reversing effects of HBO of SNT-induced changes in some of the other markers studied by the authors. Alternatively, the authors cite a work by Chazalviel and colleagues published in Med Gas Res. The authors should further
cite another work by Chazalviel and colleagues published in Brain - which better explains one way by which HBO could provide neuroprotection - or replace one by another.

Response: It was corrected.

Reviewer #2:

ABSTRACT

- The methods should be better explained in the Abstract. The biochemical parameters were assessed only in spinal cord, but the abstract suggests that biochemical and immunohistochemical parameters were assessed in DRG and spinal cord. The MDA results are not consistent with what is described in the results of the manuscript.

Response: It was corrected.

- When did HBO therapy start after surgery?

Response: It was corrected.

- There is a disagreement between the conclusion of the abstract and the conclusion of the manuscript. Please, consider showing a similar conclusion

Response: It was corrected.

MATERIAL AND METHODS

- On Page 6, line 21, the authors informed that HBO therapy began immediately after nerve transection. Was there no anesthesia recovery period before beginning the treatment?

Response: It was corrected.

- In the manuscript there is information that "The pressure was gradually raised to and maintained at 2.0 atmospheres absolute". What was the increase rate of the pressure in the chamber? What was the rate of decompression?

Response: It was corrected.
- Was the same time point used to deliver HBO therapy? Was the rat adapted to the HBO chamber before beginning the treatment?

Response: It was corrected.

- Optical densitometry should be clearer. Were the measurements obtained in the ipsilateral or contralateral side to injury?

Response: It was corrected.

- How was the protein assessed?

Response: It was corrected.

RESULTS

I think that more information about histological results should be provided in the Results. What was the diameter of the positive cells in DRG? In what cell type was the change located? Where were the changes located in spinal cord? What was the diameter of the positive cells in spinal cord? Information should be provided for all histological techniques.

Response: It was corrected as much as possible. It should be noted that the size of the cells changes in the process of cell degeneration (chromatolysis is characterized by swelling of the nerve cell body, apoptosis was initially described by its morphological characteristics such as cell shrinkage); therefore, measuring cell size does not seem to be a very accurate and appropriate method. Also, the total amount of protein expression is measured by densitometry.

DISCUSSION

The discussion about oxidative parameters indicated that HBO treatment decreased MDA levels and increased catalase and superoxide dismutase activities in spinal cord after sciatic nerve transection. However, there is difference in MDA levels in SNT + Pre and SNT + Post-HBO groups. Why has this occurred? I personally think that the authors should show a brief discussion clarifying these differences. It is also important for the authors to briefly discuss the differences between SNT group and SNT + Pre and SNT + Post-HBO groups.
Response: It was corrected as much as possible. (It should be noted that only one of the biochemical factors is different between pre- and post treatment groups, while this difference has not been made among other measured biochemical factors. Meanwhile, lipid peroxidation and subsequent MDA production is a progressive process and therefore pre-treatment with HBO which acts as a scavenger of free radicals is more effective in reducing MDA).

- While there was difference in MDA levels in SNT + Pre and SNT + Post-HBO groups, the results were similar in immunoreactions in these groups. Why has this occurred? I think it would be interesting if the authors showed a relationship between immunohistochemical and biochemical results.

Response: It was corrected as much as possible (It should be noted that only one of the biochemical factors is different between pre- and post treatment groups, while this difference has not been made among other measured biochemical factors. Meanwhile, lipid peroxidation and subsequent MDA production is a progressive process and therefore pre-treatment with HBO is more effective in reducing MDA).

- The conclusion should give more prominence to the results of the study.

Response: It was corrected.

FIGURE LEGENDS

- I personally think that the word "staining" is more appropriate than "expression" for reporting immunohistochemical results.

Response: It was corrected.

- The figure legends lack essential information for the reader. They should be expanded to include the description of the HBO group that is seen in each figure. In addition, it should include the description of the spinal cord region that is seen in each figure.

Response: It was corrected.
Thanking you most sincerely for your time and consideration.

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