Author’s response to reviews

Title: Exogenous hydrogen sulfide alleviates surgery-induced neuroinflammatory cognitive impairment in adult mice by inhibiting NO signaling

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Author’s response to reviews:

Dear Editors and Reviewers:

Thank you for your letter and for the reviewers’ comments concerning our manuscript titled "Exogenous hydrogen sulfide alleviates surgery-induced neuroinflammatory cognitive impairment in adult mice by inhibiting NO signaling" (BANE-D-19-00596). Those comments are all very valuable for revising and improving our paper, as well as providing important guiding significance to our research. We have studied your comments carefully and have made corrections, which we hope meet your approval. The main corrections to the paper and responses to the reviewer’s comments are as follows:

Reviewer reports:

Ali Dabbagh (Reviewer 1):

I think the manuscript has been designed in an excellent manner and well written with interesting findings. Just 2 minor comments:

1- in the abstract, the methods section is mis-typed with the contents of the result

Response: Thank you for your comment. We separated the Methods and Results in the Abstract.
2- in page 18, I think this is too early to draw such conclusion now and it should be written more cautiously

Response: Thank you for your comment. We revised the Conclusion as follows:

In conclusion, we found that the long-acting hydrogen sulfide donor GYY4137 had an ability to reversed the cognitive deficits caused by carotid artery exposure surgery. NO signaling pathways might participate in this process. The results indicate that exogenous to H2S may be a promising therapy for POCD.

Kong Eric You-Ten, MD PhD FRCPC (Reviewer 2):
1. Abstract: Methods is a repeat of the Results?
Response: Thank you for your comment. We separated the Methods and Results in the Abstract.

2. P2 L21: To Investigate the effects and mechanisms…
Response: Thank you. We have changed the text.

3. P2 L23-29: This paragraph is a repeat of the results??
Response: Thank you for your comment. We separated the Methods and Results in the Abstract.

4. Methods: P5 L100-103: Group description is confusing and unclear:
Did the “YY4137 group (intraperitoneal injection 50 mg/kg GYY4137 in phosphate buffered saline (PBS) 1 h before surgery was performed)” had the surgery with exposed carotid or the sham surgery.

Did the “ L-NAME group (water intake with 15 mg/kg in drinking water on Day 1 before surgery lasting to Day 3 post-surgery)” had the surgery with exposed carotid or the sham surgery.

Response: Thank you for your comment. Some information was missing in this section and we have added the intervention methods for each group.

GYY4137 group: intraperitoneal injection of 50 mg/kg GYY4137 in phosphate-buffered saline (PBS) 1 h before sham surgery
L-NAME group: water intake with 15 mg/kg L-NAME in the drinking water from Day 1 before sham surgery to Day 3 post-surgery.

Why was the GYY4137 given only on preop day 1, whereas, the L-NAME given for 4 days (preop Day to Postop Day 3).

Response: The treatment times differed because the drug delivery method and the efficacy effect times were different between GYY4137 and L-NAME. GYY4137 is a long-acting hydrogen sulfide donor (PMID: 29607941), while L-NAME is not.

5. Results: Figure 2 D: Can you please provide an explanation to why the level of iNOS with S+G+L appears to be as high as S (surgery group) and not decreased.

Response: We apologize for the error. The experiment was repeated several times and the WB figure in the paper was one of them. However, it was not the proper WB to include in the paper. We have replaced the figure with another one.

6. The results showed that L-NAME and GYY both reduced IL1, TNF and NO. This implies that these drugs are anti-inflammatory in general and are not that specific. It is unclear to conclude that NO is the cause of improving spatial learning and memory capacity, or if it was simply the reduction of inflammation from decreased of all inflammatory cytokines (IL1, TNF and NO).

Response: Thank you for your professional suggestion. We did not conduct targeted research and could not confirm the current conclusion. Therefore, we revised the conclusion as follows: The long-acting hydrogen sulfide donor GYY4137 had an ability to reversed the cognitive deficits and inflammation caused by carotid artery exposure surgery. NO signaling pathways might participate in this process. The results indicate that exogenous H2S may be a promising therapy for POCD.

7. It would have been more conclusive on the role of NO in surgery-induced POCD if NO or iNOS knock-out mouse was used.

Response: Thank you. This suggestion is very important to us and we will continue our research in subsequent experiments.

Jonathan Ball (Reviewer 3): Thank you for the invitation to review this manuscript.
The authors describe a well designed series of experiments to investigate the mechanism of, and possible therapies for, POCD. Using an established mouse model of extracranial surgery they produced a predictable neuroinflammatory insult with reliable effects on memory and cognitive function assessed using established techniques. Using appropriate controls they investigated the effects of an established slow H2S donor and an NO synthase inhibitor. They found that both of these drugs equally reduced the surgically induced neuroinflammatory insult but found no increased efficacy by co-administration. They conclude that POCD / surgery induced neuroinflammation is partially mediated by increased levels of endogenous NO and / or reduced levels of endogenous H2S. Furthermore, they propose that the drugs used might offer prophylactic protection against POCD.

I have no suggestions for the authors regarding improvements to their manuscript.

Response: Thank you very much for your recognition of our manuscript.

We earnestly appreciate the Editors’ and Reviewers’ warm work, and we hope that our corrections meet with your approval.

Yours sincerely,

Changkun Li