Author's response to reviews

Title: Effects and mechanism of dexmedetomidine on neuronal cell injury induced by hypoxia-ischemia

Authors:

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Author’s response to reviews:

Dear editor and reviewers:

Thanks very much for your letter and for the comments concerning our manuscript entitled “Effects and mechanism of Dexmedetomidine on neuron cell hypoxia ischemia injury” (ID: BANE-D-17-00119). All the comments are valuable and constructive for revising and improving our paper. We have studied the comments carefully and revised the manuscript accordingly which we hope meet with approval. We would like to re-submit this revised manuscript, and hope it is acceptable for publication in the journal. Please do not hesitate to contact us with any additional questions or concerns.

The responses to the comments and corrections in the paper were stated point-by-point as follows.

Wei Yin (Reviewer 1):

1. The authors cited the references inappropriately, for example the no. 1,2,3,4, and 5 in MS was indeed old or not displaying the latest progress.

Response: Many thanks for the professional and helpful comments. All the references were checked carefully and inappropriate references had been deleted. Reference 1, 2, 3, 4 and 5 had been replaced by the latest references. We hope the revised contents could meet with approval, if not, do not hesitate to contact us for further revision. Thanks again for your serious comments.

2. Considering well explored of the neuroprotein of dexmedetomidine in many in vivo and on vitro models, the conclusion of authors is not solid in MS because the authors only provide the preliminary results on apoptosis, oxidative stress or inflammatory signals/pathway using a single
OGD model in PC12 cell line, the authors should supply more data using at least primary cultures.

Response: Many thanks for reminding us the limitations in our study. Accordingly, primary hippocampal neurons were prepared from rats following the method described previously. Then, primary neuronal cells were divided into three groups, named Control, OGD, OGD+DMED groups. Subsequently, the levels of TNF-α and IL-6 were estimated by using an immunoassay-based automated system. Meantime, the content of Nox2 was assessed by a Nox2 ELISA kit and catalase (CAT) activity was determined by using chemical assay kits. These results were elaborated in Figure 6, in which levels of TNF-α, IL-6 and Nox2 as well as CAT activity were enhanced after OGD, whereas the enhancements were all ameliorated by DMED treatment. The related descriptions in the whole manuscript had been revised accordingly. We hope the revised contents could meet with approval.

Revised Figure 6

3. Most importantly, the authors did not provide the critical experimental counter evidences to illustrate the relationship between the apoptosis or cell viability of PC12 cell and the apoptosis, oxidative stress or inflammatory signals/pathway studied in MS.

Answer: Thank you very much for your professional and constructive comments. Accordingly, we used Notch inhibitor (DAPT) and NF-κB inhibitor (SN50) to inhibit these two pathways respectively, followed by assessments of cell viability, apoptosis and levels of LDH, MDA, SOD and GSH-Px. Results in Figure 5 illustrated that cell viability at 6 h after OGD treatment was further increased by DMED treatment, and was further increased by DAPT or SN50 treatments. Conversely, the cell apoptosis and oxidative stress were decreased by DMED treatment, and were further decreased by DAPT or SN50 treatments. These results implied that DMED might affect PC12 cells through NF-κB and Notch signaling pathways. The related descriptions in the whole manuscript had been revised accordingly. We hope the revised contents could meet with approval.

Pradip Kamat (Reviewer 2): The Manuscript by Ya-Jun Liu et al entitled "Effects and mechanism of Dexmedetomidine on neuron cell hypoxia ischemia injury" is written well and added some of the additional information from the neuroprotective effects of Dexmedetomidine on hypoxia induced neuronal injury. However, manuscript needs to address some of the issue before publication.

1. Please make sure Authors wrote abbreviation corrects throughout the manuscript. Such as Notch1 written Norch1 in abstract.

Answer: Thank you very much for reminding us the incorrect spelling. The related “Norch1” has been replaced by “Notch1”. In addition, we checked the whole manuscript and revised “MTMD” and “GSX-Px” with “DMED” and “GSH-Px”. We hope the revised manuscript could meet with approval. If not, do not hesitate to contact us for further revisions. Thanks again.
2. Author needs to provide the quantitative and graphical representation of all the western blot assay.

Answer: Thank you very much for your serious and professional comments. Yes, it is really needed to provide quantitative results of the western blot assay. Accordingly, we performed additional western blot assays and quantified the intensity of the bands using Image Lab™ Software. The statistical results had been shown in Figure 1D, 3D, 4D and 4H. We hope the supplementations could meet with approval.

3. Needs to provide source of funding. I see author received no funding from any agency but have to acknowledge such as institution or department.

Answer: Thank you very much for your appreciated comments. This research received no specific grant from any funding agency in the public, commercial or not-for-profit sectors.

4. Author should show the flowcytometric graphs/histogram/cytogram for apoptotic and necrotic cells assay.

Response: Thank you very much for your professional comments. We are terribly sorry for the incorrect description of cell apoptosis assay in the manuscript. Actually, cell apoptosis was measured by using TUNEL staining, not flow cytometry. We had revised the related contents in the Methods Section. The graphs of TUNEL staining had been shown in Figure 1B. We hope the revised contents could meet with approval, if not, do not hesitate to contact us for further revision. Thanks again for your serious comments.

5. In method section author mentioned that they have used 30mg of protein for western blot analysis which is wrong ad should be corrected.

Response: Thank you very much for your serious and professional comments. We are so sorry for the mistake of the amount of protein in western blot analysis. In actual, the amount of proteins for each lane was 30 μg, not 30 mg. The amount in the Methods Section had been revised. We hope the response could meet with approval.

6. Authors ignored the citation of the some of the published reports on the effects of Dexmedetomidine on neuronal impairment such as A) "Neuroprotective effects of dexmedetomidine against hyperoxia-induced injury in the developing rat brain" by Stefanie Endesfelder Published et al February 3, 2017. B) Effect of dexmedetomidine on hippocampal neuron development and BDNF-TrkB signal expression in neonatal rats by Jie Lv, et al Neuropsychiatr Dis Treat. 2016; 12: 3153-3159. Published online 2016 Dec 9. doi: 10.2147/NDT.S120078 PMCID: PMC5158139 and many other reports on Dexmedetomidine.

Answer: Thank you very much for your professional comments. We had read these two papers carefully, and cited them in the Introduction Section of the manuscript. We hope the revised manuscript could meet with approval.
We tried our best to improve the manuscript and made some changes in the manuscript accordingly. These changes will not influence the content and framework of the paper. We earnestly appreciate for reviewers’ warm work, and hope that the correction will meet with approval.

Once again, thank you very much for your comments and suggestions.

Sincerely,

Dr. Wei-Fu Lei