Author’s response to reviews

Title: Electric vagal nerve stimulation inhibits inflammation and improves early postoperation cognitive dysfunction in aged rats

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Version: 1 Date: 30 Mar 2019

Author’s response to reviews:

March 28, 2019

Dr. Zhongcong Xie,
Handling Editor,

Dear Prof. Zhongcong Xie,

Firstly, we appreciate you and editorial members of BMC Anesthesiology for your valuable suggestions on our manuscript: BANE-D-19-00116: Electric vagal nerve stimulation inhibits inflammation and improves postoperation cognitive dysfunction in aged rats. Based on your comments and suggestions, we have revised carefully this manuscript and listed responses to the reviewer’s comments one to one as follows:

1. All revision comments of editors and reviewers have been followed. The spellings have been again rechecked. Furthermore, some mistakes in the grammar and typos, and misnomers have been corrected. All changes to the manuscript are indicated in the text using track changes.
2. The responses to Fang Zhang’s (Reviewer 1) Comments:

(1) Question 1: This study was designed to investigate if electric vagal nerve stimulation plays a role on inflammation and postoperation cognitive during aged rats. The authors found that serum inflammatory cytokines of TNF-α and IL-6 increased by the operation/anesthesia, but these cytokines could be decreased by the vagal nerve stimulation. The level of TNF-α protein and mRNA expression of NF-κB in hippocampus were also eliminated by the vagal nerve stimulation compared to S group. The Morris Water Maze results showed the escape latency of postoperation in the S group was significantly longer than the C group (P<0.05), and the times of crossing platform in the S group was lower than that of the C group (P<0.05).

The authors used in vivo studies to evaluate cytokines expression both in serum and hippocampus, cognitive function. Various techniques such as western blot and PCR were conducted to study cytokines expression levels. These methods significantly strengthened this paper. This is a well-written manuscript that examines the effects of electric vagal nerve stimulation on inflammation and postoperation cognitive during splenectomy. However, there are some concerns on the methods and data interpretation, need at least to be well discussed.

The methods on the electric vagal nerve stimulation are sparse. These really need to be substantially extended for the data presented to be appropriately interpreted by the reader.

Response: Thank you very much for this good suggestion. Vagal nerve stimulation (VNS) is a therapy to refractory epilepsy, depression, and so on. In our hospital, many patients receive this therapy every. Studies with effects of VNS against inflammatory reaction were started by Borovikova and colleagues in 2000. Their work was published in Nature. But anti-inflammation treatment of VNS has not been universal in clinical, it focuses on animal experiments now. We have studied it’s anti-inflammation effects for many years. For example, we confirmed VNS provided protection to myocardial ischemia-reperfusion injury. In our article, we provided detail method and parameters of VNS in the part of method. At same time, several reference articles were provided. In fact, we provided basic stimulation parameters. For every animal, stimulation parameters should be regulated constantly with changes of heart rate. At last heart rate was kept in appropriate range. Clinically, when patients receive VNS therapy for epilepsy, the detail stimulation parameters will be regulated with patients’ reaction too. If anyone is interesting to VNS, please contact with correspondence author without hesitate.
(2) Question 2: Please provide justification on the why 1-7 days were selected as the observed time point? Since cognitive function impairment is a long-term process. Why the authors think 1-7 days after splenectomy could cause cognitive function damage?

Response: Thank you very much for this good question. Evidence has shown that POCD is associated with both short and long term cognitive impairment, although results of previous studies strongly suggest it is a long term process. The progress and time course of POCD varies greatly due to its heterogeneous etiologies. For example, Kim and colleague found postoperative delirium might take place two days after surgery (Kim S, et al. doi: 10.1016/j.jcrc.2017.08.011.). In fact we found many elderly patients were associated with POCD on postoperative 1 day of hip/knee surgery. Some studies confirmed that POCD might occur 3 to 7 days after surgery. And inflammation played more important role to cause POCD (Zhu YZ, et al. doi: 10.1097/MD.00000000000004082. Chen K, et al. doi: 10.12659/MSM.894384.). There is no contradiction between these results with long term POCD. POCD may be a long term process, but it can take place early or late after surgery. That is why observation time points in our study were selected early after surgery. We explained this reason in the part of discussion. For accurate expression of content of our study, we revised our title “Electric vagal nerve stimulation inhibits inflammation and improves early postoperative cognitive dysfunction in aged rats”

(3) Question 3: The authors stated that "The NF-κB expression of the S group was increased significantly compared to the C and SV groups. There was similar expression between the C and SV groups. That showed VNS might reduce the pro-inflammation gene transcription." NF-κB, as a key transcriptional factor, plays a critical role in modulating inflammatory responses via regulating the expression of pro-inflammatory mediators. When inactive, NF-κB resides in the cytoplasm and forms a multiprotein complex with an inhibitory subunit, inhibitor of NF-κB (IκB). When activated by external stimuli, IκB phosphorylation, and degradation, the liberated NF-κB then enters the nucleus and activates transcription of multiple inflammatory response genes by interacting with κB elements in the promoter region. Thus, increased NF-κB activation is considered as an important pathogenic factor in many inflammatory disorders. In this study, the authors only tested total NF-κB expression, and this result can't gain the conclusion that "VNS might reduce the pro-inflammation gene transcription".

Response: Thank you very much for your constructive suggestion. We revised some sentences about NF-κB to avoid misunderstanding. In this revised manuscript, VNS decreased pro-inflammatory cytokines in serum, levels of TNF-α and NF-κB in hippocampus. At the same time, we added the part of limitation in this revised manuscript to explanation.
(4) Question 4: The pictures are not clearly and beautiful enough for publication.

Response: Thank you for your suggestions. Transformation of these pictures into word format may cause deformation or fuzzy. We provided primitive WB pictures again.

3. The responses to Yiying Zhang’s (Reviewer 2) Comments:

(1) Question 1: In this review, the author attempted to evaluate effects of vagal nerve stimulation on postoperation cognitive dysfunction in aged rats. These observational studies gave a general overview of the association between the Control, splenectomy group, splenectomy + vagal nerve stimulation group in the level of TNF-α protein, mRNA levels of NF-κB, and Morris Water Maze studies. They found that the inflammation caused by the operation and general anesthesia can be partly inhibited by the electric vagal nerve stimulation. The work presented is overall very important and interesting, however, some issues may need to be addressed before publication.

The author may need to clarify whether vagal nerve stimulation group alone would infect above factors.

Response: Thank you very much for your good question. Anti-inflammation effect of VNS has been studied for many years. And it’s anti-inflammation has been confirmed, although it’s clinical application has not been widely. In previous studies, anti-inflammation effect of VNS has been demonstrated mostly by comparing normal experimental animals with chronic inflammatory or infectious animals. Recently, it was confirmed that VNS was safe to pregnancy rat, because it did not impact on production and release of cytokines in brainstem of newborn (Judkins A, et al. doi: 10.1038/pr.2017.265.). That mean for normal body, VNS alone would not cause inflammatory response. So in our study, we did observe effects of VNS on POCD without comparing the difference between control group and VNS alone group.

(2) Question 2: The author also needs to clarify the increase of TNF-a levels in hippocampus brain tissue is due to the increase of blood serum or due to the brain tissue itself. There are some figures missing Y-axis indications.
Response: Thank you very much for your good question. TNF-α is one of the main inflammatory cytokines involved in initiating and propagating inflammatory response. Although levels of TNF-α in periphery and central nervous system of healthy adults are maintained at very low levels, its levels are significantly elevated in blood and brain due to external stimuli. TNF-α is able to be synthesized in brain, meanwhile directly damage blood brain barrier and induce inflammatory cells infiltration in hippocampus. So in our study the increased level of TNF-α in hippocampus might be from brain tissue itself and/or blood serum. But these did not disturb the explanation of results of our study. In future study, we will track the origin of TNF-α in brain.

According your constructive suggestion, we revised figures.

4. Our revised paper is written using a word processing program and has been saved in the format of MS Word for Windows. All supplemental and revised contents have been interposed using a red-color text. Because some contents have been supplemented due to revision requirements, word number of text in the revised paper has been decreased from 3996 in original paper to 3863 in the revised paper. Because of revision requirements, two new reference (Ref. 30,31) have been added in revised paper. Thus, there are 32 references in revised paper. However, there are still one table and 7 figures in revised paper. Yin Bao and Yuliang Guo were added as authors because they took part in revising this article. They gave constructive suggestion for this study and English writing. We provided the change of authorship request form according with BMC Anesthesiology policy.

5. We are still very pleased to submit this revised manuscript to BMC Anesthesiology. We solemnly declare that (1) each author contributes to the design and conduct of this work; (2) the manuscript has been written, read, and approved by all authors; (3) all authors attest to the validity and legitimacy of data, and agree to the modifications made on this new revision; (4) this manuscript has not been published, either in whole or in part, and is not under consideration for publication elsewhere; and (5) there is no financial support and potential conflicts of interest for the work. In consideration of BMC Anesthesiology taking action in reviewing and editing our submission, all authors undersigned hereby transfers, assigns or otherwise conveys all copyright ownership to the editorial board of BMC Anesthesiology if this manuscript is published. If our manuscript is published in the journal, we agree that the reviewer reports and your responses to the reviewers will be published alongside the article.
6. We do not know whether this new revised manuscript will achieve the sufficient level for publication in BMC Anesthesiology. Should you require any further revision and information with your warm heart to inform us at your convenience, we shall be happy to do it. Thank you and looking forward to receiving an early favorable reply.

Sincerely,

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