Author's response to reviews

Title: Electroacupuncture improves cerebral blood flow and attenuates moderate ischemic injury via Angiotensin II and its receptors-mediated mechanism in rats

Authors:

Jing Li (jlipre@163.com)
Jiaojun He (jjhepre@163.com)
Jingjun Cui (jicuipre@163.com)
Ying Ma (ymapre@163.com)
Xuezhu Zhang (xzzhangpre@163.com)
Yuanhao Du (yhdupre@163.com)

Version: 2
Date: 28 June 2014

Author's response to reviews: see over
Dear Editors and Reviewers,

Thank you for your kind work and for the reviewers’ comments concerning our manuscript entitled “Electroacupuncture improves cerebral blood flow and attenuates moderate ischemic injury via Angiotensin II and its receptors-mediated mechanism in rats”. Those comments are all valuable and very helpful for revising and improving our paper, as well as the important guiding significance to our researches. We have studied comments carefully and have made correction which we hope meet with approval. Revised portion are marked in the paper. The main corrections in the paper and the responds to the reviewer’s comments are as flowing:

Responds to the reviewer’s comments:

**Reviewer: 1**

1. In this manuscript, author observed expression of Angiotensin II and its receptor in brain section. Author also observed DAG and IP3 expression in brain. Author did immunohistochemistry and western blot of Ang II and Elisa for DAG and IP3. The problem is what nucleus did author study? There are many nucleuses in brain. The function of nucleus is very complicated. Did the author did all this experiment on whole brain tissue?

**Response:** In our study, the immunohistochemistry and western blot were performed on whole brain tissue to determine the expressions of Ang II, AT₁R, and AT₂R, as well as CAM and Gq, while ELISA for DAG and IP3 were performed on cerebral artery.
2. In this manuscript, there are 3 groups, control group, Model group and Electroacupuncture group. I think non-acupoint electroacupuncture group or control acupuncture point is necessary as an electroacupuncture control.

**Response:** Electroacupuncture (EA) has been previously reported to produce beneficial effects on stroke patients [Systematic review of resuscitation needling technique for wind stroke], and our recent study has also demonstrated that EA at GV26 (Shuigou) can cause active angiogenesis after MCAO insult and is an important driving force of angiogenesis during cerebral ischemia [Angiotensin AT2 receptor protects against cerebral ischemia-induced neuronal injury]. Moreover, the aim of the present study was to explore the potential mechanism mediating the beneficial effects of EA from the respect of Ang II and its receptors-mediated signal transduction pathways, and therefore we did not include an electroacupuncture control in this study.

3. Author mentioned in methods section about neurological behaviors were evaluated with Zea Longa’s scale. But author did not show neurological deficit score. I want to know whether electroacupuncture can improve animals’ neurological behaviors. If so, this demonstrated electroacupuncture can cure MCAO somehow. It is better to show this data in the manuscript.

**Response:** We have added the data of neurological deficit score in the manuscript.

4. In the method session, there is no reagent information. For example, what is the host source of the antibody of Ang II, Cam and Gq? Which company do you buy the antibody from. What is the antibody concentration you used? A good manuscript
should include these details so that the other researcher can repeat what you did.

**Response:** We have added the detailed information of reagents used in our study, as marked in red in the revised manuscript.

5. Statistical analysis: in the whole manuscript, the author did not mention whether the mean ± SE or mean ± SD was used.

**Response:** Thank you for your kind reminds, we used mean ± SD in this study, and the corresponding information has been added to the statistical analysis section.

6. Figure legends are not complete. Author should describe every figure by conveying as much information as possible about what the Figure tells the reader: the treatment applied or the relationship displayed and so on.

**Response:** We have added detailed information in the figure legends.

7. Figure 1. I was confused by time phase 0-5 hour. Author can mark the information of each bar on the bar body. In Figure 1 A, I can only see 2 time points of model group from 1-5 hour.

**Response:** Thanks, according to your comments, the data information in Fig. 1 was transformed into the Table to make the data more clearly, and the detailed information could be seen in the Table 2.

8. Figure 2, 3 and 4. The images of control group from 1 hour -24 hour are the same images. In different time phase, the images of control group should also be different because of different time point. And it is better to do a statistic analysis in different time phase. Histogram figure can help reader to better understand in which time point Ang II reached a peak and how was the effect of electroacupuncture.
**Response:** In this study, the immunohischemical analysis of the control tissue sample was carried out only at 1 h time point following MCAO, and we have deleted the corresponding data from our manuscript.

9. Figure 1, 5, 6 and 7, there are no standard bar in the figures. There are no * to mark the significant difference, either.

**Response:** In view of the quality of figures after addition of standard bar and *, we converted the data information of the fig. 1, 5, 6 and 7 to the corresponding Tables (Table 1, 2, 3, and 4), and statistic marker (*) were added in appropriate.

10. Figure 7. Author first described DAG level and then described IP3 level; however in Figure 7 author first showed DAG level and second showed IP3 level. It is better to keep consistent.

**Response:** We have made the corresponding changes in the revised manuscript.

**Reviewer: 2**

1. All IHC images of Control groups are same during 1h to 24h in each Figure. Furthermore, in Model group or in EA group IHC image’s cell sizes are different in comparison with other time images after MCAO in each Figure. Are these IHC images really same levels or positions?

**Response:** Thanks for your comments, the immunohischemical analysis of the control tissue sample was carried out only at 1 h time point following MCAO, and we have deleted the corresponding data from our manuscript.

2. No statistically-significant differences have been shown in the all result’s graph. It
is necessary to show the significant differences in all graphs.

Response: The corresponding revisions have been made to better interpret the data.

3. Figure 6. EA group (A), CaM and Tublin bands from 15h to control are very similar to EA group, Gq and Tublin bands from 1h to 9h, and from 15h to control. Please explain why different sample (Gq and Tublin data; 1h-9h and 15h-control) blots by western blotting are very similar images. Furthermore, EA group, CaM and Tublin bands from 15h to control are very similar to EA group, Gq and Tublin bands from 1h to 9h. Why?

Response: Thanks for your kind reminds, we sincerely say sorry for our mistakes, and we have made the corresponding revisions as shown in Fig. 4.

Miner Comments

Response: Thanks, we have made some revisions.

Reviewer: 3

Response: Thank you for your time and comments.

Reviewer: 4

1. In Methods part, Laser doppler detection: Using the Laser doppler detect the rCBF, the detailed methods should be stated. The original value should be listed as soon as possible. The original unit was “mV”?

Response: Thanks, we have added some detailed information about Laser doppler detection, as described in the revised manuscript. The original value that expressing as
percentages of basal values of control group were listed in Table 2, and the original unit was “PU”.

2. You would investigated the effects of electroacupuncture on AT1R/AT2R mediated the signaling, such as the expressions of CaM Gq. Therefore, you should give the AT1R/AT2R antagonist and detect the change of CaM Gq et al.

**Response:** In view of the complex of issues concerning basic mechanisms of MACO insult and EA intervention, we did not give the AT1R/AT2R antagonist in our study. Thank you very much for your valuable comments and suggestion, and it is of important guiding significance to our future researches.

3. Same to the upper suggestion, should give the AT1R/AT2R antagonist and detect the change of DAG and IP3.

**Response:** Thank you for your valuable comments and suggestion, and it is of important guiding significance to our future researches.

4. In Figure 2, 3, 4, should add the scal value.

**Response:** According to your comments and that of other referees, we have converted our figure data to the Tables, as presented in the revised manuscript.

Thank you and best regards.

Looking forward to hearing from you.

Yours sincerely,

Yuanhao Du,
yhdupre@163.com

Tianjin University of Traditional Chinese Medicine, Tianjin, China