Reviewer's report

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

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Reviewer: Meiyan Jiang

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Major Compulsory Revisions

This manuscript led by Dr. Du described MCAO induced significant increases in expression of Angiotensin II and its receptor-mediated signal pathway. These enhanced expressions were significantly attenuated by electroacupuncture intervention at GV26, followed by reduced vasoconstriction and improved blood supply in ischemic region, and ultimately conferred beneficial effects on cerebral ischemia. It is an interesting topic and good design proposal. The author did a lot of work to approve their hypothesis. It would be beneficial to study out electroacupuncture mechanism and effect of MCAO, but there are quite a few points need to be more robust.

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?

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 a electroacupuncture control.

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.

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.

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

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.

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 helps reader to better understand in which time point Ang II reached a peak and how was the effect of electroacupuncture.

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

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

**Level of interest:** An article whose findings are important to those with closely related research interests

**Quality of written English:** Needs some language corrections before being published

**Statistical review:** No, the manuscript does not need to be seen by a statistician.

**Declaration of competing interests:**

I declare that I have no competing interests.