Reviewer's report

Title: Electroacupuncture at different frequencies (5Hz and 25Hz) ameliorates cerebral ischemia-reperfusion injury in rats by activating p38 MAPK-mediated anti-apoptotic signaling pathways

Version: 2 Date: 25 March 2015

Reviewer: Toru none Kawada

Reviewer's report:

Minor essential revisions.

1. Some descriptions are difficult to understand and may need to be improved.

1a. What is the definition of penumbra? Was the averaged penumbra region defined based on the ischemic injury in the Model group and was the anatomically same area selected from other groups to perform the cellular analysis? Or otherwise did the authors define the penumbra in each animal? In the latter case, the selection of penumbra can be somewhat arbitrary and may need some criteria such as the distance from the ischemic core region.

1b. Page 6, lines 14-16. After 30 min of MCAo, the suture was carefully removed to restore blood flow, and the right distal MCA was exposed through a cranial burr hole. Was the burr hole made after MCAo? Please describe the procedure along the time line.

1c. Page 7. The authors applied electroacupuncture to the Baihui and Fengfu acupoints. Does this mean that the current was passed between the Baihui and Fengfu acupoints? Can the polarity of stimulation affect the results? The electrodes consisted of 0.5-mm stainless steel wires. Does 0.5 mm indicate the diameter of the wire? Were the animals tethered to the stimulator during electroacupuncture? How was it possible for animals be freely moving (Page 8, line 15)? We do not usually use the words "freely moving" if the animals are tethered.

1d. The rats in the EA-5Hz group were subjected to MCAo and simultaneously received EA at acupoints at a frequency of 5 Hz (EA-5Hz) for 25 min. They were then subjected to 30 min of ischemia followed by reperfusion. "simultaneously" can mean that EA was performed during MCAo. "then" can mean that the ischemia and reperfusion was performed after EA. Was EA performed on the same day of MCAo? In that case, what was the time difference between MCAo and the first EA? The descriptions are difficult to understand and may need to be improved with the time line in mind.
2. It seems that the neurological deficit scores decreased with time in the EA group. Did the 7-day treatment provide the maximum improvement? In other words, is it possible to further reduce the neurological deficit scores by continuing the EA treatment? Some discussion may be added.

Discretionary revisions

1. Page 5, line 13. The rationale for the use of 5 Hz and 25 Hz stimulation may need to be discussed because in the background section the cited papers used lower frequencies (2/15 Hz).

2. Page 12, line 4. All variables were approximately normally distributed ... Is it possible to talk about distribution with only 5 data points?

3. Figures seem to be small even when I enlarged them on the screen. Labels are illegible. Could the resolution be improved?

4. Possible minor corrections are as follows.
   Page 11. RT (room temperature?) needs to be defined.
   Page 20, line 2. expression was marked[ly] downregulated.
   Page 21, line 19. where it interacts with

5. Figure 1 legends.
   Sham, Sham group; Model, Model group; EA-5Hz, EA-5Hz group;
   These descriptions add nothing and can be omitted.

Level of interest: An article of importance in its field

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.