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: 18 March 2015
Reviewer: Jakob Troppmair

Reviewer’s report:

The paper by Chin-Yi Cheng et al. entitled “Electroacupuncture at different frequencies (5Hz and 25Hz) ameliorates cerebral ischemia-reperfusion injury in rats by activating p38 MAPK-mediated anti-apoptotic signaling pathways” addressed the benefit of pretreating experimental animals (rats) with electroacupuncture of two different frequencies (5Hz, 25Hz) on the cerebral ischemia/reperfusion injury induced by middle artery occlusion (MCAo). Ischemia time was kept at 30 minutes; reperfusion was done for 7 days. The authors mainly studied the activation of p38 MAPK and the expression of proteins involved in the activation of the intrinsic cell death pathways, both of which have been implicated in the induction of neuronal cell death. Animals in the EA group received treatment daily up to day 7, when they were sacrificed. EA performed at nonacupoints served as a control. Neurological parameters were assessed on days 1, 3 and 7.

Overall the authors present data, which suggest a protective effect for EA in their model. However, the dissection of the cellular signaling pathways shown and the conclusions drawn are not convincing and data yet have to be provided to achieve sufficient novelty for this paper. In particular p38 activation has to be shown as the ratio of p-p38MAPK/p38MAPK as it is commonly done to really address the activity status of this pathway. Also a cytosolic location of p38MAPK cannot be implicated from an immunoblots. In the case of pCREB the total CREB levels have to be shown as changes in pCREB phosphorylation intensity may
result from upstream signaling but also from increased expression of total CREB.
In general the value of signaling analysis restricted to day 7 has to be
questioned, as most pronounced changes in signaling pathway activity usually
are observed at during ischemia and the early reperfusion in other settings of
ischemia/reperfusion. Functional impairment is already maximal at day 1 arguing
that e.g. underlying cell death causing it already has happened. Also lysates form
infarcted and non-infarcted areas may be compared.

Other major issues
Figure 1/2A: Since EA treatment proceeds the damage, the question arrives
whether EA pretreatment results in smaller infected areas or whether it is
somehow linked to better recovery as analysis is only done on day 7.
Figure 3: The increase in p38 is striking (A), but the blot shown does not seem to
be truly representative if compared to (D). To gain insights into the activity of
signaling pathways the important parameter is the ratio of pMAPK/MAPK,
pMAPK /actin ratios do not provide any relevant information. Thus to arrive at
conclusions that p38 signaling is activated in these samples p-p38/p38 ratios
have to be calculated.
Also in the analysis of intracellular (signaling) proteins a possible effect of EA in
the SHAM setting has to be considered.
Figure 4: In the case of pCREB the ratio pCREB/CREB would be informative. A
rationale should be provided why these proteins were analyzed.
The data on the Bcl-2 family proteins suggest effects of the procedure on the
Bcl-2 protein rheostat. These data also suggest that an intrinsic cell death
pathway may be activated. However, this is not supported by data shown in
Figure 7 (e.g. Cytochrome c release). Thus also remains elusive how activation
of caspase 3 may have been achieved.
Figure 9: also changes in total CREB have to be considered.

Major compulsory revisions:
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Figure 3: The increase in p38 is striking (A), but the blot shown does not seem to be truly representative if compared to (D). To gain insights into the activity of signaling pathways the important parameter is the ratio of pMAPK/MAPK, pMAPK/actin ratios do not provide any relevant information. Thus to arrive at conclusions that p38 signaling is activated in these samples p-p38/p38 ratios have to be calculated.

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**Level of interest:** An article of limited interest

**Quality of written English:** Acceptable

**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.