

Effects of electroacupuncture on postoperative cognitive dysfunction: a preclinical study (abridged secondary publication)

YS Ho *, RCC Chang, GTC Wong, WF Yeung, HQ Zhang

KEY MESSAGES

1. Electroacupuncture reduces cognitive impairment and phosphorylation of tau in a mouse model of postoperative cognitive dysfunction.
2. The protective effects of electroacupuncture are comparable to that of ibuprofen.

Hong Kong Med J 2022;28(Suppl 6):S45-7

HMRF project number: 15161201

¹ YS Ho, ² RCC Chang, ³ GTC Wong, ¹ WF Yeung, ⁴ HQ Zhang

¹ School of Nursing, The Hong Kong Polytechnic University

² Laboratory of Neurodegenerative Diseases, School of Biomedical Sciences, LKS Faculty of Medicine, The University of Hong Kong

³ Department of Anaesthesiology, The University of Hong Kong

⁴ School of Chinese Medicine, Hong Kong Baptist University

* Principal applicant and corresponding author: janice.ys.ho@polyu.edu.hk

Introduction

Postoperative cognitive dysfunction (POCD) is a common complication following surgery and hospitalisation. After general anaesthesia and surgery, patients may experience impairment in attention, concentration, executive function, memory, visuospatial ability, and psychomotor speed over a period ranging from weeks to years. These changes in cognition can put patients at higher risks of postsurgical complications (eg, misunderstanding wound care and drug treatment instruction).

No specific pathophysiological mechanism can fully explain POCD, owing to its multifactorial nature. Possible explanations include inflammatory responses induced after surgical procedures, excessive neuronal apoptosis, increased oxidative stress, free radical injury, and synaptic changes. Among these, inflammation seems to have a major role in POCD. Both systemic- and neuroinflammation, particularly in the hippocampus, triggered by peripheral surgery trauma or anaesthesia have been proposed for the cognitive deficits. Elevated levels of pro-inflammatory cytokines (such as IL-6, IL-1 β , and TNF- α) after surgery may be related to POCD. Morphological changes and activation of microglia after surgery have been reported in preclinical studies. It is proposed that microglia mediate multiple aspects of neuroinflammation and can trigger cognitive dysfunction through multiple signalling pathways.

In our previous study, ibuprofen (a non-steroidal anti-inflammatory drug) could attenuate systemic inflammation and cognitive impairment in mice that had undergone laparotomy. Ibuprofen is commonly used for postoperative pain management, but it can cause gastric bleeding and renal damage

and hence not suitable for prolonged use. Non-pharmacological approach for POCD is preferred because of adverse effects of medications, especially in older patients with comorbidities.

Acupuncture and electroacupuncture (EA) have been used to treat neurological and mental disorders. Acupuncture before surgery has been reported to reduce the incidence of POCD and suppress systematic inflammation. This study aims to assess the effects of postoperative EA on cognition and related pathology of mice following laparotomy under general anaesthesia.

Methods

12-week-old male C57BL/6N mice (weighing 25 \pm 3 g) were randomly assigned to four groups: sevoflurane alone (control), laparotomy alone, laparotomy + EA, and laparotomy + ibuprofen (positive control). Under anaesthesia with sevoflurane for 20 minutes, a longitudinal midline incision was made in the abdomen. The intestine was exteriorised and generally rubbed for 1 minute and then put back into the abdominal cavity. The muscle layers and skin were then closed and sutured. Analgesic was given for 3 days after laparotomy.

EA treatment was started 2 days after laparotomy to allow sufficient rest. Mice were physically restricted using a custom-made nylon net. Acupuncture needles were inserted horizontally at the Baihui (DU20) and Zusanli (ST36). Electrical stimulation was generated using an EA apparatus set to disperse waves of 1 and 20 Hz. The 7 days and 14 days protocols were used, in which EA treatment lasts for 20 minutes daily for 5 or 12 days, respectively. These time points represent the early and middle postoperative periods. Mice in the

control, laparotomy alone, and ibuprofen groups were fixed in place for 20 minutes using similar apparatus but without EA. Mice in the ibuprofen group were fed with ibuprofen (60 mg/kg/day) for 5 or 12 days.

Behavioural changes were assessed using the (1) novel object recognition test for hippocampal-dependent memory, (2) open field test for general locomotor activity and anxiety levels, (3) Y-maze test for associated memory, and (4) puzzle box test for executive functions and cognitive ability.

Mice were euthanised after assessment. The plasma and brain were harvested; the brain was dissected into the frontal cortex and hippocampus. Real-time polymerase chain reaction, western blot analysis, immunohistochemical staining (according to the Accu-OPTIClear protocol), and Milliplex cytokine assays were performed to assess pathological changes in mice.

Results

Electroacupuncture attenuated cognitive impairment induced by laparotomy

To understand the short- and medium-term effects of EA, cognitive performances of mice were assessed at different time points in the 7-day and 14-day protocols. In the Y-maze test, the number of errors in early and medium time points significantly increased in mice in the laparotomy-alone group than in the control group, whereas those received EA made fewer errors than those in the laparotomy-alone group. This suggested an improvement in associated memory. In the novel object recognition test, mice in the laparotomy-alone group displayed reduced ability to differentiate the novel object from the old object, which suggested an impairment in recognition memory, whereas mice that had received EA for 12 days showed significant improvement. In the puzzle box test, mice in the laparotomy-alone group showed significantly impaired problem-solving ability, short-term memory, and long-term memory during the middle postoperative period, but these cognitive changes were attenuated by EA. In most behavioural tests, the effects of EA were similar or slightly worse than that of ibuprofen.

For the general health condition of mice after laparotomy, an immediate weight loss was observed, which persisted for 2 weeks and could not be reversed by EA. The locomotor activity was reflected by the total distance travelled in the arena in the open field test. The anxiety- or depression-like behaviour was reflected by the time spent in outer zone during the open field test. There was no significant difference in the anxiety- or depression-like behaviour between groups. These suggested that the cognitive changes were unlikely to be caused by diminished physical ability or anxiety or depression levels.

Electroacupuncture attenuated laparotomy-induced tau phosphorylation

Tau is an essential protein for maintaining the stability of microtubules. Post-translational modification of tau, such as increased phosphorylation, can occur in pathological situations including Alzheimer disease and results in tau aggregation and generates toxicity. To determine if EA can reduce such pathological changes, we used western blot analysis to assess changes of the tau phosphorylation. Laparotomy induced phosphorylation of tau (AT180, AT8, p-Tau 404, p-Tau 396) in the hippocampus and frontal cortex of mice 14 days after surgery. These changes were partly attenuated by EA. The effects of EA on attenuating tau phosphorylation slightly differed between the hippocampus and the frontal cortex.

Western blot analysis was used to examine any change of tau- and stressed related kinases in the early postoperative period (7 days post-surgery). We found that EA attenuated the activation of GSK3 β (at tyrosine 216) and reduced the phosphorylation of JNK after laparotomy in both the hippocampus and the frontal cortex. However, there were no significant changes in the JAK/STAT signalling proteins.

We examined the effects of EA on synaptic proteins, which play key roles in neurotransmission. In the hippocampus, there was no significant change in the levels of synapsin-1, synaptophysin, or NMDAR2B receptor 14 days after laparotomy. EA also did not show any significant effect on the expression of these proteins. In the frontal cortex, a significant reduction of NMDAR2B was found 14 days after laparotomy. However, EA could not reverse this reduction. This suggested that cognitive impairment after laparotomy might not be related to the expression levels of the detected synaptic proteins.

Effects of electroacupuncture on attenuating neuroinflammation

To study the effects of EA on neuroinflammation, we assessed the morphological changes of microglia (detected by Iba-1) and astrocyte (detected by GFAP) in the hippocampus of the mice. There were increased levels of Iba-1 and GFAP in CA1 region of the hippocampus 14 days after laparotomy. Microglia changed their morphology to amoeboid-like shape, with retraction of the fine processes. In astrocytes, the processes showed more ramification (hypertrophy). All these were attenuated in mice that received EA treatment. To determine if cytokines were involved, we examined expression of mRNA for inflammatory cytokines in the hippocampus and frontal cortex 7 days and 14 days after laparotomy. There were no significant changes in the levels of IL-1 β , TNF- α , MCP-1, IL-6, IL-10 and IL-8 in the brain in the two time points. However, there were

mild yet significant increased levels of TNF- α and IL-10 in the hippocampus 14 days after laparotomy. EA attenuated the increase of IL-10. In the peripheral circulation, there were increased levels of IL-6 and IL-10 in the plasma 7 days after laparotomy. These changes seemed to be attenuated by EA despite not significantly.

Discussion

Postoperative EA could attenuate the cognitive impairment after laparotomy. This effect was not prominent at the early postoperative phase, in which EA improved the performance of mice in the Y-maze test but not the novel object recognition test. This may be related to the number of EA received before the tests were conducted. When the number of EA treatment was increased in the 14-day protocol, a positive treatment effect of EA was found in both tests. These suggests that a single/few EA treatment is unlikely to attenuate the cognitive impairment. This is not consistent with the finding in a study that a single session of EA during or after surgery is sufficient to reduce the incidence of POCD in patients.¹ It seems that EA before/during surgery provides more beneficial effects than EA after surgery. Our findings cannot be compared directly with those of other animal studies, as different POCD models such as partial hepatectomy or splenectomy are used.²⁻⁴ We believe that laparotomy without removal of any parts of internal organs is a relatively milder surgery. Our data suggested that EA could suppress stress kinases and reduce tau-pathology, but its effect on attenuating neuroinflammation was less prominent.

Conclusion

EA can attenuate cognitive dysfunctions and some neuropathological changes in the brain of mice after laparotomy. The protective effect of EA is slightly lower than that of ibuprofen. EA can attenuate the

activation of microglia and astrocyte in the brain. However, other mechanisms, both peripherally and in the brain, may also exist to explain the observed benefits. Postoperative EA can be a viable option for the management of POCD.

Funding

This study was supported by the Health and Medical Research Fund, Health Bureau, Hong Kong SAR Government (#15161201). The full report is available from the Health and Medical Research Fund website (<https://rfs1.fhb.gov.hk/index.html>).

Acknowledgements

We thank the technicians in the University Research Facility in Life Sciences (ULS) of The Hong Kong Polytechnic University for providing excellent support in the Milliplex cytokine assays. We thank Mr. Krit Lee for supporting us the Accu-OPTIClear processing protocol and confocal imaging.

References

1. Ho YS, Zhao FY, Yeung WF, Wong GT, Zhang HQ, Chang RC. Application of acupuncture to attenuate immune responses and oxidative stress in postoperative cognitive dysfunction: what do we know so far? *Oxid Med Cell Longev* 2020;2020:9641904.
2. Yuan S, Zhang X, Bo Y, Li W, Zhang H, Jiang Q. The effects of electroacupuncture treatment on the postoperative cognitive function in aged rats with acute myocardial ischemia-reperfusion. *Brain Res* 2014;1593:19-29.
3. Feng PP, Deng P, Liu LH, et al. Electroacupuncture alleviates postoperative cognitive dysfunction in aged rats by inhibiting hippocampal neuroinflammation activated via microglia/TLRs pathway. *Evid Based Complement Alternat Med* 2017;2017:6421260.
4. Liu PR, Zhou Y, Zhang Y, Diao S. Electroacupuncture alleviates surgery-induced cognitive dysfunction by increasing α 7-nAChR expression and inhibiting inflammatory pathway in aged rats. *Neurosci Lett* 2017;659:1-6.