Electroacupuncture enhances motor recovery performance with brain-derived neurotrophic factor expression in rats with cerebral infarction

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ABSTRACT
Objective Electroacupuncture (EA) is a traditional medicine in patients with post-stroke rehabilitation. Brain-derived neurotrophic factor (BDNF) is a potent growth factor involved in recovery following cerebral injury. The aim of the present study was to investigate whether EA increases BDNF levels and facilitates functional recovery.

Methods Occlusion of the middle cerebral artery was performed in rats (N=12) followed by reperfusion. EA was applied at the GV20 (Baihui) acupoint. Motor and sensory functions were monitored on the Garcia scale for 2 weeks. Expressions of BDNF and receptor tyrosine kinase B (trkB) were determined by immunoblotting and immunohistochemistry.

Results Improvement of Garcia scores, particularly in motor performance, were noted in the group with EA stimulation (p<0.05). With EA application, BDNF was elevated in the ischaemic hemisphere with increased numbers of BDNF(+) cells. Increased expression of trkB was also detected.

Conclusion These results indicate that EA at GV20 improves motor recovery and stimulates BDNF/trkB expression in rats with cerebral ischaemia.

METHODS
A total of 12 Sprague-Dawley male rats with right middle cerebral artery occlusion (MCAo) were prepared.21 Over 3 days, they were grouped into either the EA(+) or EA(−) group. EA was applied every 2 days for 2 weeks. Treadmill exercise was performed for both groups.22–25 The Garcia scale assessments were performed on days 2, 7 and 14, after which half the rats were killed to evaluate BDNF expression. Protocols for the care and use of number of animals were approved by the Catholic University animal care committee.

Longa’s method with minor modifications was used as the animal model of cerebral infarction.21 Rats were anesthetised with 5% isoflurane in oxygen/nitrogen (N2/O2 (30%/70%)) and maintained at 2% isoflurane during the surgical procedure. Rectal temperature was kept at (37.0±0.5°C) with a heating pad (Harvard Apparatus, Holliston, Massachusetts, USA). The right common, external and internal carotid arteries were exposed. A 3-0 monofilament nylon suture was inserted to occlude the MCA. After 2 h,
the suture was withdrawn to allow reperfusion. The acupoint was chosen according to the International Standard Scheme by the China Acupuncture Association, using the text drafted by the China Institute of Livestock Husbandry Medicine. The EA protocol was according to the procedure described by Inoue et al. The scalp was sterilised and an acupuncture needle (0.3 mm diameter, 26 mm long) was inserted into the ‘Baihui (GV20)’ acupoint, and threaded down between the galea aponeurotica and the periosteum along the cranial bone to the point that corresponds to Qubin (GB7) on the side opposite to the paretic limbs (figure 1). A ring electrode was placed in the paretic limb. EA was applied for 5 min. Using a bipolar waveform, a current of 3 Hz pulses was applied for bursts of 5 s, with 2-s intervals. The intensity was increased until the limb and the ear twitched.

Treadmill exercise (Columbus Instruments, Columbus, OH, USA) was given for 10 min on postoperative days 4, 6, 8, 11 and 13. Garcia’s scale (total score ranging from 3 to 18) measures (1) spontaneous activity; (2) symmetry in the movement of four limbs; (3) forepaw outstretching; (4) climbing; (5) body proprioception and (6) response to vibrissae touch. Items ‘1 to 4’ are for motor performance and ‘5 and 6’ are the sensory component. The assessment was performed by blinded investigators. For evaluating behaviour, two trained persons participated. Animals were always tested at 10.00 each day.

Following the end of study period, each group was divided into two, half for the protein analysis and the other half for the histochemistry.

Protein extract from the brain was performed on day 14. Rats were decapitated under 15% urethane and brains were divided into right and left hemispheres. A total of 10 volumes of cold homogenisation buffer (50 mM Tris, 120 mM NaCl, pH 7.4) had protease inhibitors added (Complete Mini, Gibco, Grand Island, New York, USA). Following determination of the protein concentrations by the Bradford method (Bio-Rad, Richmond, California, USA) and anti-γ-actin antibody (Sigma-Aldrich, St. Louis, Missouri, USA) were incubated overnight at 4°C. Peroxidase-conjugated anti-rabbit IgG (1:3000, Vector laboratory, Burlingame, CA, USA) was used for secondary antibody and signals were detected by enhanced chemiluminescence (Supersignal, Pierce, Rockford, IL, USA). Films were exposed from 10 to 30 min.

For immunohistochemistry, rats were anesthetised with 15% urethane, the heart injected with cold saline and then fixed in 4% paraformaldehyde in phosphate-buffered saline (PBS). Coronal sections were cut at 30 μm thickness (Leica, Wetzler, Germany). They were blocked in 10% normal goat serum, 1% bovine serum albumin, 0.2% Triton X-100 and 1% H2O2 in PBS. Anti-BDNF or anti-trkB antibody (Santa Cruz) was incubated for 40 h at 4°C. Fluorescein isothiocyanate (FITC)-conjugated anti-rabbit IgG (Vector, USA) was used as secondary antibody. Confocal microscopy (LSM 510; Carl Zeiss) or a fluorescence microscope (Olympus, Hamburg, Germany) was used. A total of 20 microscopic fields at ×400 were evaluated grading from 0 to 4+ with BDNF(+) cells (0 (0), 1+ (1–10), 2+ (11–20), 3+ (21–30) 4+ (>30) per field).

SPSS V.15.0 (SPSS, Chicago, Illinois, USA) was used for statistical assessment, and a p value<0.05 was considered to be significant. Immunohistochemistry evaluation by microscopy was performed blinded.

RESULTS
Mean Garcia scores are shown in table 1 and figure 2. At 14 days, overall Garcia scores and motor scores were significantly improved in the EA group compared with the control group.

Table 1  Garcia scale scores

<table>
<thead>
<tr>
<th>Group</th>
<th>Day 2</th>
<th>Day 7</th>
<th>Day 14</th>
</tr>
</thead>
<tbody>
<tr>
<td>Garcia (total)</td>
<td>EA(+)</td>
<td>15.3±2.7</td>
<td>16.4±1.6</td>
</tr>
<tr>
<td></td>
<td>EA(−)</td>
<td>14.7±2.5</td>
<td>15.1±2.3</td>
</tr>
<tr>
<td>Motor</td>
<td>EA(+)</td>
<td>9.8±1.9</td>
<td>10.7±0.5</td>
</tr>
<tr>
<td></td>
<td>EA(−)</td>
<td>9.2±1.8</td>
<td>8.3±0.4</td>
</tr>
<tr>
<td>Sensory</td>
<td>EA(+)</td>
<td>5.4±0.9</td>
<td>5.7±0.7</td>
</tr>
<tr>
<td></td>
<td>EA(−)</td>
<td>4.3±1.4</td>
<td>4.3±1.4</td>
</tr>
</tbody>
</table>

*p<0.05. EA(+), electroacupuncture; EA(−), without electroacupuncture.
The results of histochemistry and immunoblotting are shown in figure 3. BDNF protein levels increased with EA, and were double those of the EA(−) group (p<0.05). The number of BDNF(+) cells also increased with EA ((p<0.05 by Wilcoxon-signed rank test) as indicated by staining for BDNF in the cytoplasm and cellular processes (figure 3). The level of BDNF and the difference in Garcia score were correlated (p<0.05, Pearson correlation coefficient \( r = 0.88 \)).

TrkB was also increased by EA (figure 4).

**DISCUSSION**

Our results indicated that EA increased the expression of BDNF in an ischaemic rat model, which was associated with improved motor recovery.

EA has been reported to increase neurotrophic factors such as insulin-like growth factor 1, basic fibroblast growth factor, glial derived neurotrophic factor or receptors, transient receptor potential cation channel, subfamily M, member 7, N-methyl-d-aspartate (NMDA NR1), NR1, glutamate-binding NMDA receptor subunit 1 and aquaporin 4.25–31–37 EA also alters the permeability of the blood–brain barrier that leads to increased levels of nerve growth factor.38 Nitric oxide, tumour necrosis factor \( \alpha \), interleukin 6 (IL-6), IL-3, inositol triphosphate, diacyl glycerol, free radicals or excitatory amino acids were reported to be increased in the experimental model of cerebral ischaemia.38–44 Increase in glucose transporter 1,45–46 tolerance to ischaemia by matrix metalloproteinase type 9, or affecting mitochondrial membrane potential47–48 may also contribute to the recovery induced by EA. The present study has provided additional evidence of the beneficial effect of EA on BDNF expression with functional recovery.

Acupuncture needling and the electrical stimulation may activate multiple signalling pathways.10 This raises a concern that the present result was not mediated by acupuncture, but by electrical stimulation. Brain stimulation activates multiple efferent pathways. The effect of EA is a combined procedure, involving acupuncture and electrical stimulation. There are reports that acupuncture needling induced an alteration in the evoked potential property.46–49 In addition, acupuncture promotes cell proliferation after transient cerebral ischaemia.47–54 Like acupuncture, EA also induces proliferation of neural stem cells in the subependymal zone and hippocampus with nestin expression.21–34–55 This proliferation effect resulted in reduced infarction volume.27–48 These findings suggest that the effect of EA cannot be attributed to the electrical stimulation alone. Therefore, EA, as well as acupuncture, can be effective in modulation of the neural tissue, which results in functional recovery. In addition, the proliferation of stem cells from EA also suggests possibility that its effect may not be transient. It would be necessary to determine the long-term outcome using EA.

Altered evoked potential50–54 and cell proliferation in cerebral ischaemia by acupuncture needling alone50–53 support the effect of acupuncture. Proliferation of nestin(+) neural stem cells34–52–56 and reduction in infarction volume by EA51 suggest that effect may not be mediated by electrical stimulation alone.

In this study, motor recovery was marked, whereas sensory recovery was not. Previous studies of acupuncture have been conducted in patients with motor or speech disturbance.57–58 Whether EA is mainly effective for the motor system is unknown, and requires standardised functional evaluation of sensory recovery.

Frequency and duration of EA in this study followed previous protocols,23–25 and treadmill test were given for both groups since exercise has been routine procedure for post-stroke rehabilitation.59–60 Therefore, the effects of EA frequency, EA duration or treadmill exercise could not be
Figure 4 Tyrosine kinase B (trkB) expression. Immunoreactivity for trkB increased in the right ischaemic hemisphere (arrows) with electroacupuncture (Rt: right, Lt: left, bar=50 μm). determined in this study. The effect of other acupoints such as GV20 or different duration of EA remain to be explored.

In conclusion, EA increases the expression of BDNF associated with motor recovery, indicating the efficacy of EA in the recovery from cerebral ischaemia.

Contributors M-WK developed the idea, protocol, design and wrote the paper. YCC performed the electroacupuncture and treadmill exercise. HJC evaluated the behavioural scores. M-SF, Y-MH, Y-AC, L-SM, MK and IM discussed experimental design. S-IP performed experimental set-up. JL performed the western blot and W-SI performed immunohistochemistry with imaging analysis. JYC analysed the data. MK discussed the idea, reviewed the protocol, supervised the experiments and wrote the manuscript.

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Competing interests None.

Provenance and peer review Not commissioned; externally peer reviewed.

REFERENCES


Summary points
- Electroacupuncture (EA) is used to assist functional recovery after stroke.
- In a rat model, EA increased neurotrophic factor in association with motor recovery.


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