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 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.

BACKGROUND

The incidence of stroke is increasing annually, and results in significant morbidity and disabilities, particularly among people older than 65.1–14 Two Cochrane Reviews report that acupuncture in patients with stroke reduced the number of patients who died or required institutional care.5,6 Fluorodeoxy glucose positron emission tomography (FDG-PET) showed increased cerebral blood flow during acupuncture in patients with vascular dementia.7 Acupuncture enhanced learning and memory in rats with multiple infarctions, implicating a role for recovery from cerebral ischaemia.9,10

For patients with stroke, electroacupuncture (EA) has been frequently used as rehabilitation strategy in Asia.11 Requests for EA have been also increasing for individuals with other musculoskeletal problems.12,13 Nevertheless, there have been debates regarding the effect of EA, warranting further accumulation of evidence that accounts for the underlying mechanism.14,15

Neurotrophic factors play various roles in proliferation and maturation of neurons.1,16 They mediate neural regeneration in cerebral ischaemia.17,18 Brain-derived neurotrophic factor (BDNF) is a potent candidate in the recovery from cerebral ischaemia.19–22 Altered BDNF plasma levels or association of BDNF genotypes in stroke potentially reflect that it may be involved in the physiological response to stroke in humans.19,20,23 However, whether EA affects BDNF is unknown.

The present study’s aim was to investigate whether EA can facilitate recovery from cerebral ischaemia associated with BDNF expression.

METHODS

A total of 12 Sprague-Dawley male rats with right middle cerebral artery occlusion (MCAo) were prepared.24 Over 5 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.25–27 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 (80%/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 acu-
point was chosen according to the International Standard
Scheme by the China Acupuncture Academy,26 using the
text drafted by the China Institute of Livestock Husbandry
Medicine.26 The EA protocol was according to the proce-
dure described by Inoue et al.24 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 corre-
sponds to Qubin (GB7) on the side opposite to the paretic
limbs (figure 1).24 A ring electrode was placed in the paretic
limb. EA was applied for 5 min.24 Using a bipolar wave-
form, 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.24 26

Treadmill exercise (Columbus Instruments, Columbus,
OH, USA) was given for 10 min on postoperative days 4,
6, 8, 11 and 13.21 27 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.21 The assess-
ment was performed by blinded investigators. For evaluat-
ing behaviour, two trained persons participated. Animals
were always tested at 10.00 each day.28–30

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
14.21 27 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), 50 μg were separated by sodium dodecyl sulfate-
polyacrylamide gel electrophoresis (SDS-PAGE) using a
10% polyacrylamide gel with 0.05% bis-acrylamide.24 27
Anti-BDNF (Santa Cruz Biotechnology, Santa Cruz,
California, USA) or 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 chemilu-
minescence (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 anti-
body. 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. TrkB was also increased by EA (figure 4).

**Figure 3** Brain-derived neurotrophic factor (BDNF) expression. Immunofluorescence staining shows that BDNF(+) cells (arrows) are detected more in the ischaemic right hemisphere with electroacupuncture (EA) (Rt EA(+) >Rt EA(−)). Lt EA(−) means left hemisphere with right middle cerebral artery occlusion without EA application. (Rt: right, Lt: left, bar=50 µm). Immunoblot shows the increased level of BDNF by EA. Relative intensity comparing to the internal control reveals that EA enhanced BDNF by twofold (*p<0.05).

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Provenance and peer review
None.

Competing interests
None.

Provenance and peer review
Not commissioned; externally peer reviewed.

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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.

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).


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