Electro-Acupuncture Improves Survival and Migration of Transplanted Neural Stem Cells in Injured Spinal Cord in Rats

Ya-Yun Chen, MD. Research Fellow.
Division of Neuroscience, Department of Histology and Embryology, Zhongshan School of Medicine, Sun Yat-sen University, Guanzhou, China.

Wei Zhang, MD. Research Fellow.
Division of Neuroscience, Department of Histology and Embryology, Zhongshan School of Medicine, Sun Yat-sen University, Guanzhou, China.

Yu-Lin Chen, MD. Associate chief physician.
Department of Acupuncture of the 1st Affiliated Hospital, Sun Yat-sen University, Guanzhou, China.

Shui-Jun Chen. Technician.
Division of Neuroscience, Department of Histology and Embryology, Zhongshan School of Medicine, Sun Yat-sen University, Guanzhou, China.

Hongxin Dong, MD., PhD. Assistant Professor.
Department of Psychiatry, Washington University School of Medicine, St Louis, Missouri, USA.

Yuan-Shan Zeng, MD., PhD. Professor.
Division of Neuroscience, Department of Histology and Embryology, Zhongshan School of Medicine, Sun Yat-sen University, Guanzhou, China.

( Correspondence: Professor Yuan-Shan Zeng, M.D., PhD. Tel: 011-86-20-87331452, E-mail address: zengysh@mail.sysu.edu.cn)

(Received: November 20, 2007; Accepted with revisions: May 1, 2008)

ABSTRACT

This study investigated whether electro-acupuncture (EA) would improve the survival and migration of neural stem cells (NSCs) transplanted in injured spinal cord as well as the potential mechanisms. T10 spinal cord segments of 50 adult Sprague-Dawley (SD) rats were completely transected, and then NSCs were immediately transplanted into the transected site of the experimental animals, while control animals were sham operated without transplantation. Five days post-operation, electro-acupuncture treatment on GV9 (Zhiyang), GV6 (Jizhong), GV2 (Yaoshu) and GV1 (Changqiang) acupoints was applied for 14 days (EA+NSCs 14d) and 30 days (EA+NSCs 30d). ELISA and immunohistochemical staining were used to assess the content of neurotrophine-3 (NT-3) and the characteristics of transplanted NSCs. We found that the number of transplanted NSCs the survived in EA+NSCs14d group was significantly increased as compared to that of the NSCs30d group (5825.20 ± 819.01 vs 4781.40 ± 500.49, P<0.05).
Immunostaining indicated that some transplanted NSCs developed into microtubule association protein 2 (MAP2) positive cells and many of them developed into glial fibrillary acidic protein (GFAP) positive cells in the NSCs30d group. Further, the migration length of transplanted NSCs toward caudal tissue in the injured site was longer in the EA+NSCs30d group than that in NSCs30d group (5.98±0.79 mm vs 3.96±1.72 mm; P<0.05). Also NT-3 in injured spinal cord tissue was 23% increased in the EA+NSCs14d group. These results suggest that the combination of EA and NSCs improves the survival and migration of NSCs in injured spinal cord in rats.

KEY WORDS: Electro-acupuncture; Neural stem cells; Stem cell transplant; cell migratory orientation; Nerotrophine-3; Spinal cord injury

INTRODUCTION

Traditionally, the central nervous system is thought of as incapable of significant self-repair or regeneration in adult mammals. It has recently been demonstrated, however, that neural stem cells (NSCs), defined by their capability for long-term self-renewal, multipotency, and ability to give rise to new neurons and neuroglial cells, remain in the human brain and spinal cord into adulthood [1, 2, 3]. Multipotent NSCs have been isolated from both the embryonic and adult brain and spinal cord [4, 5]. NSCs that can be maintained in vitro in an actively proliferating state and that can differentiate into mature neurons and neuroglial cells are attractive candidates for use as transplants to repair damaged CNS tissue. For reducing the magnitude of spinal cord injury and diseases, transplantation of NSCs has been an increasingly attractive strategy [1]. One critical issue to be solved in this paradigm is the poor survival and integration of transplanted cells in host tissue. Permanent functional deficit with spinal cord injury is in part due to severe nerve cell death. Therefore, increasing the amount of differentiation of transplanted NSCs into neurons is important to successfully compensate for lost neurons.

Electro-acupuncture (EA), a traditional Chinese medicinal therapy, has been used for the treatment of spinal cord injury as it has been shown to significantly improve traumatic paralysis in both clinical practice and experimental animals [6, 7, 8, 9, 10]. Our preceding results have shown that the combination of electro-acupuncture and transplanted NSCs may promote the functional recovery of the hind limb locomotion in rats whose spinal cord have been completely transected[11]. There is strong evidence suggesting that acupuncture, specifically EA, is beneficial for protection against spinal cord injury. A possible protective mechanism of EA is up-regulation of the content of neurotrophine-3 (NT-3) [12, 13]. NT-3 plays an important role in the survival of neurons and axonal elongation during embryonic development [14]. NT-3 also supports injured neuronal survival and axonal regeneration when delivered to the site of an injured spinal cord [14, 15]. In the present study, we investigated the effects of EA on the survival and appropriate migration of NSCs transplanted in an injured spinal cord as well as NT-3's role in these processes.
MATERIALS AND METHODS

Isolation and Culture of Neural Stem Cells for Transplantation

Cultured NSCs were obtained from the hippocampal tissue of newborn Sprague-Dawley (SD) rats following the procedure described in detail in our earlier study [2]. Briefly, hippocampal tissue from newborn SD rats was dissected in D-Hank’s balanced salt solution (HBSS) and digested with 0.25% trypsin (Fig.1C). The cell suspension was centrifuged at 1000rpm for 5min. The final volume was adjusted to a cell density of $5 \times 10^5$ viable cells/μl of culture medium containing Dulbecco’s modified Eagle’s medium (DMEM)/F12 (1:1 Gibco), B27 (Gibco) supplement (2ml/100ml) and Basic fibroblast growth factor (bFGF) (20ng/ml, Sigma). The cells were planted onto 75ml culture flasks with canned necks. Half of the medium was replaced every three days. Typically, the cells grew in suspending neurospheres which were passaged by mechanical dissociation approximately once each week and reseeded at approximately $5 \times 10^5$ cells/μl for transplantation (Fig. 1A and B).

Fig. 1 Schematic diagram indicates the incised dorsal skin of the rat (A), exposed complete transected spinal cord (B), and the procedure of transplanting neural stem cells into the transected site of spinal cord (C).

To enable the detection of NSCs in vivo, expanded cultures of secondary passaged clones were labeled with fluorescence Hoechst 33342, which was added to the culture medium at 1μg/10ml 1h before transplantation. Neurospheres of NSCs were triturated using a fire-polished glass pipette and washed three times by centrifugation and re-suspension in HBSS. The pellet was then re-suspended in 1ml of HBSS for cell counting and the final volume was adjusted to a cell density of $5 \times 10^5$ viable cells/μl of DMEM/F12 medium.
Animals

A total of 50 female SD rats (200–230g, approximately 2–2.5 months old) were randomly divided into 6 groups of animals who: 1) received spinal cord injury alone (14 d), 2) received spinal cord injury and EA treatment over 14 days (EA+14d), 3) received spinal cord injury and transplanted NSCs over 14 days (NSCs14d), 4) received spinal cord injury and EA therapy as well as transplanted NSCs over 14 days (EA+NSCs14d), 5) received spinal cord injury and transplanted NSCs over 30 days (NSCs30d), and finally 6) received spinal cord injury and EA therapy as well as transplanted NSCs over 30days (EA+NSCs30d).

Spinal cord surgery and NSCs transplantation

The animals were anesthetized with 1% pentobarbital (40mg/kg, IP). A laminectomy was carried out at the T8-T10 level to expose the T10 spinal segment (Fig. 1A). The dura was cut and the T10 segment was transected completely. A piece of Gelfoam (1x1x1mm), which was pre-injected with cell suspension containing both NSCs (5x10^5 cells/µl, 5µl, prelabeled with Hoechst 33342), was placed in the transected site of the spinal cord in the NSCs14d, EA+NSCs14d, NSCs30d and EA+NSCs30d groups. In the control 14d, EA+14d and EA+30d groups, only 5µl DMEM/F12 media was injected into a piece of Gelfoam (1x1x1mm) and then transplanted into the transected site. The dura was sutured and the muscle and skin were closed in layers. After surgery, all animals received an intraperitoneal injection of penicillin and then returned to their cages. All procedures were approved in accordance with the Public Health Service Guide for the Care and Use of Laboratory Animals (Fig.1).

Electro-acupuncture (EA) therapy

Five days after spinal cord operation, the EA14d, EA+NSCs14d, and EA+NSCs30d groups were treated with EA every other day. EA stimulation was targeted simultaneously at two groups of well-established acupuncture points. The acupuncture points of the first group were two Ashi points on DU meridian. Two Ashi points ["GV9 (Zhiyang)" and "GV6 (Jizhong)"] were respectively located at the interval of T7–T8 and T11–T12 processus spinosus (Fig. 2A), nearby the transected site of spinal cord. The acupuncture points of the second group, namely, were ["GV2 (Yaoshu)" and "GV1 (Changqiang)"] on DU meridian (Fig. 2A). The two pairs of needles were connected with the output terminals of an electro-acupuncture apparatus (Medical Electronic Apparatus Factory, Jiangsu, China, Model KWD-808II, (Fig. 2C and D)). Alternating strings of densesparse frequencies (60 Hz for 1.05 s and 2Hz for 2.85 s alternately) were used; total duration of EA stimulation was 15min. The two groups of acupuncture points have been targeted because both are the key needling points in acupuncture treatment following a clinical spinal cord injury in human (Fig. 2B).

Enzyme-linked immunosorbent assay (ELISA)
ELISA was utilized to measure the content of Neurotrophine-3 (NT-3) of the injured spinal cord tissue in all 14d groups. The animals were anesthetized with 1% pentobarbital, 40mg/kg, and were perfused with 0.9% NaCl solution (containing Heparin) and 0.1M phosphate buffer (pH 7.2). Spinal cords were quickly dissected, from which the cranial segment (T2–T9) and caudal segment (T11–L5) located at the injured site (T10) were obtained. Each spinal cord segment (50mg) was measured and ground in ice bath to prepare tissue homogenate of which the supernatant liquid was extracted for ELISA analysis. All reagents and working standards were prepared following the manufacture’s instructions (Boster). In brief, 100μl of standard or diluted solution in all the supernatant liquids for testing was added in the 96-well plates packed with neurotrophine-3 antibody, gently mixed and incubated at 37°C for 90min. Each well was aspirated and then 100μl of the diluted biotinylated anti-NT-3 antibody was added to each well. Subsequently, the plate was incubated for 60min at 37°C. After aspiration and washing, 100μl of diluted ABC compound was added to each well and incubated for 30min at 37°C. After 100μl tetramethylbenzidine (TMB) was added at 37°C for 10min, the reaction was stopped by 100μl stop solution. The optical density (OD) of each well was determined within 30min using a microplate reader (Model 680, Bio-Rad, USA) set to 450nm.

**Fig. 2:** Diagram of dorsal view of the rat shows the rat acupoints (A), which are corresponded to the equivalent acupoints in human (B). (C) The EA procedures in conscious rats. (D) The wave-form of the pulse on the oscilloscope.
Tissue processes for immunofluorescence

After 14 or 30 days of NSCs transplantation and/or EA treatment, the rats were anesthetized and perfused transcardially with 4% paraformaldehyde in 0.1M PBS. T8-T12 spinal cord segments containing the injury sites were dissected, placed into 4% paraformaldehyde for 4h, then placed into 0.1M PBS containing 30% buffered sucrose for 48h at 4°C. Horizontal 30μm sections of the spinal cord tissues (T8-T12) were cut on a freezing microtome. The sections were immunofluorescently stained following previously described procedures [12]. Briefly, sections were incubated with appropriate primary antibody, mouse anti-rat microtubule-associated protein2 (MAP2, 1:200, Boster), rabbit anti-rat glial fibrillary acidic protein (GFAP, 1:200, Boster) and rabbit anti-rat tyrosine protein kinase C (TrkC, 1:200, Boster) overnight at 4°C and then washed three times (5min each) in 0.01M PBS. Then sections were incubated with biotinylated secondary antibody at 37°C for 30min and incubated with strept-avidin-biotin-peroxidase complex (SABC)-Cy3 and SABC-FITC (diluted 1:100; from Boster). Sections were washed three times (5min each) in PBS and then coverslipped.

Quantification of surviving neural stem cells

A cell count was conducted in 10 horizontal spinal cord sections from each animal through the injury site. The surviving NSCs (labeled by Hoechst 33342) were counted in 4 unit areas (0.16mm²/each) distributed with equal distance among the area 10mm rostral and 10mm caudal to the injury site per section. The total number of labeled cells from total 10 sections was calculated and considered surviving cells with which comparisons were made between groups.

Migration length of surviving neural stem cells

A cell migration length was conducted in 10 horizontal spinal cord (T8~T12 segments) sections from each animal through the injury site. The migration length of surviving NSCs (labeled by nuclear fluorescent Hoechst 33342) was measured with a measuring scale, from injured site to cranial and caudal directions respectively (under the fluorescence microscopy with magnification 10×20). The total surviving NSCs and the migration length of surviving NSCs were compared statistically between the experimental and control groups using one-way analysis of variance (ANOVA) and post-hoc Student’s t test.

RESULTS

EA increased survival of NSCs transplanted in injured spinal cord

Increased transplanted NSCs labeled by nuclear fluorescent Hoechst 33342 at the transected site of spinal cord and the tissue adjacent to the injured area were observed under the fluorescence microscopy in all groups selected. Transplanted NSCs were mainly distributed at the injured site, and some migrated into neighborhood spinal cord tissue.
Number of surviving NSCs was significantly increased in the EA+NSCs14d or EA+NSCs30d groups as compared to that in the NSCs14d or NSCs30d groups (Table 1). However, the number of surviving NSCs in the NSCs14d or EA+NSCs14d groups was significant increased as compared to that in the NSCs30d or EA+NSCs30d groups (Table 1).

Table 1. Comparison of the number of surviving NSCs transplanted in injured spinal cord (means±SE)

<table>
<thead>
<tr>
<th>groups</th>
<th>n</th>
<th>NSCs number of cranial segment</th>
<th>NSCs number of caudal segment</th>
<th>total NSCs number of cranial and caudal</th>
</tr>
</thead>
<tbody>
<tr>
<td>NSCs14d</td>
<td>5</td>
<td>2202.40±521.70</td>
<td>2580.60±569.60</td>
<td>4783.00±357.50</td>
</tr>
<tr>
<td>NSCs30d</td>
<td>5</td>
<td>1452.80±162.71</td>
<td>1764.00±825.63</td>
<td>3216.80±868.41</td>
</tr>
<tr>
<td>EA+NSCs14d</td>
<td>5</td>
<td>2505.60±603.71</td>
<td>3319.60±329.92</td>
<td>5825.20±819.01</td>
</tr>
<tr>
<td>EA+NSCs30d</td>
<td>5</td>
<td>2001.20±455.20</td>
<td>2780.20±268.53</td>
<td>4781.40±500.47</td>
</tr>
</tbody>
</table>

* t test: a1 VS a2, b1 VS b2, P>0.05; c1 VS c2, d1 VS d2, a3 VS b3, a3 VS c3, b3 VS d3, c3 VS d3 P<0.05

EA promoted differentiation of NSCs transplanted in injured spinal cord

Thirty days after transplanted NSCs, some microtubule-associated protein2 (MAP2) positive cells (Fig. 3A) were found at the transected site of spinal cord and the tissue nearby injured area in the EA+NSCs30d and NSCs30d groups. The nuclei of these cells (Fig. 3B) were labeled by nuclear fluorescent Hoechst 33342. Interestingly, some

Fig. 3. Distribution of microtubule-associated protein2 (MAP2) positive stained cells
into the injured site of spinal cord. 30 days after transplanted NSCs, small amounts of cells showed MAP2 positive staining (arrows, A) and their nuclei were labeled by fluorescent Hoechst 33342 (arrows, B). 30 days after EA and transplanted NSCs, some cells showed MAP2 positive staining and neurite growth (arrow, C). Their nuclei were labeled by fluorescent Hoechst 33342 (arrow, D). Scale bar=10μm.

Transplanted NSCs showed long neurites as labeled by MAP2 immunofluorescence (Fig. 3C and D) at the injured site of spinal cord in the EA+NSCs30d group.

However, significantly increased glial fibrillary acidic protein (GFAP) positive cells (Fig. 4A) were found at the transected site of spinal cord and the tissue nearby injured area in the NSCs30d and EA+NSCs30d groups. The nuclei of these cells (Fig. 4B) were labeled by Hoechst 33342. This suggests that certain transplanted NSCs differentiated into astrocyte-like cells. There were many processes as labeled by GFAP immunofluorescence at the injured site of spinal cord in the NSCs30d groups. These processes showed enlargement, elongation and branching (Fig. 4C and D). But, in the EA+NSCs30d group, less GFAP positive processes were observed.

Fig. 4. Distribution of glial fibrillary acidic protein (GFAP)-positive stained cells into the injured site of spinal cord. 30 days after EA and transplanted NSCs, some cells showed GFAP positive staining (arrows, A), and their nuclei were labeled by fluorescent Hoechst 33342 (arrows, B). 30 days after transplanted NSCs treatment, major cells showed GFAP positive staining (arrows, C) and grow long processes. Their nuclei were labeled by fluorescent Hoechst 33342 (arrows, D). Scale bar=10μm.
In addition, tyrosine protein kinase C (TrkC) immunofluorescence labeling showed some positive cells located at the injured site of spinal cord and the tissue near the injured area in the NSCs30d and EA+NSCs30d groups, and the nuclei of these cells were also labeled by nuclear fluorescence (data not shown).

**EA stimulated the migration of NSCs transplanted in injured spinal cord**

It was found that transplanted NSCs migrated from the injured site of spinal cord to the host tissue rostrally and caudally 30 days after transplantation. The migrating cells labeled by nuclear fluorescence showed a blue fluorescent zone under fluorescent microscopy when they migrated toward host tissue. After measuring the migration length of transplanted NSCs, we found the migration length of transplanted NSCs at caudal host tissue was longer than that at rostral host tissue in EA+NSCs14d group. Interestingly, this phenomenon was more obvious in EA+NSCs14d group than that in EA+NSCs30d group (Table 2). However, in NSCs14d and NSCs30d groups, these migrating cells seemed to move equally through the rostral and caudal host tissues (Table 2). These results indicate that EA may influence the migratory direction of transplanted NSCs at the injured site of spinal cord toward host tissue.

**Table 2. Comparisons of migration length (mm) of NSCs transplanted in injured spinal cord**

<table>
<thead>
<tr>
<th>groups</th>
<th>n</th>
<th>migration length to rostral segment</th>
<th>migration length to caudal segment</th>
</tr>
</thead>
<tbody>
<tr>
<td>NSCs14d</td>
<td>5</td>
<td>4.54±1.50 ±1</td>
<td>4.88±1.30 ±2</td>
</tr>
<tr>
<td>NSCs30d</td>
<td>5</td>
<td>4.38±1.03 ±1</td>
<td>3.96±1.72 ±2</td>
</tr>
<tr>
<td>EA+NSCs14d</td>
<td>5</td>
<td>3.42±1.18 ±1</td>
<td>7.74±1.38 ±2</td>
</tr>
<tr>
<td>EA+NSCs30d</td>
<td>5</td>
<td>2.80±0.38 ±1</td>
<td>5.98±0.79 ±2</td>
</tr>
</tbody>
</table>

*t test: a1 VS a2, b1 VS b2 P > 0.05
c1 VS c2, d1 VS d2 P < 0.05
One-way ANOVA: a2 VS b2, c2 VS d2 P > 0.05

**EA increased neurotroDhine-3 expression in the tissue nearby injured site of spinal cord**

Fourteen days after operation, we measured NT-3 content in cranial segment T2~T9 and caudal segment T11~L5 nearby the injured site (T10 segment). We found NT-3 at the cranial segment was not significantly different from that at the caudal segment in each group of animals (Table 3). However, NT-3 level at the cranial and caudal segments was significant increased in the EA+NSCs14d group. It is about 23% increase in EA+NSCs14d group as compared to NSCs14d group. (Table 3). NT-3 level at the cranial and caudal segments in the EA14d group had no significant difference from that in the NSCs14d group. NT-3 level at the cranial and caudal segments was lowest in the control group (Table 3).
Our results showed that EA therapy or transplanted NSCs may enhance NT-3 level in the tissue nearby the injured site of spinal cord; however, combination of EA therapy and transplanted NSCs may significantly promote NT-3 content.

Table 3. Comparisons of the NT-3 (pg/ml) in the transection site of spinal cord

<table>
<thead>
<tr>
<th></th>
<th>n</th>
<th>cranial segment</th>
<th>caudal segment</th>
<th>cranial and caudal segments</th>
</tr>
</thead>
<tbody>
<tr>
<td>EA 14d</td>
<td>5</td>
<td>167.16±43.03</td>
<td>190.60±43.13</td>
<td>178.88±42.45</td>
</tr>
<tr>
<td>NSCs 14d</td>
<td>5</td>
<td>229.93±54.79</td>
<td>229.34±55.88</td>
<td>229.63±52.17</td>
</tr>
<tr>
<td>EA+14d</td>
<td>5</td>
<td>231.83±18.12</td>
<td>257.00±16.95</td>
<td>244.52±21.46</td>
</tr>
<tr>
<td>EA+NSCs 14d</td>
<td>5</td>
<td>267.35±13.27</td>
<td>296.42±46.38</td>
<td>281.88±35.62</td>
</tr>
</tbody>
</table>

\( P > 0.05; \)

One-way ANOVA:

\( a1 VS a2; b1 VS b2; c1 VS c2; d1 VS d2 \)

\( a3 VS b3; a3 VS c3; a3 VS d3; b3 VS d3; c3 VS d3 \)

\( P < 0.05; \)

\( P > 0.05 \)

DISCUSSION

In this study, we evaluated the effects of EA on the survival and migration of NSCs in injured spinal cord. Our results indicate that the number of surviving NSCs was significantly increased in the EA+NSCs 14d group as compared to the NSCs 14d group. However, the number of surviving NSCs was not significantly increased in the EA+NSCs 30d as compared to the EA+NSCs 14d. These results suggest that EA promotes NSCs survival limited to a critical time period, most likely prior to 30 days as suggested in our experiment. Furthermore, the number of surviving NSCs in the injured spinal cord may decrease as time increases. Nevertheless, in the EA+NSCs 30d group, some viable cells developed microtubule association protein 2, although many glial fibrillary acidic protein positive cells also appear at that time. The results suggest that some transplanted NSCs may differentiate into neuron-like cells. To explore the potential mechanism of the beneficial effect of EA on NSCs transplantation in injured spinal cord, we measured the NT-3 content and found that NT-3 was significantly increased in the EA+NSCs 14d group. This suggests that the combination of EA and NSCs transplantation increases NT-3 which may improve the survival and migration of the NSCs in injured spinal cord in rats.

NSCs transplantation is one approaches for studying spinal cord injury. Permanent functional deficits with spinal cord injury is in part due to severe neuronal death. Therefore, it is critical for transplanted NSCs to differentiate and migrate to injured regions in order to effectively compensate for the loss of neurons. Despite great efforts are being made in this field, one persistent difficulty lies in the maintenance, differentiation, and proliferation of transplanted NSCs into appropriate tissue. In our study we found that EA therapy promotes NSC survival, differentiation, and proper migration into the host tissue located at the injured area of the spinal cord. Interestingly, we noted that the migration length of transplanted NSCs at caudal directions was longer than that at rostral directions in the EA+NSCs 14d group. This may be due to the selection of the acupoints used. Acupoints of
the first group are two Ashi points “GV9” and “GV6” on DU meridian, located at the interval of T7–T8 and T11–T12 processus spinosus, near the transected site of spinal cord. However, the acupoints of the second group, “GV2” and “GV1” on DU meridian, are located at the caudal end nearby the transected site of spinal cord [11]. Therefore, only one acupoint is located at the rostral region nearby the transected site of spinal cord, while three are at the caudal region. This distribution of the two groups of acupoints may contribute to the direction the transplanted NSCs migrated. The pulsating electromagnetic field of EA considerably could cause de-polarization of neurons, which in turn could increase enzymatic activity, enhance axonal transport, and promote axonal regeneration of injured neurons [16, 17]. Therefore, the migratory orientation of NSCs may be significantly affected by the pulsating electromagnetic field of EA.

Neurotrophine-3 (NT-3) is one significant family member of neurotrophic factors and plays an important role during nervous system development, neuronal survival and differentiation, and injured neuronal repair through a signal transduction pathway [20]. It has been reported that NT-3 mRNA transcription and NT-3 protein expression of neuroglial cells and neurons were increased in dorsal root ganglion when incised parts of dorsal roots of rats were treated with EA [12]. Our study has demonstrated that the combination of EA and NSCs transplantation can significantly enhance NT-3 content of the tissue neighboring the completely transected site of rat spinal cord. We also found that NT-3 content in the EA+NSCs transplanted tissue was significantly increased, which corresponded to the highest number of surviving NSCs. It is possible that EA promoted NT-3 expression in neuroglial cells and neurons adjacent to the injured site of spinal cord, leading to the observed increase in NT-3 levels in tissue. It is also possible that transplanted NSCs themselves express NT-3 or stimulate NT-3 expression in neuroglial cells and neurons in injured spinal cord. One recent study has reported that transplanted NSCs secrete some neurotrophic factors in host tissue [19]. We propose that the enhanced NT-3 content in injured spinal tissue was the result of a synergistic effect of EA therapy and NSCs transplantation. On the other hand, we found that transplanted NSCs are capable of expressing tyrosine protein kinase C (TrkC). TrkC is an NT-3 receptor [20]. Generally, TrkC is mainly expressed in the cerebral cortex, cerebellum and hippocampus. NT-3 plays a neurobiological role by TrkC phosphorylation which initiates intra-cellular signal transduction [21, 22]. The significance of TrkC expression with NSCs needs to be further confirmed.

In summary, our study demonstrated that EA may promote the survival and migration of NSCs transplanted in injured spinal cord and subsequent differentiation into neuron-like cells by NT-3 regulation. These results strengthen the evidence of beneficial effects of EA on NSCs transplantation in the injured spinal cord. The underlying mechanisms of NT-3 may play an important role in regulating the survival and differentiation of NSCs. Clarification of these processes may bring important information regarding EA, NSCs, and spinal cord injury therapy.
ACKNOWLEDGMENT

This research was supported by the Chinese National Natural Science Foundation 30270700 and 30472132 to Y.S. Zeng.

REFERENCES


