Electroacupuncture upregulated platelet derived growth factor expression in spared dorsal root ganglion of cats

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Abstract
A bilateral spared dorsal root ganglion model was established in healthy adult cats by bilateral resection of \(L_1\)–\(L_5\) and \(L_7\)–\(S_2\) dorsal root ganglia. \(L_6\) dorsal root ganglia were spared. Zusanli (ST36) and Xuanzhong (BL39) or Futu (ST32) and Sanyinjiao (SP6) were alternatively electro-stimulated on the right leg. Immunohistochemical staining of anti-serum platelet-derived growth factor demonstrated that the number of total neurons and medium-small sized platelet-derived growth factor positive neurons was significantly decreased on the 7th day following injury. After 7 days of acupuncture, the total number of positive and large neurons staining for platelet-derived growth factor on the acupuncture side significantly increased compared to the non-acupuncture side. After acupuncture for 14 days, the total positive and medium-small sized neurons significantly increased compared with the non-acupuncture side. Results indicate that acupuncture promoted the synthesis of platelet-derived growth factor in spared dorsal root ganglia.

Key Words
platelet-derived growth factor; acupuncture; dorsal root ganglion; immunohistochemistry; cat; traditional Chinese medicine; neural morphology; neural regeneration

Research Highlights
(1) A bilateral spared dorsal root ganglion model was successfully established by bilateral resection of \(L_1\)–\(L_5\) and \(L_7\)–\(S_2\) dorsal root ganglia and reserving bilateral \(L_6\) dorsal root ganglia in healthy adult cats.
(2) After alternate electroacupuncture at Zusanli (ST36) and Xuanzhong (BL39) or Futu (ST32) and Sanyinjiao (SP6) on the right leg, platelet-derived growth factor was expressed in spared dorsal root ganglion on the acupuncture side. The number of large and small-medium sized neurons was also significantly increased on this side.

Abbreviations
DRG, dorsal root ganglion; PDGF, platelet-derived growth factor

INTRODUCTION
Studies have shown that the expression of neurotrophic factors changes after nervous system injury\(^1\)–\(^4\). Changes have been found in spared dorsal root ganglia after partial dorsal root rhizotomy, which is related to a spinal cord central process of collateral sprouting and repair of spinal cord injuries\(^5\)–\(^16\). The pioneering work showed that removal of adjacent dorsal root ganglion
(DRG) could trigger sprouting of central processes from spared DRG neurons to the denervated territory within the dorsal horn\cite{11-14}. Subsequent studies revealed that acupuncture could enhance intraspinal sprouting of the spared afferents. Adult mammalian spinal cord is known to be plastic, and this plasticity can be promoted by electroacupuncture\cite{15-20}. Recent studies indicate that electroacupuncture promotes the expression of nerve growth factor, neurotrophin, and epidermal growth factor in dorsal roots after partial dorsal root rhizotomy or the application of acupuncture. The present study investigated the expression of PDGF in spared DRG after partial dorsal root rhizotomy and acupuncture. The present study tested 7 cats as a comparison. The electroacupuncture group was subjected to bilateral L₄–S₃ dorsal root rhizotomy, followed by Xuanzhong (BL39) or Futu (ST32) and Sanyinjiao (SP6) electroacupuncture on the right leg until immunostaining. Left sided L₄ electroacupuncture on the right leg until L₄ Quantitative analysis of experimental animals RESULTS application of acupuncture in cats. after partial dorsal root rhizotomy and subsequent investigation the expression of PDGF in spared DRG after partial dorsal root rhizotomy or the application of acupuncture. Platelet-derived growth factor (PDGF) is a member of the neurotrophic factor family. PDGF has been shown to play a neuroprotective role in nervous system injury\cite{21-23}. However, few studies have reported expression of PDGF in dorsal roots after partial dorsal root rhizotomy and acupuncture could enhance intraspinal sprouting of the spared afferents. Adult mammalian spinal cord is known to be plastic, and this plasticity can be promoted by electroacupuncture\cite{24-26}. PDGF expression in L₄ DRG after bilateral dorsal root rhizotomy PDGF was mainly expressed in small-to medium-sized neurons in L₄ DRG in each group. Expression was also noted in large neurons. PDGF positive immunoreactive products were present mainly in the cytoplasm, and in the partial fibers, without pronounced satellite cell staining (Figure 1, Table 1).

PDGF expression in spared DRG after partial dorsal root rhizotomy and acupuncture PDGF was expressed in neurons in spared DRG. These neurons were small-to-medium (13–56 μm) and large (57–100 μm) in size, as previously classified\cite{27}. The changing trends in electroacupuncture and non-electroacupuncture sides were the same after partial dorsal root rhizotomy, but the control group remained unchanged over the 2 weeks. The number of PDGF positive neurons and small-to-medium-sized neurons on both sides of the model group decreased significantly as compared with that in control DRG at 7 days ($P < 0.05$).

![Figure 1 Platelet-derived growth factor in L₄ dorsal root ganglion following immunohistochemical staining (× 100).](image)

| Type of positive neurons | Side of spinal cord | Electroacupuncture group after acupuncture (day) |
|--------------------------|---------------------|-----------------------------------------------|
|                          | Control group       | 7                                              |
|                          | Electroacupuncture group | 14                                           |
| Total                    | Left                | 21.55±3.41                                    |
|                          | Right               | 12.80±2.19                                    |
| Large                    | Left                | 22.65±3.17                                    |
|                          | Right               | 14.71±3.12                                    |
| Medium-small             | Left                | 4.36±0.79                                     |
|                          | Right               | 4.68±0.83                                     |
| Small                    | Left                | 18.07±3.02                                    |
|                          | Right               | 16.80±3.36                                    |
| Total                    |                      | 20.19±1.78                                    |

Electroacupuncture group was electro-stimulated on the right side. Data are expressed as the mean ± SEM, and evaluated by one-way analysis of variance and least significant different-$t$ test with post-hoc statistical analysis.

a$P < 0.05$, vs. left side (non-acupuncture side); b$P < 0.05$, vs. control group.

On the 14th day, there were few differences between

Table 1  Number of platelet-derived growth factor positive neurons (×100-fold field of view) in normal and acupuncture groups
number in the spared DRG after partial dorsal root rhizotomy and in the normal DRG. The number of positive large neurons remained unchanged in each period. After the application of acupuncture, however, there were overall more PDGF positive neurons, particularly large neurons, than those on the non-electroacupuncture side on the 7th day ($P < 0.05$). Furthermore, there were more PDGF positive neurons and small-to-medium-sized neurons than those on the non-electroacupuncture side on the 14th day ($P < 0.05$).

**DISCUSSION**

**Significance of PDGF expression in normal DRG**

The present study illustrated that there were PDGF positive neurons in L6 DRG in healthy adult cats, suggesting that adult cat DRG express PDGF, which is most likely related to the survival of DRG neurons. Fruttiger et al. have reported that PDGF is produced by neuronal cell bodies via axoplasmic transport. This observation reveals that the produced PDGF does not have a nutritional function in DRG neurons, but plays an important role in spinal cord trough axoplasmic transport. In the same way, the positive reaction of partial fibers observed in this study also supports the transportation of PDGF in neuritis.

**Significance of PDGF expression in spared DRG after partial dorsal root rhizotomy and acupuncture**

The present study observed that the total number of PDGF positive neurons, specifically small-to-medium-sized neurons, significantly decreased compared with that in normal DRG on the 7th day post injury. The number of neurons then returned to normal on day 14. This observation most probably resulted from two reasons: early spinal injury induced by partial dorsal root rhizotomy inhibits the function of spared DRG; in addition, PDGF synthesis is spared in DRG, but its expression is reduced when it is transported to the spinal cord where it participates in early spinal injury neuroprotection, in a similar way to brain-derived neurotrophic factor, neurotrophin-3 and glial cell-derived neurotrophic factor. This occurred in favor of the spinal cord central process collateral sprouting for compensation of the removal of partial afferent nerve fibers, and in addition to partial PDGF transportation to the spinal cord for early spinal injury neuroprotection. Results from the present study revealed that there were, in total, more PDGF positive neurons, particularly large neurons on the electro-acupuncture side on day 7 compared to the non-electro-acupuncture side. The total number of PDGF positive neurons continued to be greater on the electro-acupuncture side on day 14 when compared to the control side, however small-to-medium-sized neurons were more abundant at this time point. This observation suggests that the application of acupuncture promoted expression of large neurons on the 7th day and small-to-medium-sized neurons on the 14th day. Considering the projection arrangement disparity between the large neurons and the small-to-medium-sized neurons, the need for spinal cord central process collateral sprouting and PDGF after spinal injury may not be the same. Alternatively, acupuncture may have played a different role in PDGF expression in different neurons. The present study concluded that acupuncture has great importance in spinal plasticity by promoting spared DRG to synthesize more PDGF. The specific mechanism of how this occurs still remains to be investigated in further studies. Nevertheless, PDGF expression was involved in promoting plastic changes in L6 DRG and associated deafferented spinal cord following electroacupuncture.

**MATERIALS AND METHODS**

**Design**

A randomized, controlled, animal study.

**Time and setting**

This experiment was performed at the Kunming Medical College Institute of Neuroscience, China from 2007 to 2008.

**Materials**

A total of 15 adult male cats, weighing 3–3.5 kg, 12–18 months old, were provided by the Medical Animal Experimental Center of Kunming, China.

**Methods**

**Establishment of a bilateral spared dorsal root model**

Animals (10 cats) were anesthetized by intraperitoneal injection of 3.5% pentobarbital sodium (1.3 mL/kg) for the surgical removal of DRG. The L1–5 and L7–S2 DRG were removed at the respective intervertebral foramina on the left side, sparing the L6 DRG. Another five cats were subjected to sham surgery, i.e. DRG was exposed but not subjected to rhizotomy. After the operation, the cats were individually housed under standard conditions of humidity and temperature with 12-hour light/dark cycles and allowed free access to food and water.

**Electroacupuncture procedure**

Electroacupuncture was performed immediately after
model establishment. Acupuncture points were selected according to results of our previous study\(^\text{[31]}\). Zusanli (1.5 cm below the anterior portion of the fibula head)/ Xuanzhong (1.5 cm above the anterior portion of the lateral malleolus) and Futu (2–3 cm above the lower edge of the patella)/Sanyinjiao (1.5 cm above the posterior edge of the medial malleolus).

Electroacupuncture was performed daily with a HB-EDT-type II electronic acupuncture instrument (Yuehua Medical Factory of Guangdong, Shantou, China). Two needles were placed into each alternate group of two points as positive and negative electrodes, respectively, on a daily basis in the right leg at a frequency of 98 Hz for 30 minutes. This study was a self-controlled experiment, and acupuncture was only performed on the right side.

**Sampling**

Cats were anesthetized intraperitoneally with 3.5% pentobarbital sodium (1.3 g/kg), and perfused with 4% paraformaldehyde for fixation. Both sides of the L6 DRG were obtained by dissection, fixed, immersed overnight in 0.1 M PBS containing 20% sucrose, frozen and then cut into 20 μm thick slices in a cryostat (Leica, Wetzlar, Germany). Five slices were obtained discontinuously in each ganglion in each sample.

**Immunohistochemistry for PDGF expression in spared DRG**

Left L6 DRG in the control and model groups was obtained by dissection. These specimens were postfixed for 12 hours, immersed overnight in 0.1 M PBS containing 20% sucrose, frozen and cut into 20 μm thick sections in a cryostat. After rinses with 0.05 M PBS and soaking in PBS containing 3% H\(_2\)O\(_2\) for 30 minutes at room temperature to quench the endogenous peroxidase activity, sections were immersed in PBS containing 5% goat serum and 0.3% Triton at room temperature for 30 minutes. Sections were then incubated with rabbit anti-PDGF polyclonal antibody (1:200) for 2 hours, and avidin-biotinylated peroxidase complexes (1:100) for 30 minutes at room temperature to quench the endogenous NT-3 from adult cat spared dorsal root ganglion on ganglionic neurons. Sichuan Da Xue Xue Bao Yi Xue Ban. 2003;34(2):245-247.

**Immunoreactive cells, photographs of DRG were collected experimental data. Wei Zhao and Guixiang Liu provided technical instruction.**

**Statistical analysis**

Data were expressed as mean ± SEM, and evaluated by one-way analysis of variance and least significant different t-test with post-hoc statistical analysis using SPSS 12.0 (SPSS, Chicago, IL, USA). A value of \(P < 0.05\) was considered statistically significant.

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**Author contributions:** Xifeng Wang was responsible for study design, assessment, manuscript authorization and the funding process. Lianshuang Zhang conducted the experiments and statistical analysis. Xiaobo Xu wrote the manuscript and collected experimental data. Wei Zhao and Guixiang Liu provided technical instruction.

**Conflicts of interest:** None declared.

**Ethical approval:** All experimental protocols were approved by the Institutional Animal Care and Use Committee of Kunming Medical College, China.

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