Evident Improvement of the Motor Functions of Rear Limbers of SCI Rats Through Electrically Acupuncturing the Acupoints

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ABSTRACT: Purpose: Observe the effect and the therapeutic mechanism of the treatment on SCI rats through electrically acupuncturing the Acupoints. Method: set up a model with 40 SCI rats which are divided into an Experimental Group and a Control Group, with each group consisting of 20 rats. On the 4th day after the model is built, the Experiment Group began to receive treatment by electroacupuncture (EA) which will be conducted for one time each day with each EA treatment lasting for 20 minutes, and the treatment will continue for 6 weeks; for the Control Group, sham EA treatment will be given for the same length and times. The rehabilitative effect on the motor functions of rear limbs of the two groups is observed and the nerve growth factor (NGF) protein expression inspected. Result: for the period between the 4th and 6th week, the rehabilitative effect on the motor functions of rear limbs of the Experimental Group is better than that on the Control Group and the BBB evaluation differences are significant ($P < 0.05$ or $P < 0.01$); after the period of the six weeks ends, all the rats in the two groups are killed at the same time. Inspection of NGF and BDNF protein expression show that the Experimental Group is obviously better than the Control Group and their differences are significant ($P < 0.01$). Conclusion: Treatment by electrically acupuncturing the Acupoints can promotes the expression of NGF and BDNF protein, thereby is conducive to the recovery of the motor functions of rear limbs of the rats, while the improvement of motor functions of rear limbs is linked with the expression of growth factors.

KEYWORDS: Electroacupuncture; Acupoint Stimulation; Motor Function; Spinal nerve; Recovery

1 INTRODUCTION

Spinal cord injury (SCI) due to acute exercises and external explosive power is a common disease and often causes high disability rate [1,2]. Relevant study shows that limb paralysis or dyskinesia caused by the SCI of mammals is mainly related to the injury of spinal nerves. After the spinal nerves are injured, the new nerve cells created by endogenous repair are very few and unable to start the regeneration of functional axons, therefore, their recovery is difficult[3,4].Electrically acupuncturing treatment combines electrical stimulation with acupuncturing and the products of the relevant study on it are elaborated from the theoretical and practical perspective. Most of the studies on it believe that the main reason for recovery of the limb functions is connected with the regeneration and rebuilding of the spinal nerves [5, 6]. This artilce, by setting up a model of SCI rates and treating them by electrically acupuncturing their Acupoints, observes the recovery of their limb motor functions and the effect on their NGF expression.

2 DATA AND METHODS

2.1 Experiment Materials

Use Wistar healthy rats (bought from the animal breeding center of the Medical School of Zhengzhou University, male, clean, 8-10 weeks old with a weight of 55-175g); After the model of SCI rates is successfully built, breed the Experiment Group and the Control Group in different cages (qualification certificate No. of the animal room: YYDZ No.4104022) with a temperature of about 24℃ and sound ventilation. They are cared for by special
persons and receive injection of penicillin of 8x104U in the abdomen each day for three consecutive days. Meanwhile, the rates are cleaned and disinfected two times each day to avoid any infection and their bladders are squeezed each day to make them urinate 3 times a day until the function of the bladder is recovered. Feed the rates once for every 8 days and the food is mainly taken by free eating by the rates assisted by injection of fluid food through their mouths. No rate of the two groups dies during the nursing period. Used also are such devices and medicine as SDZ-IV type electro-acupuncture (produced by Suzhou Medical Products Co. Ltd), NGF & BDNF antibody (bought from Santa Cruz company), Benzylpenicillin Sodium for injection (produced by CSPC Pharmaceutical Group Limited, Batch No.:09119209) and normal saline.

2.2 Building of SCI Rats Model

Table 1. Basic Data Comparison of the Two Groups of Rats (\(\bar{x} \pm s\)).

<table>
<thead>
<tr>
<th>Groups</th>
<th>Sex</th>
<th>Number</th>
<th>Weight(g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control Group</td>
<td>Male</td>
<td>20</td>
<td>164.52±8.24</td>
</tr>
<tr>
<td>Experimental Group</td>
<td>Male</td>
<td>20</td>
<td>166.30±7.93</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Age(weeks)</th>
<th>Before BBB (mark)</th>
<th>Modelingevaluation</th>
<th>After Modeling BBB evaluation (mark)</th>
</tr>
</thead>
<tbody>
<tr>
<td>9.26±1.05</td>
<td>21</td>
<td>1.36±0.20</td>
<td></td>
</tr>
<tr>
<td>9.31±1.02a</td>
<td>21</td>
<td>1.34±0.23b</td>
<td></td>
</tr>
</tbody>
</table>

Note: Basic Data Comparison of the Two Groups of SCI Rats, \(P > 0.05\)

Take 50 of the said Wistar rats (male and clean) and record their age (in terms of weeks) and weight. Use special laminectomy rongeur to bite T8 and T9 spinal process and vertebral plate to reveal the dura mater. Use an aneurysm clip to directly clip the T9 spinal cord (about 0.5s) (method: use the improved aneurysm clip to bite T9 spinal process and vertebral plate with a calibrated force of 35g. Open the aneurysm clip with a clip holder, then abruptly release the holder so that the spinal cord is stricken with the abrupt force for about 0.5s. During the process, the position of the aneurysm clip must be correct to rapidly squeeze the spinal cord to cause acutely complete injury to set up the SCI model. The method can keep the completeness of the spinal dura mater and, after the spinal cord is injured, the change to the anatomical structure and neurological function is extremely similar to the contusion-type spinal cord injury. When the force increases from 2g to 98g, the more powerful the force of the clip is, the fewer the residual axons in the injured area are, and the less satisfactory the recovery will be. So, the calibrated force in this study is 35g to cause the effect of spasitic wag of the tails and paralysis of the rear limbs of the rats. Take 40 rats for which the model is successfully built and randomly divide them into two groups with each group consisting of 20 rats. A comparison between the basic data of the two groups of SCI rats shows no evident difference, \(P > 0.05\). See table 1.

2.3 Therapeutic Method

Experimental Group: Electrically acupuncture the Acupoints of the rats immobilized and start the treatment on the 4th day after the model is built. Process of treatment: the rats lie prostrate with their heads and limbs fixed. Choose “Jiaji Acupoint” at the between 2 vertebra of the injured section as the Acupoint for electrical acupuncturing and stab the two acupuncture needles (with a length of 25mm and a diameter of 0.2mm) of the therapeutic apparatus into the said Acupoint with the positive pole into the heads and the negative pole into the tails. The dilatational waves with a frequency of 10/50Hz shall be adopted and the current intensity causes no more than the vibration of the rear limbs of the rats. The rats are treated in this way once a day with each treatment lasting for 20 minutes.

Control Group: Electrically acupuncture the Acupoints of the rats immobilized and the Acupoint shall be same as that of the Experiment Group but without electrical acupuncturing. The process, method and length of the treatment for this Group shall be same as those for the Experimental Group.

On the 4th day after the model is built, both groups began to receive treatment by electroacupuncture (EA) which will be conducted for one time each day with each EA treatment lasting for 20 minutes, and the treatment will continue for 6 weeks.

2.4 Effect Evaluation

Evaluation of Motor Functions of Rear limbs Use the BBB System for Motor Functions Evaluation to evaluate the motor functions of the rear limbs of the two groups of rats. The evaluation of the motor functions of the rear limbs of the rats shall be conducted by the same experimenter successively using the Single Blind Method with reference to BBB rating scale respectively in the first, second, third, fourth, fifth and sixth week after the first EA treatment begins. BBB rating scale divides the motors of the rear limbs of rats into 22 grades, with zero mark representing total paralysis and 21 marks representing total recovery of the motor functions of the rear limbs.

NGF and BDNF Inspection After the sixth week of treatment, all the rats in the two groups are decapitated and their chests are opened. 200ml normal saline at 4°C is injected through the heart to wash off the blood in the vessels before 450 ml PFA phosphate buffer (pH7.2-7.4) at concentration of 4% and 4°C is injected. Thirty minutes after the said injections, take the T8 and T9 spinal cord into the
PFA phosphate buffer at concentration of 4% and incubate overnight in the sucrose solution at concentration of 4% at 4°C. Embed them in paraffin and continuously section them with cryostat microtome with a thickness of 25 μm. Operation on the chemical staining of the immune system shall be conducted in strict accordance with the instructions on the Kit and make negative control with PBS in the replacement of primary anti-bodies. Take 5 sections from each rat at random. Use an image processing system composed of image analysis cards (produced by Beijing Tiani Company), BM586 and Olympus Bx60 microscope to observe such sections and calculate the quantity of positive reactions cells with SPssl3.0 software. The averages of the NGF and BDNF positive cells of each group is respectively calculated.

2.5 Statistical Treatment

Conduct statistical treatment with SPssl3.0 software and the measurement data shall be expressed with \((\bar{x} \pm s)\). Make inter-group comparison of BBB ratings of the two groups of rats at the same time after each week the rats are treated and compare the NGF and BDNF inspection data of the two groups of rats after the same time after their treatment. Use inspection for comparison of measurement treatment and \(X^2\) inspection for comparison of enumeration data and \(P < 0.05\) demonstrates the statistical significance of the difference.

3 RESULT

3.1 Comparison of BBB Ratings

Before the modeling, the rats score an average of 21 in term of BBB rating. On the third day (the day preceding the start of the EA treatment) after the modeling, all rats in the two groups are nearly paralyzed and the scores of the Control Group and Experimental Groups in BBB rating are 1.36±0.20 and 1.34±0.23 respectively. During the period between the first week and the third week after the EA treatment, a stab in the rear legs of the two groups of rats shows that there is an evident retraction reaction but the inter-group difference is not conspicuous. The weekly inspection after the fourth and the following weeks shows that the motor functions of the rear limbs of the Experimental Group become increasingly evident and are better than those of the Control Group. For the fourth, fifth and sixth week of EA treatment, the difference between the scores of the two groups has statistical significance \((P<0.05\) or \(P<0.01\)). See table 2.

3.2 Comparison of NGF and BDNF Inspections

The treatment stops after the EA treatment is conducted for 6 weeks and all the rats are decapitated on the first day of the 7th week, then inspect the two groups in terms of their NGF and BDNF protein expression to find out the effect of EA stimulation treatment on NGF and BDNF expression by comparing the data of NGD and BDNF protein expression. The comparison shows that, after 69 days of treatment by electrically acupuncturing the Acupoints, the NGF and BDNF expression quantity of the Experimental Group is obviously higher than that of the Control Group, \(P<0.01\) for both of them. And the difference has has statistical significance. See table 3.

<table>
<thead>
<tr>
<th>Groups</th>
<th>1st week after EA Treatment</th>
<th>2nd week after EA Treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control Group</td>
<td>2.40±0.40</td>
<td>4.63±1.04</td>
</tr>
<tr>
<td>Experimental Group</td>
<td>2.47±0.46</td>
<td>5.02±1.21</td>
</tr>
</tbody>
</table>

Table 2. Comparison of the BBB Ratings of Motor Functions of the Rear Limbs of the Two Groups of Rats during the EA Treatment \((n=20, x \pm s)\).

<table>
<thead>
<tr>
<th>Treatment</th>
<th>3rd week</th>
<th>4th week after EA</th>
<th>5th week after EA</th>
<th>6th week after EA</th>
</tr>
</thead>
<tbody>
<tr>
<td>EA</td>
<td>6.49±1.17</td>
<td>7.92±1.44</td>
<td>8.38±1.40</td>
<td>9.12±1.50</td>
</tr>
<tr>
<td>EA Treatment</td>
<td>7.26±1.35</td>
<td>7.43±1.61</td>
<td>13.49±1.59</td>
<td>15.32±1.63</td>
</tr>
</tbody>
</table>

Note: Comparison between the Experimental Group and the Control Group, \(^aP<0.05\); \(^bP<0.01\)

Table 3. Comparison of the Two Groups of Rats in NGF and BDNF Positive Cell Count after 6 Weeks of EA Treatment \((n=20, x \pm s)\).

<table>
<thead>
<tr>
<th>Groups</th>
<th>NGF (piece)</th>
<th>BDNF (piece)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control Group</td>
<td>38.51±5.46</td>
<td>109.22±11.50</td>
</tr>
<tr>
<td>Experimental Group</td>
<td>81.37±6.42</td>
<td>227.61±16.04</td>
</tr>
</tbody>
</table>

Note: Comparison between the Experimental Group and the Control Group, \(^aP<0.01\)

4 DISCUSSION

Limb paralysis or rear limb dyskinesia caused by SCI is a globally knotty problem in clinical medicine. Most studies on this subject believe that, after the nerve system is injured, a lot of nerve cells are lost and new nerve cells are hard to generate to build new synaptic connection, thus making it hard for the patients to recover [7-9]. So, how to prompt the regeneration of new nerve cells to build new synaptic connection is the key to clinical treatment. EA treatment is a modern physical treatment developed on the basis of traditional Chinese medicine and has the double functions of electrical stimulation and acupuncture. The relevant study shows that, EA treatment can not only improve the blood circulation to accelerate metabolism and repair...
of the injured body, but conduct to resist free radicals, mitigate the secondary injury and reduce the content of Ca2+ so that the injured nerves are protected, the death of cells controlled and the excitability of nerve cells recovered [10-13].

In this study, Treatment by electrically acupuncturing the Acupoints is conducted on rats suffering actual spinal cord injury and the NGF and BDNF protein expression and the recovery of motor functions of rear limbs are observed to discuss the effect and the relevant treatment mechanism of EA stimulation. In the inspection of the 4th, 5th and the 6th week after the EA treatment begins, it is found that the motor functions of the rear limbs of the Experimental Group (group receiving EA treatment) is increasingly better than those of the Control group (group receiving sham EA treatment), P<0.05 or P<0.01 and the difference in their ratings has the statistical significance. After 6 weeks of EA treatment, the inspection of the two groups of rats in NGF and BDNF protein expression show that the NGF and BDNF protein expression is evidently better than that of the Control Group, P<0.01 for both cases and the difference is conspicuous. NGF has proved to be a special protein able to accelerate and maintain the growth, subsistence, differentiation and performance of functions and save the injure the nerve cells [14, 15]. BDNF is an important neurotrophic factor and has the similar homology to NGF. It not only plays an important role in maintaining the physiological function in the development of central nervous system, but induces the oriented growth of the nervous process [16,17]. So, it can be seen that NGF and BDNF are important materials interfering with the nerve repair system and playing an important role in the growth and repair of nerves. That is to say, the expression effect of NGF and BDNF protein quantity directly affect the nerve repair. So, EA stimulation of the SCI rats can obviously increase the NGF and BDNF protein expression to promote the regeneration of nerve cells to build new synaptic connection; thereby conducive to the recovery of the motor functions of the rear limbs.

So, this study shows that acupoints stimulation by electrical Acupuncture is conducive to the expression of NGF and BDNF protein the recovery of motor functions of rear limbs of acute SCI rats; while the recovery of motor functions are connected with NGF and BDNF expression that prompts the regeneration of nerve cells to build new synaptic connection; furthermore, the EA treatment is convenient and safe without any side effect, therefore is worthy of deeper study and gradual application in clinical practice.

REFERENCES
