Basic Investigations

Experimental Study on Mechanical Vibration Massage for Treatment of Brachial Plexus Injury in Rats

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Objective: To investigate the curative effect of the self-made mechanical vibration massage instrument for treatment of brachial plexus injury in rats and to explore its mechanism. Methods: Brachial plexus injury models were made in 144 Wistar rats and one week after natural healing of the wound, they were randomly divided into 3 groups, mechanical vibration treatment group (MV group), nerve growth factor treatment group (NGF group) and model group, 48 rats in each group. Then again, each group was randomly divided into 4 subgroups, 7-day group, 14-day group, 21-day group and 28-day group, 12 rats in each subgroup. The MV group were treated by mechanical vibration at acupoints on three-yang and three-yin channels of the hand with the mechanical vibration massage instrument; The NGF group were treated with injection of NGF into muscle major on the affected side; And the model group were normally fed with no treatment. After treatment for 7, 14, 21 and 28 days, the diameter of both forelimbs were measured, the electrophysiological examination on the brachial plexus in vitro and the ultrastructure observation with electron microscope on the affected side were carried out, the motor nerve conduction velocity (MNCV) and motor nerve action potential (MNAP) of the brachial plexus on the affected side, NGF content of submaxillary gland as well as muscular Na⁺, K⁺-ATPase activity were determined respectively.

Results: The different rates of the forelimb diameter in the MV group and the NGF group on the 14th d, 21st d and 28th d were better than those in the model group (P<0.05 or P<0.001), and in the MV group were better than those in the NGF group on the 21st d and the 28th d (P<0.05). MNCV in the MV group and the NGF group on the 21st d and 28th d was better than that in the model group (P<0.05 or P<0.001), and in the MV group was better than that in the NGF group on the 28th d (P<0.05). MNAP in the MV group and the NGF group on the 14th d, 21st d and 28th d was better than that in the model group (P<0.05 or P<0.001), and in the MV group was better than that in the NGF group on the 21st d and 28th d (P<0.05). The NGF mean gray index of submaxillary gland in the model group was higher than that in the MV group on the 7th d (P<0.05); in the NGF group and the model group was higher than that in the MV group on the 21st d and 28th d (P<0.05). Na⁺, K⁺-ATPase activity in the model group and the MV group was higher than that in the NGF group on the 14th d and in the MV group was higher than that in the model group on the 28th d (P<0.05). Conclusion: As compared with the NGF group and the model group, mechanical vibration treatment can effectively accelerate repair of injured brachial plexus, slow down atrophy of skeletal muscle, and promote secretion of NGF in submaxillary gland.

Key Words: mechanical vibration massage; brachial plexus injury; nerve growth factor; Na⁺, K⁺-ATPase activity
Brachial plexus injury is a frequently encountered disease and it severely affects human life quality. In the present paper, the curative effect of a self-made mechanical vibration massage instrument for treatment of brachial plexus injury in rats and its mechanism were investigated.

**MATERIALS AND METHODS**

**Grouping of Experimental Animals**

Brachial plexus injury models were made in 144 Wistar rats, weighing 160±10 g, and one week after natural healing of the wound, they were randomly divided into 3 groups, mechanical vibration treatment group (MV group), nerve growth factor treatment group (NGF group) and model control group, 48 rats in each group. Then again, each group were randomly divided into 4 subgroups, 7-day group, 14-day group, 21-day group and 28-day group, 12 rats in each subgroup.

**Main Reagents**

4% Paraformaldehyde solution, nerve growth factor (NGF), SABC kit and DAB color kit.

**Mechanical Vibration Massage Instrument:**

The mechanical vibration massage instrument was developed and manufactured by imitating the principle of one-finger massage of TCM. In animal experiment, it provided quantitative frequency, amplitude, duration and acting force for mechanical massage.

**Preparation of Rat Brachial Plexus Injury Model**

After the rat was anaesthetized with intraperitoneal injection of 10% chloral hydrate (12.5 ml/kg), the four limbs were fixed on a operation table in a dorsal position, it was shaved and routinely sterilized on the area around right sternoclavicular articulation within diameter of 5 cm with iodine tincture, and then the iodine was removed. An incision of 1.5 cm in length was made on the right infraclavicular part with a scissors at an angle of 15 degree with the clavicle (The top was at first third lateral to the clavicle and the base margin at the lateral to manubrium sterni).

The brachial plexus nerve was explored and separated to the lateral root of the intervertebral foramina, it was clamped twice with a ophthalmic micro-hemostat (with a soft plastic film casing pipe in its one tip for preventing over injuring the neurolemma), one buckle each time, once for 30 s. The nerve root of the brachial plexus was injured 1.5 mm in width with intact nerve perilemma and with axon almost completely split. Finally, penicillin powder was applied to the wound and the skin was sutured. Then the skin around the wound was disinfected once again. The modeling was completed. Seven days after the wound healed, the model rats were randomly grouped and treated.

**Treatment of Various Groups**

**MV group:** The rat in the MV group was fixed on a operation table in a dorsal position and the brachial plexus injury area (Ashi point) and “Quchi (LI 11) ”, “Hegu (LI 4) ” were vibrated in order with the massage instrument in a force of about 150±20 Gf (vibration frequency 2 times/s, amplitude 2 mm), 1 min for each point, in total 3 minutes, once daily; And then the three yin and three yang channels of the affected limb were continuously vibrated with above methods, five times for each channel, once each day.

**NGF group:** The rats in the NGF group were treated with injection of rat nerve growth factor (0.4 µg/kg) into musculus pectoralis major on the affected side, once each day.

**Model group:** After modeling, the rats in the model group were maintained in the same condition, with normal feeding and without treatment.

**Indexes and Methods of Observation**

The 7, 14, 21 and 28 days after treatment, the following observation and detection were made in order and respectively in the groups.

**Measurement of Limb Diameter**

After anaesthetized with intraperitoneal injection of 10% chloral hydrate (12.5 ml/kg), and before removing the brachial plexus, the diameters of
bilateral forelimbs (below the foreknee joint, corresponding to the largest medial and lateral transverse distance below human elbow joint, the highest point of brachioradial muscle) were respectively measured and the different rate (Diff rate) of the forelimbs was calculated according to the following formula, so as to judge the decree of muscular atrophy of the affected limb:

\[
\text{Diff rate} \, (\%) = \frac{(\text{Diameter of healthy forelimb (mm)} - \text{The diameter of affected forelimb (mm)})}{\text{Diameter of healthy forelimb (mm)}} \times 100\%
\]

Detection of MSCV and MSAP of Brachial Plexus in Vitro

Right brachial plexus sample was placed in Ren’s solution at 37.8°C for 10 minutes, and the motor nerve conduction velocity (MNCV) and the motor nerve action potential (MNAAP) of the brachial plexus were recorded with a Medlab-u/4 cs (V6.0) bio-signal collection and process system made by Nanjing Meiyi Science and Technology Ltd, Co. The stabilized brachial plexus sample was placed on the electrodes in a nerve shielding box. The central end was connected with the stimulation electrode and the peripheral end to the leading electrode. The efferent electric pulse acted on the central end of the nerve sample through the stimulation electrode, stimulation intensity 5V, wave duration 3.1 ms, main cycle 1s, interval 10 ms, pulse number 1, time delay 40 ms, cycle number continuous. The results were recorded respectively and related data were stored, and neuromyograms were collected for judging recovery degrees of injured nerve function.

Determination of the NGF Content in Submaxillary Gland

Contents of NGF in the submaxillary gland were detected with in situ hybridization method.

Measurement of the Na⁺, K⁺-ATPase Activity of Biceps Brachii on the Affected Limb

Na⁺, K⁺-ATPase activities of biceps brachii on the affected side were detected with Ubain method.

Statistical Process

All experimental data were entered into a computer and processed with SPSS statistical software. Enumeration data were analyzed with \( \chi^2 \) test and measurement data with \( t \)-test for comparison of independent samples between two groups, and ranked data with rank sum test for comparison of several independent samples.

RESULTS

Comparison of the Different Rate between both Forelimbs among the Groups (Table 1)

The different rates between the two forelimbs within the 28 days in all groups gradually increased at the early stage and gradually decreased at the later stage, reached to the peak on the 21st days in the model group and the NGF group, and on the 14th days in the MV group. The different rates in the MV group and the NGF group on the 14th, 21st and 28th days were better than those in the model group (\( P<0.05, \) or \( P<0.01 \)), and in the MV group was superior to those in the NGF group on the 21st and 28th days (\( P<0.05 \)); There were no significantly differences among other groups (\( P>0.05 \)).

<table>
<thead>
<tr>
<th>Group</th>
<th>The 7th day ( \bar{x} \pm s )</th>
<th>The 14th day ( \bar{x} \pm s )</th>
<th>The 21st day ( \bar{x} \pm s )</th>
<th>The 28th day ( \bar{x} \pm s )</th>
</tr>
</thead>
<tbody>
<tr>
<td>Model</td>
<td>10.337±0.804</td>
<td>13.546±0.889</td>
<td>15.345±1.188</td>
<td>14.019±1.071</td>
</tr>
<tr>
<td>NGF</td>
<td>8.249±1.744</td>
<td>10.076±1.611*</td>
<td>11.476±0.859*#</td>
<td>10.277±1.154*#</td>
</tr>
<tr>
<td>MV</td>
<td>9.692±0.589</td>
<td>9.982±2.621*</td>
<td>7.833±1.618**</td>
<td>6.694±0.368**</td>
</tr>
</tbody>
</table>

Note: Compared with the model group, *\( P<0.05 \), **\( P<0.01 \); Compared with the MV group, #\( P<0.05 \).

Comparison of the Motor Nerve Conduction Velocity among the Groups (Table 2)

The motor nerve conduction velocity (MNCV) in the model, NGF and MV groups within the 28 days increased gradually; MNCV in the MV group and the NGF group on the 21st and 28th days were faster
than that in the model group \((P<0.05\) or \(P<0.01\)), and in the MV group was faster than that in the NGF group on the 28th day \((P<0.05)\); There were no significantly differences among other groups.

**Table 2. Comparison of the motor nerve conduction velocity among the groups (m/s, \(n=12\), \(\bar{x} \pm s\))**

<table>
<thead>
<tr>
<th>Group</th>
<th>The 7th day</th>
<th>The 14th day</th>
<th>The 21st day</th>
<th>The 28th day</th>
</tr>
</thead>
<tbody>
<tr>
<td>Model</td>
<td>4.420±0.552</td>
<td>6.229±1.413</td>
<td>7.050±0.126</td>
<td>9.502±2.241</td>
</tr>
<tr>
<td>NGF</td>
<td>6.974±0.281</td>
<td>9.479±2.185</td>
<td>11.812±1.129</td>
<td>12.455±0.464</td>
</tr>
<tr>
<td>MV</td>
<td>5.306±0.740</td>
<td>8.316±2.258</td>
<td>11.817±1.395</td>
<td>15.138±1.22</td>
</tr>
</tbody>
</table>

Note: Compared with the model group, \(*P<0.05\), \(**P<0.01\); Compared with the NGF group, \(#P<0.05\).

**Comparison of the Motor Nerve Action Potential (MNAP) among the Groups (Table 3)**

MNAPs in the model, NGF and MV groups increased gradually within the 28 days. MNAPs in the MV and NGF groups were higher than that in the model group \((P<0.05\) or \(P<0.01\)) on the 14th, 21st and 28th days, and in the MV group was higher than that in the NGF group on the 21st and 28th days \((P<0.05)\). There were no significant differences among other groups \((P>0.05)\).

**Table 3. Comparison of motor nerve action potential among the groups (mv, \(n=12\), \(\bar{x} \pm s\))**

<table>
<thead>
<tr>
<th>Group</th>
<th>The 7th day</th>
<th>The 14th day</th>
<th>The 21st day</th>
<th>The 28th day</th>
</tr>
</thead>
<tbody>
<tr>
<td>MV</td>
<td>6.503±1.580</td>
<td>12.212±3.103</td>
<td>18.801±2.126</td>
<td>22.294±1.220</td>
</tr>
</tbody>
</table>

Note: Compared with the model group, \(*P<0.05\), \(**P<0.01\); Compared with the MV group, \(#P<0.05\).

**Comparison of Average Gray Scale Values of Submandibular Gland NGF among the Groups (Table 4)**

The cytoplasm of NGF positive cells in all of the groups showed pale brown, and the average gray scale values in the model, MV and NGF groups gradually increased within the 28 days. The average gray scale value in the model group was higher than that in the MV group on the 7th day \((P<0.05)\), and in the NGF group and the model group were higher than that in the MV group on the 14th day \((P<0.05)\), and in the NGF and MV groups were higher than that in the model group on the 21st and the 28th days \((P<0.05)\); There were no significant differences among other groups \((P>0.05)\).

**Table 4. Comparison of average gray scale values of submandibular gland NGF among the groups \(n=12\), \(\bar{x} \pm s\))**

<table>
<thead>
<tr>
<th>Group</th>
<th>The 7th day</th>
<th>The 14th day</th>
<th>The 21st day</th>
<th>The 28th day</th>
</tr>
</thead>
<tbody>
<tr>
<td>Model</td>
<td>2108.26±221.22</td>
<td>2948.80±332.95</td>
<td>3096.90±416.56</td>
<td>3404.98±320.73</td>
</tr>
<tr>
<td>NGF</td>
<td>1473.31±208.34</td>
<td>2889.82±181.69</td>
<td>4066.62±522.786</td>
<td>4956.04±128.33</td>
</tr>
<tr>
<td>MV</td>
<td>1286.71±143.64</td>
<td>2180.00±253.53*</td>
<td>3924.76±335.87*</td>
<td>4865.87±425.33*</td>
</tr>
</tbody>
</table>

Note: Compared with the model group, \(*P<0.05\); Compared with the MV group, \(*P<0.05\).
Table 5. Comparison of Na\(^+\), K\(^+\)-ATPase activities in biceps brachii on the affected limb among the groups (mol/s, n=12, \( \overline{x} \pm s \))

<table>
<thead>
<tr>
<th>Group</th>
<th>The 7th day</th>
<th>The 14th day</th>
<th>The 21st day</th>
<th>The 28th day</th>
</tr>
</thead>
<tbody>
<tr>
<td>Model</td>
<td>54.66±9.05</td>
<td>59.98±4.82(^*)</td>
<td>47.68±8.54</td>
<td>37.99±7.61</td>
</tr>
<tr>
<td>NGF</td>
<td>53.45±6.07</td>
<td>51.18±3.67</td>
<td>46.81±6.45</td>
<td>40.67±8.01</td>
</tr>
<tr>
<td>MV</td>
<td>55.38±3.12</td>
<td>58.73±1.65(^*)</td>
<td>50.74±3.89</td>
<td>44.67±7.91(^*)</td>
</tr>
</tbody>
</table>

Note: Compared with the model group, \(^*\)P<0.05; Compared with the NGF group, \(^*\)P<0.05.

DISCUSSION

Effect of Mechanical Massage Treatment on Muscles of Limbs

Mechanical vibration massage treatment has obvious effect on muscular atrophy induced by nerve root injury.\(^1\) It can dilate capillary, increase volume of blood flow, so as to greatly improve blood supply and nutrition in local tissue; It can make the wall of micrangium rhythmically flatten and restore, accelerating flow of blood; And it can promote contraction and extension of muscle fibers, strengthen muscular tension, elasticity and tolerance, so, it can prevent and cure muscular atrophy.

Effect of Mechanical Massage on Secretion of NGF

Benign stimulation of mechanical vibration massage can activate the response of nerve immune and neuroendocrine systems, and transmit the signals to the submandibular gland through complicated ways, promoting secretion and storage of NGF in the submandibular gland.\(^2,3\) Finally, NGF is transported to brachial plexus root injury area through digestive, circulative and nerve systems.

Effect of Mechanical Massage on Repair of Injured Nerves

Mechanical vibration massage can effectively promote the repair of myelin sheath and axes of injured brachial plexus in the rat. It can effectively improve blood circulation of the injured myelin sheath, promote proliferation of SC and survival of the cell body of injured neurons, so as to form a necessary regenerative micro-environment early for repair of nerve, and it induces stress responses of immune and neuroendocrine systems in the rat, promotes secretion of NGF in this gland, and it can improve peripheral nerve units and excite peripheral nerves, so as to accelerate their conduction reflection.\(^1,4,5\)

Effect of Mechanical Massage on Na\(^+\), K\(^+\)-ATPase Activities

Na\(^+\), K\(^+\)-ATPase activity on the surface of muscular cell membrane is an important limited factor for excitability and contractile strength of muscular cells. After skeletal muscles lose nervous innervation, generation of ATP is hindered, so Na\(^+\), K\(^+\)-ATPase activity decreases. Under the mechanical massage stimulation, the muscular cells cultured in vitro show increases in stress-related gene expression and protein synthesis, leading to adaptability reconstruction of structures and contractile characters of the muscular cells, which are closely related with activation of Na\(^+\), K\(^+\)-ATPase, and influences the distribution and functional activity of Na\(^+\), K\(^+\)-ATPase on the surface of muscular cell membrane.\(^6-12\)

In brief, mechanical vibration massage can promote the regeneration and recovery of the brachial plexus, and effectively slow down the decrease of Na\(^+\), K\(^+\)-ATPase activities induced by the nerve injury, preventing muscular atrophy, and it promotes the generation of submandibular gland NGF, providing a favorable environment for regeneration of nerve cells.

REFERENCES

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