**Optimal Electrical Stimulation Frequency to Improve the Muscle Endurance in Spinal Cord Injury Rabbit**

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**Introduction**

Functional Electrical Stimulation (FES) has been applied to patients with central nervous system disorders such as spinal cord injury (SCI) or stroke in order to strengthen paralyzed limbs or assist ambulation. However, clinical application of the FES was limited due to muscle fatigue phenomenon and unresponsiveness of muscle to continuous electrical stimulation [1]. After SCI, there is noticeably marked muscle atrophy, and also a reduction in the proportion of fatigue-resistant Type I fibers [2], which aggravates muscle fatigability and limit the clinical applications of FES.

There are several reports showing certain types of electrical stimulation can improve muscle fatigability by increasing the proportion of fatigue-resistant muscle fibers. It was demonstrated by Hudlicka et al. that long-term electrical stimulation of fast muscles could affect muscle contractile properties to resemble those of slow muscles irrespective of the frequency of stimulation (10, 40 or 60 Hz) when the total number of stimulation was comparable, and the duration of stimulation was long enough, (2 weeks or longer) [3]. But optimal frequency parameters of electrical stimulation to retard muscle fatigue and to improve muscle endurance in SCI patients are still controversial. There has been much success in the use of FES, but little has been done to develop optimal protocol for endurance training [4]. This study was undertaken to find an electrical stimulation frequency, which optimally improves muscle endurance in spinal cord injured rabbit.

**Method**

**Experimental setting**

Twelve white New Zealand rabbits weighing 3.0 kg were taken to get spinal cord injury by transecting their spinal cords at T10 or T11 spinal level [5]. Complete paraplegia was obtained in all 12 rabbits. Hind limb spasticity was observed within 2 days after experimental spinal cord injury. Ankle clonus and increased knee jerk were found in all rabbits. Electrical stimulation was applied to the motor point of the tibialis anterior muscle for 1 hour per day for 2 weeks. Electrical stimulation parameters were set to have 0.3-millisecond duration sine wave, 6 seconds on-off duty cycle and constant voltage at 10 V. Three kinds of stimulation frequencies (10, 20 and 40 Hz) and sham control stimulation were applied, for a total of four groups (10Hz, 20Hz, 40Hz and a control group).

**Outcome measurements**

After 2 weeks of electrical stimulation, isometric ankle dorsiflexion (DF) torque was measured using a push-pull strain gauge during 30 seconds of electrical stimulation of tibialis anterior muscle in decubitus posture with knee (90° flexion) and ankle joints (45° plantar flexion) immobilized (Fig.1). Peak isometric torque of ankle dorsiflexion and muscle fatigue indices were compared among the four groups. The muscle fatigue index was calculated by taking the ratio of the decrement of the isometric DF torque after 30 sec of electrical stimulation compared with the initial torque. (Muscle fatigue index (%) = (initial torque – torque after 30 seconds stimulation) / Initial torque x 100)

Tibialis muscles were isolated after the biomechanical test and snap-freezeed to get ATPase staining at pH 9.4. Numbers of type I muscle fibers (stained white) and type II muscle fibers (stained dark) were counted under a
light microscope at x100 magnification in two different fields of the same specimen. The proportion of type I muscle fibers was calculated by taking the percentage of the number of type I muscle fibers in the total number of muscle fibers counted in those sampled fields. The cross sectional areas of type I and type II muscle fibers in each tibialis anterior muscle were also measured using an image analysis software (Image Pro Plus®).

Fig 1. Experimental setting for isometric torque measurement.  Fig 2. Measurement of isometric torque of ankle dorsiflexor

Results

Mean peak isometric torque were 54.88±38.52 x 10^{-3} Nm in the control group, 95.94±90.28 x 10^{-3} Nm in the 10 Hz group, 47.75±34.23 x 10^{-3} Nm in the 20 Hz group and 57.80±52.41 x 10^{-3} Nm in the 40 Hz group. There were no significant differences among 4 groups.

Muscle fatigue index were 59.92±17.99 in control group, 60.26±19.19 in 10 Hz group, 50.60±31.86 in 20 Hz group and 26.22±13.73 in 40 Hz group, and there was significant difference between control group and 40 Hz group and between 10 Hz group and 40 Hz group (p<0.05 by Mann-Whitney test), but no significant differences among the 4 groups (by Kruskal Wallis test).

The proportion of the type I muscle fiber were 4.24±1.72% in the normal group, 1.27±0.46% in the control group, 1.07±2.89% in the 10 Hz group, 1.70±2.46% in the 20 Hz group and 3.33±1.32% in the 40 Hz group. The proportion of the type I fiber was reduced in all four groups 2 weeks after spinal cord injury. However, there was a statistically significant difference among all groups (p<0.05 by Kruskal Wallis test) and the 40 Hz group showed less decline in proportion of type I muscle fiber than control or 10 Hz group (p<0.05 by Mann-Whitney test).
The cross sectional areas of type I fiber were 3725.0±1035.5 µm² in the normal group, 1787.9±1184.7 µm² in the control group, 2063.0±1454.6 µm² in the 10 Hz group, 1849.9±811.0 µm² in the 20 Hz group and 1945.5±1105.9 µm² in the 40 Hz group. Those of type II fiber were 3398.7±969.3 µm² in the normal group, 2028.5±1071.8 µm² in the control group, 1789.1±737.4 µm² in the 10 Hz group, 1929.1±1257.6 µm² in the 20 Hz group and 1835.0±1090.2 µm² in the 40 Hz group. There were no significant differences of cross sectional area of type I and type II fibers among all groups.

Table 1. Outcome measurements

<table>
<thead>
<tr>
<th>Group</th>
<th>Peak torque (10⁻³ Nm)</th>
<th>Fatigue Index (%)</th>
<th>Proportion of type I muscle fiber (%)</th>
<th>Cross sectional area (µm²)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Type I</td>
</tr>
<tr>
<td>Normal</td>
<td></td>
<td></td>
<td></td>
<td>3725.0±1035.5</td>
</tr>
<tr>
<td>Control</td>
<td>54.88±38.52</td>
<td>59.92±17.99</td>
<td>1.27±0.46</td>
<td>1787.9±1184.7</td>
</tr>
<tr>
<td>10 Hz</td>
<td>95.94±90.28</td>
<td>60.26±19.19</td>
<td>1.07±2.89</td>
<td>2063.0±1454.6</td>
</tr>
<tr>
<td>20 Hz</td>
<td>47.75±34.23</td>
<td>50.60±31.86</td>
<td>1.70±2.46</td>
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</tr>
<tr>
<td>40 Hz</td>
<td>57.80±52.41</td>
<td>26.22±13.73</td>
<td>3.33±1.32</td>
<td>1945.5±1105.9</td>
</tr>
</tbody>
</table>

*: The 40Hz group showed a significantly (p<0.05) higher value than the control and 10Hz groups by Kruskal-Wallis and Mann-Whitney tests.

Discussion

It was demonstrated that 2 weeks of electrical stimulation following spinal cord injury altered the proportion of type I fiber in comparison with no electrical stimulation after spinal cord injury, suggesting electrical stimulation can retard the atrophy of fatigue-resistant type I fibers. This finding was noticed especially in 40 Hz frequency group, which showed lower muscle fatigue index than other groups, though the difference was not statistically significant.

Traditionally, low electrical stimulation frequency was thought to induce transformation of type II muscle fiber into type I muscle fiber. For example, the result of Grimby et al. was different from that of this study in terms of the electrical stimulation frequency to give higher proportion of type I muscle fiber. They found lower frequency had better effect for developing fatigue resistance [5]. According to Hudlicka et al., the important factor to increasing fatigue resistance was not the frequency but the number of stimulation pulses [3]. Because we used the same stimulation duration in each different frequency group, the higher frequency group must have had a higher number of stimulation pulses. This factor might have affected the results of our data.

It can be assumed that the duration of electrical stimulation was too short to alter the mechanical properties of muscle fibers, considering there was no significant difference in any of the biomechanical parameters. The duration of electrical stimulation was limited by the survival of spinal cord injured rabbits, which was for 2 to 3 weeks after spinal cord injury.

We did not evaluate the effect of duty cycle. Petrofsky reported that the most effective parameters to improve muscle endurance was duty cycle with 6 seconds on and 6 seconds off, a duration of 30 minutes daily and at every other day of the week [4]. Further study of electrical stimulation with various duty cycles is required.

Electrical stimulation for 2 weeks after spinal cord injury had no significant effect on muscle fatigue index and muscle strength. But high frequency electrical stimulation (40 Hz) applied at an early stage of spinal cord injury was more effective in preserving muscle endurance than low frequency stimulation (10Hz). The benefits of high frequency electrical stimulation in preserving muscle endurance must be verified by further studies.
References