Electrophysiological and Histological Investigation on the Gradual Elongation of Rabbit Sciatic Nerve

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Abstract

A basic study using animal models was performed to investigate whether the sciatic nerve retains physiological functions and normal morphology after the gradual elongation associated with adjacent bone elongation. Electrophysiological and histological studies were performed on the elongated sciatic nerve of rabbit accompanied by the femur bone elongation. Compound action potentials evoked by electrical stimulation of the sciatic nerve were recorded and histological specimens of elongated nerve fibers were obtained immediately after final bone elongation from 4 rabbits (immediate group). Three rabbits were allowed to recover for 8 weeks after the bone elongation (maintained group). Three rabbits without bone elongation were used as controls of the immediate and maintained groups (control group). In the immediate group, the average amplitude of evoked nerve potentials were 30.38 ± 1.58 mV before elongation and diminished significantly to 18.35 ± 1.25 mV immediately after elongation (P<0.01). The amplitude of evoked potentials was not significantly different between before (30.30 ± 0.61 mV) elongation and after elongation (27.47 ± 1.63 mV) in the maintained group. The axonal area of the myelinated nerve fibers of the proximal region of the sciatic nerve in the immediate group was significantly decreased after elongation (P<0.01). The decrease in the area of the distal region was greatest in the control group and was followed by that in the maintained group and the immediate group (P<0.05, 0.01). These results suggest that the sciatic nerve shows dysfunction immediately after elongation, but can recover electrophysiologically and histologically several weeks after elongation.

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Key words: peripheral nerve elongation, animal model, nerve evoked potential, axonal area of myelinated nerve fiber

Introduction

When soft tissue of the extremities is severely injured by trauma, the peripheral nerves may be cut or severely crushed. Clinically, neurorrhaphy is performed to repair injured nerves', but when primary suture is not possible, 1- or 2-stage autologous nerve grafting may be performed. However, this method is disadvantageous for nerve

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regeneration from various aspects. Thus, several methods for reconstructing nerve defects other than nerve grafting have recently been investigated, but their clinical effects have not been verified through basic studies. On the other hand, basic studies of compensatory indirect elongation of healthy peripheral nerves have shown that nerves can be elongated under conditions structurally and functionally similar to the physiological state. In addition, experimental studies have started to examine the repair of nerve defects by the direct elongation of nerves.

Severe traumatic nerve injuries are often accompanied by compound fractures, which may necessitate excision of contaminated or comminuted bone and subsequent bone elongation to repair the shortened limb. For this reason, it is important to investigate from a clinical viewpoint whether a sutured nerve regenerates when simultaneously elongated with the bone. In the present study, we designed a rabbit model of gradual bone elongation in which the sciatic nerve is cut and its proximal stump is also slowly elongated. To evaluate the functional and morphological properties of the elongated nerve, we examined the elongated sciatic nerve both electrophysiologically and histologically.

**Materials and Methods**

**Animals**

All experimental studies were performed with procedures including animal care conforming to The Guide for the Use of Laboratory Animals and were approved by the Animal Experimental Ethics Review Committee of the Nippon Medical School (No. 14:25, 15:11, 16:1).

In all experiments, 20-week-old female New Zealand White rabbits (weight 2.8 to 3.5 kg) were divided into an immediate group (5 rabbits), a maintained group (6 rabbits), and a control group (5 rabbits). After animals were excluded because of death during the bone elongation period or wound infections that made final observation impossible, the final experimental results were obtained from 4, 3, and 3 rabbits, in the immediate, maintained, and control groups, respectively.

**Development of Animal-holding Apparatus and Animal Preparation**

An H-shaped frame prepared in our laboratory was vertically fixed to the side of the experimental table where the caudal side of the animal was placed. A stainless-steel tensile spring (spring constant 4.3 N/m) was set to the frame and its total length was measured with calipers.

General anesthesia was induced by the intravenous administration of pentobarbital at a dose of 30 mg/kg, and the animal preparation was started after the animal was confirmed with pain stimulation to have been completely immobilized. An incision was made in the posterolateral aspect of the thigh through the popliteal fossa, and the soft tissue was dissected. The region was opened along the sciatic nerve from the sciatic notch, through which the nerve distributes to the muscles of the posterior thigh, to the popliteal fossa. The posterior femoral bone was exposed and ligated with 6-0 nylon thread at the knee joint level of the sciatic nerve, staying as much as possible at the depth of the epineurium, with the hip joint extended and the knee joint flexed 30 degrees. One end of the nylon thread was passed through the spring, and the thread-tying region was adjusted to prevent the thread from bending and to prevent the total length of the spring from changing. The nerve was then cut 2 mm distal to the nylon-ligated site.

**Measurement of the Physiological Tension to Fix the Nerve to the Femur**

A separate experiment was performed to set a constant tension to fix the nerve to the femur. Because the cut nerve shortened and pulled the spring, extension of the spring was measured with calipers. The experiment was performed with both the right and left nerves in 5 rabbits (10 nerves in total), and the mean spring extension was calculated as an indicator of physiological tension.

**Femur Elongation**

The posterior aspects of the femur and sciatic nerve were exposed as described above. Two 0.8-mm-diameter holes were made 5 mm apart on a line perpendicular to the longitudinal axis one-fourth the
length of the femur from its proximal end, and the sciatic nerve was cut at this level. Threads of 6-0 nylon were applied to 2 sites on the circumference of the nerve stump facing at about 180 degrees, i.e., individually passing through the holes made in the femur and exiting on the opposite side. The tension of the exiting nylon thread was adjusted to the value determined in the above experiment of physiological tension. Namely, the thread was tied to the tensile spring, which was then slowly pulled to the site corresponding to the physiological tension, and the site where the nylon thread exited the femur was marked with using a surgical marker pen. The thread was fixed to the bone with an orthopaedic stainless-steel button at the marked sites. The sciatic nerve epineurium exposed through the sciatic notch was ligated with 8-0 nylon thread as a marker for nerve elongation.

Two 2.5-mm-diameter cortical bone screws (M300; MiniRail Fixator System, Orthofix Inc., Lewisville, TX, USA) were placed 8 mm apart on the central and peripheral sides on a line perpendicular to the longitudinal axis of the femur (4 screws in total). The distance between the screws on the central and peripheral sides was 3 cm. The 4 screws were fixed to a straight line external fixator (M101; MiniRail Fixator System, Orthofix), and the femur was cut perpendicularly to the femoral longitudinal axis at the midpoint between the central and peripheral screws (Fig. 1). The evoked potential of the peripheral nerve near the nerve stump in response to sciatic nerve stimulation at the sciatic notch was recorded and the wound was closed. After this condition was maintained for a recovery period of 5 days, the bone was elongated at a rate of 2 mm per day for 14 days.

Immediately after bone elongation was completed, 5 rabbits in the immediate group were anesthetized with pentobarbital in a similar manner to that for clinical osteotomy, the posterior femoral region was opened, and the sciatic nerve was exposed. The evoked potential was recorded, and then the elongated region of the nerve was excised. In the 6 rabbits of the maintained group, the sciatic nerve was exposed 8 weeks after bone elongation was completed, and evoked potential recording and nerve sampling were performed. In the 5 rabbits of the control group, all procedures were performed in the same way as those in the experimental groups except for bone elongation; evoked potentials were recorded and nerve specimens were obtained 19 days after surgery.

**Electrophysiological Examination**

Compound nerve action potential (CNAPs) evoked by electrical stimulation of the sciatic nerve were recorded with an electromyograph (Neuropac 2, Nihon Kohden Corp., Tokyo). For the recording electrode, 0.08-mm-diameter urethane-coated stainless steel wires were used. One end of a wire was exposed for a length of 2 mm and 2 wires were fixed in a holder as bipolar electrodes 2 mm apart. The exposed parts of the wires were placed in contact with the superficial layer of the nerve 5 mm central to where the nerve was attached to the femur. For the bipolar stimulation electrode, the same wires were fixed 5 mm apart and attached to the region exposed via the sciatic notch. The sciatic nerve was stimulated at 1 Hz with a square pulse lasting 0.1 millisecond at the supramaximal intensity of the CNAP evoked on the peripheral side. Thirty-two evoked potentials were averaged, and the amplitude (absolute value of the difference between the maximum negative and positive peaks) and latency (the first negative peak) of the CNAP were
determined (Fig. 2). The CNAP was measured before and after the elongation of the sciatic nerve in each group.

**Histological Examination**

Rabbits were anesthetized with 30 mg/kg of pentobarbital. After the evoked nerve potential was recorded, the abdomen and thorax were opened. An 18-G indwelling venous needle was inserted into the abdominal aorta, and a 2.5% glutaraldehyde solution was infused for fixation. The abdominal vena cava was cut to allow the perfusion solution to flow out spontaneously. After the cessation of spontaneous respiration and subsequent cardiac arrest were confirmed, the sciatic nerve from the sciatic notch through the region fixed to the holes was excised en bloc and placed on physiological saline-soaked gauze, and its total length was measured. The excised nerve was divided into 3 parts of equal length, and 3-mm-long nerve fragments were cut out from the proximal and distal sides (Fig. 3). After post fixation in 2% osmic acid at 4°C for 2 hours, the preparations were dehydrated with ethanol and embedded in Epon-812. Ten thin sections (0.5 µm thick) stained with toluidine blue were prepared from each specimen. Three sections were randomly selected and observed under a microscope (magnification: 10 × 40), and the central 100-µm region was digitally recorded with an image-recording device (Provis AX80T, Olympus Optical Co., Ltd., Tokyo). The data were stored in a personal computer, and the axonal area of myelinated nerve fibers was measured in the images with Photoshop CS3® Extended (Adobe Systems, San Jose, CA, USA) (Fig. 4).

**Statistical Analysis**

Results are presented as mean values and standard errors of the mean (SEM). The amplitude and latency of the CNAP were compared between before and after elongation, and the significance of differences was investigated with the Wilcoxon t-test. The Kruskal-Wallis H-test was used to compare the amplitude and the latency among the 3 groups.

Unpaired t-tests were used to compare the axonal areas of the sciatic nerves between the proximal and distal region among each group. After significant mean differences were identified by one-way analysis of variance (ANOVA), Fisher’s protected
least significant differences tests were used to evaluate differences in the axonal areas of the sciatic nerves between groups.

For all statistical tests, P<0.05 was considered to indicate statistical significance.

Results

Length of Nerve Elongation

The mean extension of the spring indicating the physiological tension to fix the nerve to the femur was 3 mm in a separate experiment with 10 nerves in 5 rabbits.

Regarding the distance between the mark on the sciatic notch and the hole in the bone before elongation as the baseline, the mean elongated lengths were 61% and 60% of the baseline in the immediate and maintained groups, respectively.

Electrophysiological Examination

In the immediate group, the mean amplitude of the CNAP was significantly decreased from 30.38 ± 1.58 mV before bone elongation to 18.35 ± 1.25 mV after the elongation (P<0.01) (Table 1). The mean latency increased slightly from 2.10 ± 0.16 milliseconds before elongation to 2.25 ± 0.17 milliseconds after elongation, but the difference was not significant (Table 2). In contrast, the maintained group showed no significant difference in either the amplitude or latency between before elongation and 8 weeks after elongation, showing normal recovery (Table 1, 2). In the control group, no significant changes were noted in the amplitude or latency before elongation or immediately before specimen collection, and, furthermore, there were no significant differences in the amplitude or latency between the control and maintained groups immediately before specimen collection, showing full recovery from the functional deficit associated with nerve elongation in the rabbits of the maintained group (Table 1, 2).

Histological Examination

In each group, the axonal area of 51 to 67 nerve fibers was measured in each visual field, and the total number of nerve fibers measured in the proximal and distal regions of the elongated nerve was 633 to 694. Comparison of the mean area between the proximal and distal regions in each group revealed no significant difference in any group (Table 3).

The mean area of the proximal region of the sciatic nerve showed significant differences between the immediate and maintained groups (P<0.05) and between the immediate and control groups (P<0.01) but not between the maintained and control groups. In contrast, the distal region showed significant
An Experimental Nerve Elongation

Table 1 Amplitudes of CNAP of the sciatic nerves

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<th>Before the elongation (mV)</th>
<th>After the elongation (mV)</th>
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<tbody>
<tr>
<td>Immediate group</td>
<td>30.38 ± 1.58</td>
<td>18.35 ± 1.25*</td>
</tr>
<tr>
<td>Maintained group</td>
<td>30.30 ± 0.61</td>
<td>27.47 ± 1.63</td>
</tr>
<tr>
<td>Control group</td>
<td>29.67 ± 0.66</td>
<td>29.03 ± 1.87</td>
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*P<0.01

Table 2 Peak latencies of CNAP of the sciatic nerves

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<th>Before the elongation (ms)</th>
<th>After the elongation (ms)</th>
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<tbody>
<tr>
<td>Immediate group</td>
<td>2.10 ± 0.16</td>
<td>2.25 ± 0.17</td>
</tr>
<tr>
<td>Maintained group</td>
<td>2.00 ± 0.06</td>
<td>2.03 ± 0.09</td>
</tr>
<tr>
<td>Control group</td>
<td>2.17 ± 0.19</td>
<td>2.17 ± 0.20</td>
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Table 3 Mean axonal area of the sciatic nerves

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<th>Proximal region (µm²)</th>
<th>Distal region (µm²)</th>
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<tbody>
<tr>
<td>Immediate group</td>
<td>39.88 ± 0.86</td>
<td>37.38 ± 0.70</td>
</tr>
<tr>
<td>Maintained group</td>
<td>43.07 ± 0.82</td>
<td>40.83 ± 0.75</td>
</tr>
<tr>
<td>Control group</td>
<td>45.13 ± 1.17</td>
<td>43.37 ± 0.84</td>
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*P<0.05 compared with the immediate group.  
P<0.01 compared with the immediate group.  
P<0.01 compared with the immediate group and P<0.05 compared with the maintained group.

differences among the 3 groups (P<0.05, 0.01); the mean area was 43.37 ± 0.84, 40.83 ± 0.75, and 37.38 ± 0.70 µm² in the control group, immediate and maintained groups, respectively (Table 3). These data of the distal region indicate a partial recovery from the morphological deficit associated with nerve elongation in the rabbits of maintained group.

Discussion

Nerve grafting is frequently performed to treat peripheral nerve defects when primary suturing cannot be performed. However, nerve grafting raises problems, such as that the nerve is sutured at 2 sites, which is disadvantageous for nerve regeneration; the number and length of nerve grafts are limited; nerve graft sampling may impair the sampled site; and inconsistency of nerve bundles at the grafted site may occur. Methods other than nerve grafting for reconstructing nerve defects have been intensively investigated; for example, the artificial nerve grafting4, the homogeneous nerve transplantation10 and the nerve elongation using a tissue expander12,13 have been developed, but their effects are unstable, and basic studies are insufficient.

The elongation of healthy peripheral nerves likely facilitates physiological nerve regeneration. Basic studies of the pathological and physiological effects of indirect nerve elongation have been progressing; for example, studies have examined an elongation method involving adjustment of the near joint angle with an external fixator after neurorraphy12 and the effects of bone elongation near the nerve without manipulation of the nerve itself14. In addition, basic experiments on the repair of nerve defects by direct nerve elongation have been started11,16. Previous experimental studies have found that maximizing the elongation speed could shorten the duration of elongation, but that overly fast elongation
irreversibly impairs the elongated nerve, mainly due to dilatation of the nodes of Ranvier, and renders the nerve nonfunctional\(^8\). A study in rabbits has shown that a nerve elongation rate of 2 mm per day causes no electrophysiological or histological abnormalities\(^9\).

On the basis of these reports, we prepared an experimental model of nerve elongation at a rate of 2 mm per day and elongated the nerve with a method different from the current indirect and direct elongation methods to investigate whether a functionally normal nerve could be prepared. Our method is intermediate between the direct and indirect elongation methods and is unlike other methods previously reported. Considering the treatment of trauma accompanied by severely crushed soft tissue and open comminuted fracture, this basic experiment is based on practical conditions and is clinically relevant.

In the present study, the amplitude of the CNAP changed significantly after nerve elongation in the immediate group, but the latency did not change after elongation in any group. Changes in the amplitude were greatest in the immediate group. In the maintained group, the amplitude of the CNAP decreased slightly from 30.30 ± 0.60 mV before elongation to 27.47 ± 1.63 mV 8 weeks after the completion of elongation, but the change was not significant. These data indicate that deficits associated with nerve elongation resolved 8 weeks after the completion of elongation. That latency was unchanged suggests that elongation did not affect the conduction velocity of the CNAP in large fibers. Histological examination revealed significant differences in the proximal region between the immediate and maintained groups and between the immediate and control groups but not between the maintained and control groups. Previous studies have also revealed that functions of the elongated nerve remain incomplete immediately after elongation, but the functions recovered to near the normal state after maintenance for several weeks after elongation\(^8,10\). Similar findings were obtained in our study, suggesting that simultaneous nerve elongation and bone elongation may be clinically applicable in the future. However, a significant histological difference was observed in the distal region between the maintained and control groups. Although nerve elongation reduced the axonal area in both the proximal and distal regions, and near-normal physiological conditions returned with maintenance in the proximal region, whether recovery of the distal region can be similarly achieved by prolonging the maintenance period should be investigated.

**References**


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