Delayed Repair of Transected Nerves

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Objective: To determine if administration of brain-derived neurotrophic factor (BDNF) after peripheral nerve transection can improve the functional outcome in situations where epineurial repair must be delayed.

Design: Randomized, blinded, controlled trial.

Subjects: Thirty-four Sprague-Dawley rats.

Intervention: Sciatic nerves were transected and, after a 2-week delay, repaired with epineurial sutures. Animals were assigned to receive daily administration of lactated Ringer solution (LR [control] group); BDNF delivered at the time of nerve transection through 2 weeks after nerve repair, for a total of 4 weeks (BDNF-early group); or BDNF delivered at the time of nerve repair through 2 weeks after repair (BDNF-late group). Outcome was assessed using sciatic functional indices (SFIs) and histomorphometric analysis.

Results: The SFI maximal recovery was superior in the BDNF groups, but this difference did not reach statistical significance (SFI, −90.1 ± 9.6 [LR group], −85.7 ± 7.6 [BDNF-early group], and −84.6 ± 4.8 [BDNF-late group], where normal function is 0 and complete loss of function is −100; P = .27). The mean axon diameter tended to be greater in the BDNF groups compared with the LR group, ie, 2.43 ± 0.23 µm (LR group), 2.80 ± 0.44 µm (BDNF-early group), and 2.83 ± 0.38 µm (BDNF-late group) (P = .05).

Conclusions: The local administration of BDNF to nerves that underwent transection and then repair after a delay resulted in an increase in axonal diameters and maximal SFIs, a difference that did not reach statistical significance. The timing of BDNF administration after nerve transection did not affect neuronal regeneration.


A recent area of intense investigation has been the role of neurotrophic factors in the process of peripheral nerve regeneration after injury and repair. After nerve transection, a series of events occur during regeneration that involve the nerve cell body, the axonal fiber, the target muscle, and the surrounding chemical and cellular environment. Distal nerve endings undergo wallerian degeneration, and skeletal muscle begins to atrophy because of lack of electrical stimulation and a decrease in myotrophic factors. Nerve cell bodies enter a restorative phase that is critical for successful regeneration to occur. Trophic factors appear to play an important role in supporting the neuronal cell body and the axons during this period of regeneration.1-2

First identified by Barde et al,3 brain-derived neurotrophic factor (BDNF) is a 27-kd homodimer with transcripts found in many organ tissues, including skeletal muscle.4 Messenger RNA from BDNF is up-regulated in adult peripheral nerves after axotomy and in muscle after denervation.5,6 Brain-derived neurotrophic factor has been shown to prevent degeneration of transected motor neurons in the neonatal rat.6-8 These characteristics and actions of BDNF suggest a role in the support of peripheral motor neurons.

Attention has been directed toward the clinical utility of BDNF in the treatment of neurodegenerative disorders and peripheral nerve injury. After nerve injury and immediate repair, local administration of BDNF has been shown to improve functional outcome in the rat sciatic nerve model.9,10 In clinical situations of nerve injury, however, immediate neural repair is not always possible. Because of its neurotrophic and myotrophic characteristics, we speculated that the early administration of BDNF may be beneficial in supporting neural cell bodies and in decreasing muscle atrophy after nerve transection when immediate nerve repair is not

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METHODS AND MATERIALS

A total of 34 male Sprague-Dawley rats were assigned in a random, blinded fashion to one of the following 3 treatment groups: lactated Ringer solution (LR [control] group); BDNF delivered at the time of nerve transection through 2 weeks after the time of delayed nerve repair, for a total of 4 weeks (BDNF-early group); and BDNF delivered at the time of delayed nerve repair through 2 weeks (BDNF-late group). A color code was applied to the base of each animal’s tail so that the investigators could remain blinded to the group designation until the completion of the study. Throughout the protocol, all animals were housed in the Stanford Research Animal Facility, Stanford University, Stanford, Calif. The research design was approved by the Institutional Animal Care and Use Committee of Stanford University.

SURGICAL TECHNIQUES

The animals were anesthetized with intraperitoneal pentobarbital sodium (80 mg/kg). By use of a sterile technique, the left sciatic nerve was exposed with a muscle-splitting incision at the middle of the thigh. The sciatic nerve was transected sharply, and a single 10-0 polypropylene suture tag (Prolene; Ethicon Inc, Somerville, NJ) was placed through the epineurium of the proximal and distal nerve ends to facilitate identification of the nerve endings for subsequent neurorrhaphy. A silicone microdome injection reservoir (Micro Injection Dome, model 350-MICRO; Mentor Corporation, Goleta, Calif), shown in Figure 1, was then implanted over the dorsum of the animal. A silicone delivery cannula was tunneled from the reservoir to the nerve and sutured to the muscle immediately adjacent to the transected nerve.

Possible. Neural cell regenerative activity reaches its peak during the first 3 weeks after nerve injury, a period of time when the action of BDNF may be quite important.12,13 Our goal was to determine whether administration of BDNF after nerve injury would improve the functional outcome in a setting of delayed neural repair in a rat sciatic nerve model.

RESULTS

We were able to evaluate the data from 29 of the 34 animals studied. Five animals (15%), 2 in the LR group and 3 in the BDNF-late group, suffered automutilation of their transected nerve.14 The top of the injection dome is soft, which allowed for serial percutaneous injections of BDNF or LR delivered to the site of the transected nerve. The wounds were closed, and the animals were allowed to recover.

After surgery, the animals were housed at the Stanford Research Animal Facility, where they received rat chow and water ad libitum. The animals in the LR and BDNF-late groups received daily injections of 0.3 mL of LR into the implanted reservoir. The animals in the BDNF-early group received daily injections of 10 µg of BDNF dissolved in 0.3 mL of LR. The syringes were prepared and coded by a third party so that the investigators were blinded to their content.

Two weeks after nerve transection, all animals were reanesthetized, and their wounds were opened. The sciatic nerve ends were exposed, and the identifying sutures were removed. The nerves were then repaired, as shown in Figure 2, with 6 interrupted epineurial stitches of 9-0 polypropylene suture swaged on a tapered needle (BV 130-3; Ethicon Inc). The wounds were closed, and the animals were allowed to recover. The animals in the LR group continued to receive daily injections of LR, whereas the animals in the BDNF-early and BDNF-late groups were given daily injections of BDNF for 2 weeks.

Two weeks after nerve repair, all animals were reanesthetized, and the microdome reservoir and silicone tubing were removed. Care was taken not to disturb the neural repair. The wounds were closed, and the animals were allowed to recover.

FUNCTIONAL ANALYSIS

Recovery of sciatic nerve function was assessed using walking track measurements, which provide a means to quantify experimental foot, requiring humane killing; there were 9 animals each in the LR group and BDNF-late group, and 11 animals in the BDNF-early group. Muscle contractures of the experimental leg developed in 2 of the animals in the LR group and in 3 of the animals in the BDNF-early group. These animals were included in the functional and histological data analysis. The maximal SFI for these animals was measured at the time point before the development of muscle contracture. At the time of extraction, the
the function of the sciatic nerve.\textsuperscript{15} Animals underwent walking track analysis before nerve transection, again just before nerve repair, and every 10 days after nerve repair for a total of 60 days. Paw prints were obtained by moistening the animals’ hind paws and having them walk across broomophenol blue–treated paper.\textsuperscript{16} Measurements were obtained of paw print length, outer toe spread, and intermediary toe spread for control and experimental sides. Sciatic functional indices (SFIs) were calculated using the following formula modified by Bain et al:\textsuperscript{17}

$$\text{SFI} = \frac{-38.3 \times (\text{EPL} - \text{NPL})/\text{NPL} + 109.5 \times (\text{ETS} - \text{NTS})/\text{NTS} + 13.3 \times (\text{EIT} - \text{NIT})/\text{NIT}}{8.8},$$

where EPL indicates experimental print length; NPL, normal print length; ETS, experimental outer toe spread; NTS, normal toe spread; EIT, experimental intermediary toe spread; and NIT, normal intermediary toe spread. The SFI is a standardized formula in which normal function is 0 and complete loss of function is $-\infty$.

Sixty days after the time of nerve repair, differences in maximal functional recovery and rate of recovery were compared using linear regression analysis. Early muscle contractures in the experimental leg developed in several animals, such that interpretable data could no longer be obtained. The SFI walking track data collected until the time of muscle contracture were used in the data analysis.

### HISTOMORPHOMETRIC ANALYSIS

At the completion of the experiment, animals (including those with muscle contractures) were anesthetized, and 5-mm segments of nerve 5 mm distal to the repair site were harvested and fixed in 10% buffered formalin solution. Ten-millimeter segments from the contralateral nerves not undergoing operation in 10 rats were harvested at random to serve as controls. The animals were then killed humanely in a carbon dioxide chamber.

Histological slides were made by cutting 0.6-mm sections and staining with Bielschowsky silver stain for nerve axons. The slides were examined with a light microscope (Nikon Alphaphot 2YS2; Technical Instruments, San Francisco, Calif) equipped with a single-chip color video camera (CCD #70-5110; JEDMED, St Louis, Mo) projected onto a color monitor (#PM-1971A; NEC, Tokyo, Japan). The monitor was connected to a computer (Performa 6115 CD MacIntosh; Apple Computers, Cupertino, Calif) equipped with a program to capture images from the video screen and to digitize the analog signal for editing on the computer (Scion Frame Grabber Card and Scion Image program, Model LG3; Scion Corporation, Bethesda, Md).

The axon counts were derived from digitized images of the nerves at 40× magnification. The Scion Image program randomly selected 10 areas, each measuring 1290 μm$^2$, within each nerve section. Axon counts within these areas were then performed manually, using crosshairs to identify and mark each axon. The axon count for the entire nerve was calculated by multiplying the mean axon count per random area by the nerve cross-sectional area and dividing by 1290 μm$^2$.

The mean axon diameters were derived from digitized images of the nerves at 100× magnification. Twenty axons were chosen randomly and outlined individually on the computer screen. Using the Scion Image program, the areas of the axons were measured, and the diameters were calculated from the areas, assuming a circular geometric shape. The mean axon diameter of each nerve section was then calculated. Unless otherwise indicated, data are given as mean ± SD.

The ideal timing for peripheral nerve repair after injury has been a source of controversy. A series of studies in
the 1970s demonstrated that axonal regenerative activity peaks at 3 weeks after injury.18-20 This finding led to the concept that neurorrhaphy should be delayed for 3 weeks. More recent investigations have conclusively demonstrated, however, that despite this delayed peak of activity, clinical outcomes are clearly superior if nerve repair is accomplished earlier.21-23 Nevertheless, a number of factors, including the stability of the patient, may prevent timely performance of nerve repair.

The ability to enhance the microenvironment of a transected motor neuron through the addition of neurotrophic factors is desirable when repair must be delayed. Neurotrophic factors, including nerve growth factor (NGF) and BDNF, are a family of structurally related polypeptides that originally were discovered to have a major role in the development and support of the nervous system during embryogenesis. In models of peripheral nerve regeneration, the use of NGF has been disappointing, especially when functional recovery is measured.24,25 We chose to use BDNF, which has been shown in a number of studies to improve functional recovery after nerve repair.9-11 The expression of receptors specific for BDNF, but not NGF, in mature spinal and cranial motor neurons may explain the improved results when using BDNF in animal models of nerve regeneration.6,26

Our study differs from previous work in that we investigated the potential role of BDNF in the setting of nerve transection and delayed repair. We speculated that the addition of a neurotrophic factor during a period of regenerative activity after nerve transection would improve ultimate functional recovery when nerve repair was delayed by 2 weeks. Our data did not support the role for the early administration of BDNF, as the histological and functional results were similar regardless of the timing of BDNF administration.

Although the administration of BDNF resulted in an increase in mean axonal diameters and maximal SFIs, the data did not reach statistical significance. Since previous studies had confirmed that BDNF is effective in enhancing functional recovery of transected and immediately repaired nerves, it may be that the delay before repair resulted in irrevocable muscle contractures or loss of viable muscle endplates that were unable to be rescued by BDNF. Since we anticipated this potential problem, BDNF was delivered immediately after nerve transection in 1 group in an effort to sustain the muscle target tissue. This group, however, fared no better than the group in which BDNF administration was delayed until the nerve repair was accomplished. The daily dose of BDNF administered was based on previous studies in animal models of immediate nerve repair.9,10 It is possible that higher doses of BDNF would be beneficial during the period of delayed nerve repair, but we did not explore that potential.

The findings of a higher number of axons in the transected and repaired nerves compared with the nerves not undergoing operation are consistent with the phenomenon of “axonal sprouting,” in which a high number of smaller, poorly formed axonal sprouts grow from the transected axon seeking a distal target and increasing the over-

Table 1. Summary of Functional Results*

<table>
<thead>
<tr>
<th>Groups</th>
<th>Maximum SFI Recovery†</th>
<th>Slope of SFI Recovery‡</th>
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</thead>
<tbody>
<tr>
<td>LR</td>
<td>-90.1 ± 9.6</td>
<td>0.53 ± 0.27</td>
</tr>
<tr>
<td>BDNF-early</td>
<td>-85.7 ± 7.6</td>
<td>0.51 ± 0.23</td>
</tr>
<tr>
<td>BDNF-late</td>
<td>-84.6 ± 4.8</td>
<td>0.43 ± 0.16</td>
</tr>
</tbody>
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* Abbreviations are given in the first footnote to Table 1. Data are given as mean ± SD.
† P = .27, differences between groups.
‡ P = .56, differences between groups.

Table 2. Summary of Histologic Data*

<table>
<thead>
<tr>
<th>Groups</th>
<th>Mean Axon Count†</th>
<th>Mean Axon Diameter, µm‡</th>
</tr>
</thead>
<tbody>
<tr>
<td>LR</td>
<td>10 944 ± 2082</td>
<td>2.43 ± 0.23</td>
</tr>
<tr>
<td>BDNF-early</td>
<td>9025 ± 2611</td>
<td>2.80 ± 0.44</td>
</tr>
<tr>
<td>BDNF-late</td>
<td>9087 ± 2272</td>
<td>2.83 ± 0.38</td>
</tr>
<tr>
<td>Control nerves</td>
<td>6331 ± 984</td>
<td>3.48 ± 0.42</td>
</tr>
</tbody>
</table>

*Abbreviations are given in the first footnote to Table 1. Data are given as mean ± SD.
† P = .05, differences between treatment groups only.
‡ P = .16, differences between treatment groups only.
CONCLUSIONS

The supportive role of BDNF and other neurotrophic factors in the setting of delayed peripheral nerve repair has not been studied previously. The local administration of BDNF to nerves that were transected and then repaired after a delay resulted in an increase in axonal diameters and maximal SFIs, a difference that did not reach statistical significance. The timing of BDNF administration following nerve transection did not affect neuronal regeneration.

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REFERENCES