SCIENTIFIC ARTICLE

Controlled Delivery of Glial Cell Line–Derived Neurotrophic Factor Enhances Motor Nerve Regeneration

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Purpose To determine the effect of a motor-specific neurotrophic factor, glial-derived neurotrophic factor (GDNF) on motor nerve regeneration.

Methods We used a nerve conduit filled with a fibrin-based delivery system that provided controlled release of GDNF during nerve regeneration. The motor branch of the rat femoral nerve was used to assess motor nerve regeneration across a 5-mm gap. Four experimental groups (n=4 to n=8) were evaluated. These included GDNF with the fibrin-based delivery system (GDNF-DS), fibrin alone, empty conduit (negative control), and nerve isograft (positive control). Nerves were harvested at 5 weeks for analysis by histomorphometry and electron microscopy.

Results At 5 mm distal to the conduit or isografts, the GDNF-DS group was not significantly different from the nerve isograft group in the following histomorphometric measures: total nerve fibers, percentage of neural tissue, and nerve density. Both the GDNF-DS and isograft groups had significantly more fibers and a higher percentage of neural tissue than fibrin alone and empty conduit groups. There were no differences in fiber width among all groups. By electron microscopy, the GDNF-DS and isograft groups also demonstrated more organized nerve architecture than the fibrin alone and empty conduit groups.

Conclusions The delivery of GDNF from the fibrin-based delivery system promotes motor nerve regeneration at a level similar to an isograft in the femoral motor nerve model. This study gives insight into the potential beneficial role of GDNF in the treatment of motor nerve injuries. *(J Hand Surg 2010;35A:2008–2017. © 2010 Published by Elsevier Inc. on behalf of the American Society for Surgery of the Hand.)*

Key words Biomaterial, drug delivery, fibrin, peripheral nerve injury, tissue engineering.

DEVASTATING PERIPHERAL NERVE injuries are common. They can lead to high functional morbidity, such as loss of limb function or facial expression. Motor nerve injuries, specifically, offer a unique challenge because of the time-sensitive nature of nerve regeneration and motor end-plate reinnervation. As the duration of muscle denervation increases, motor recovery is negatively affected, often leading to less-than-ideal return of function.[1–3] For this reason, our group and others have sought new strategies to enhance motor nerve regeneration.

When a direct nerve coaptation cannot be performed owing to nerve loss or excessive tension, the gold standard is to repair the nerve using a nerve autograft. However, the harvesting of autologous nerve tissue requires additional surgical sites and prolonged surgical time, and it can result in morbidity at the harvest site, including loss of sensation and potential neuroma for-

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mation.[4,5] For these reasons, alternatives to autografting are desirable.

Since the late 1980s, nerve conduits have been used clinically as an alternative to nerve graft repairs.[6–8] Conduits can bridge the nerve gap, isolate the regenerative environment, and prevent scar tissue infiltration, and they are easily obtainable off the shelf.[5,9–12] This commercial availability avoids additional surgical sites and their associated morbidities and time constraints. Despite their benefits, conduit use and efficacy are limited, clinically, to small-diameter sensory nerves for gaps less than 3 cm.[5,6,12–15] Research has focused on enhancing nerve regeneration through a conduit by manipulating the conduit material itself, either by adding neurotrophic agents or by engineering structural scaffolds into the lumen of the conduit.[5,12,16]

Previously, we have shown that a silicone nerve conduit, filled with a fibrin-based drug delivery system that provides controlled release of nerve growth factor and glial-derived neurotrophic factor (GDNF), enhanced nerve regeneration in a rat sciatic nerve injury model.[17,18] The GDNF has also been shown to enhance nerve regeneration in other surgical models.[9,18–20] Although GDNF receptors are expressed by both sensory and motor neurons,[21] GDNF is reported to be the most potent trophic and survival factor for motor neurons.[9,22–27]

Previously, in a sciatic nerve injury model, we found that the controlled delivery of GDNF from our fibrin-based delivery system resulted in an increased number of mature nerve fibers (5–7 μm in diameter) and an organized nerve architecture similar to that of the isograft group.[18] However, the sciatic nerve contains a mixed population of both sensory and motor fibers that can be targeted with GDNF and are capable of regenerating. Although this model allowed us to explore our ability to promote peripheral nerve regeneration with our delivery system and GDNF, it was not clear whether we could specifically target motor neurons and their axons for regeneration. The ability to target motor neurons and enhance motor nerve regeneration, either by speed of regeneration or by quality of regeneration, would potentially improve functional recovery.

The purpose of the present study was to examine the effects of controlled GDNF delivery specifically on motor nerve regeneration. We used the rat femoral motor nerve model to evaluate motor nerve regeneration. Because the femoral nerve divides into the motor nerve to the quadriceps muscles and the pure sensory nerve to the skin,[28,29] this model allows us to use the motor branch to study motor nerve regeneration in isolation and examine the regenerative effects of the delivery of the most potent known motor neurotrophic factor, GDNF.

METHODS

Experimental animals

Adult male Lewis rats (Harlan Sprague-Dawley, Indianapolis, IN), each weighing 250 to 300 g, were used in this study. All surgical procedures and perioperative care measures were performed in strict accordance with the National Institutes of Health guidelines.

Experimental design

Twenty-nine male Lewis rats (250–300 g) were divided into 4 groups (groups 1–4). Four additional animals were used as isograft donors. All animals had a femoral nerve motor branch transection at 3 mm distal to the take-off of the motor branch from the common femoral nerve. The nerve was repaired with a 7-mm silicone conduit or 5-mm isograft. A 1-mm section of nerve was incorporated into each end of the conduit to create a 5-mm nerve gap. Group 1 (n = 8) served as a positive control and received a reversed, 5-mm femoral motor nerve isograft from a syngeneic donor rat. An isograft was used instead of an autograft to avoid the confounding affects of donor site morbidity from autograft harvesting. Group 2 (n = 7) received a conduit filled with the delivery system and 100 ng/mL GDNF, a dose based on *in vitro* cultures of embryonic chick dorsal root ganglions[30] and previous *in vivo* studies in a rat sciatic nerve model.[18] Group 3 (n = 7) received a conduit filled with the fibrin alone. Group 4 (n = 7) served as the untreated, negative control group and received an empty conduit.

Preparation of fibrin matrices

Fibrinogen solutions were prepared by dissolving human plasminogen-free fibrinogen in deionized water at 8 mg/mL for 1 hour and dialyzing, versus 4 L Tris-buffered saline (33 mmol/L Tris, 8 g/L NaCl, 0.2 g/L KCl) at pH 7.4 overnight to exchange salts present in the protein solution. The resulting solution was sterilized by filtration through 5.0-μm and 0.22-μm syringe filters, and the final fibrinogen concentration was determined by measuring absorbance at 280 nm. For the delivery system, a bi-domain peptide (ATIII) based on a modified version of the antithrombin III–heparin binding domain (*[AcG]NQEQVSPK*(βA)FAKLAARLYRKA, where AcG denotes N-acetyl-glycine and the transglutaminase substrate is given in italics)[31,32] was synthesized, as described earlier.[30] Fibrin matrices were prepared as described earlier.[33] Components were mixed to obtain the following final solution concentra-