SCIENTIFIC ARTICLE

Supercharge Nerve Transfer to Enhance Motor Recovery: A Laboratory Study

Scott J. Farber, MD, Simone W. Glaus, MD, Amy M. Moore, MD, Daniel A. Hunter, Susan E. Mackinnon, MD, Philip J. Johnson, PhD

Purpose To investigate the ability of a supercharge end-to-side (SETS) nerve transfer to augment the effect of regenerating native axons in an incomplete rodent sciatic nerve injury model.

Methods Fifty-four Lewis rats were randomized to 3 groups. The first group was an incomplete recovery model (IRM) of the tibial nerve complemented with an SETS transfer from the peroneal nerve (SETS-IRM). The IRM consisted of tibial nerve transection and immediate repair using a 10-mm fresh tibial isograft to provide some, but incomplete, nerve recovery. The 2 control groups were IRM alone and SETS alone. Nerve histomorphometry, electron microscopy, retrograde labeling, and muscle force testing were performed.

Results Histomorphometry of the distal tibial nerve showed significantly increased myelinated axonal counts in the SETS-IRM group compared with the IRM and SETS groups at 5 and 8 weeks. Retrograde labeling at 8 weeks confirmed increased motoneuron counts in the SETS-IRM group. Functional recovery at 8 weeks showed a significant increase in muscle-specific force in the SETS-IRM group compared with the IRM group.

Conclusions An SETS transfer enhanced recovery from an incomplete nerve injury as determined by histomorphometry, motoneuron labeling within the spinal cord, and muscle force measurements.

Clinical relevance An SETS distal nerve transfer may be useful in nerve injuries with incomplete regeneration such as proximal Sunderland II- or III-degree injuries, in which long regeneration distance yields prolonged time to muscle reinnervation and suboptimal functional recovery. *(J Hand Surg 2013;38A:466–477. Copyright © 2013 by the American Society for Surgery of the Hand. All rights reserved.)*

Key words Nerve regeneration, nerve transfer, neurorrhaphy, peripheral nerve, supercharge end-to-side.

The management of proximal second- or third-degree nerve injuries, or *incomplete* injuries, is challenging[1,2] because partial regeneration is expected, but in a delayed fashion (~1 mm/d) with the likelihood of incomplete recovery. The time necessary for muscle reinnervation and the number of regenerating axons that successfully reinnervate the muscle play a major role in motor recovery after nerve injury.[3–8] With extended denervation times, Schwann cells (SCs) in the distal nerve become less supportive of regener-

From the Division of Plastic and Reconstructive Surgery, Washington University School of Medicine, St. Louis, MO.

S.J.F. and S.W.G. equally contributed to the work for this article.

Received for publication August 21, 2012; accepted in revised form December 12, 2012.

The authors acknowledge Maryam Saheb-Al-Zamani, BS, for her contributions to this study.

This work was supported in part by a grant from the American Foundation for Surgery of the Hand and National Institutes of Health (NINDS, R01 NS03340615).

No benefits in any form have been received or will be received related directly or indirectly to the subject of this article.

Corresponding author: Philip J. Johnson, PhD, Division of Plastic and Reconstructive Surgery, Washington University School of Medicine, 660 South Euclid Avenue, Campus Box 8238, St. Louis, MO 63110; e-mail: johnsonp@wudosis.wustl.edu.

0363-5023/13/38A03-0006$36.00/0
http://dx.doi.org/10.1016/j.jhsa.2012.12.020

ating axons[9–11]; the denervated muscle begins to atrophy, resulting in profound muscle changes including apoptosis and fibrosis[3,4,6–8,12–17]; and fewer motor neurons from the proximal nerve regenerate.[4,7,11,16,18,19] Replacement of muscle fibers by adipose tissue has also been seen with chronic denervation.[16,20] Interstitial fibrosis may prevent appropriate intramuscular axonal regeneration,[4,15] leading to decreased numbers of motor end plates and, ultimately, diminished motor recovery.[21] Therapeutic strategies that decrease the duration of end-organ denervation may have a major clinical impact on motor recovery.

A "supercharge" end-to-side (SETS) nerve transfer moves an expendable, distal motor nerve donor to the side of an injured recipient nerve.[22,23] This provides both earlier reinnervation of the target muscle as well as additional regenerating motor axons to more completely reinnervate neuromuscular junctions.

We have previously shown in a model of a *complete* nerve injury (Sunderland IV- and V-degree) that axonal regeneration indeed occurs across the SETS coaptation and that the regeneration is equivalent to that of an end-to-end nerve transfer.[23] The purpose of this study was to evaluate the SETS effect in an *incomplete* recovery model[3,24–26] using histomorphometric analysis, functional muscle force testing, and retrograde labeling.

METHODS

Animals

Fifty-four adult male Lewis rats (200—250 g; Charles River Lab, Wilmington, MA) were used. Rodents were housed in a central animal care facility. Food and water were provided *ad libitum.* Animals were monitored for appropriate postsurgical recovery and weight gain. All study procedures were approved by our institutional animal studies committee.

Experimental design

Surgical procedure. All surgical procedures were performed aseptically using an operating microscope. Animals were anesthetized by subcutaneous administration of a mixture of ketamine hydrochloride (Fort Dodge Animal Health, Fort Dodge, IA) and dexmedetomidine hydrochloride (Orion Corporation, Espoo, Finland). A gluteal muscle–splitting incision was performed to expose the sciatic nerve and its trifurcation. Careful neurolysis was performed to isolate the peroneal and tibial nerves. The tibial nerve was sharply transected 5 mm distal to the sciatic trifurcation. Three experimental groups were studied (Fig. 1). Six animals per group[23] and 2 end points were evaluated: 5 and 8 weeks.

The first group was the incomplete recovery model (IRM) with SETS nerve transfer, termed SETS-IRM. To create the IRM, a 10-mm fresh tibial isograft was interposed between the 2 transected ends of the tibial nerve and coapted with 10-0 nylon suture. We have previously shown that this nerve graft model provides some, but incomplete, recovery.[3,24–26] To create the SETS nerve transfer, the peroneal nerve was sharply transected approximately 15 to 20 mm distal to the sciatic nerve trifurcation. An epineurial and perineurial window was created in the side of the tibial nerve approximately 3 to 5 mm distal to the distal isograft coaptation. The peroneal nerve was then transferred in an end-to-side fashion using interrupted 10-0 nylon suture.

The second group represented the IRM alone. The tibial transection was performed and a tibial isograft was interposed in between the 2 cut ends as described previously.

In the SETS control group, the tibial nerve was transected and both ends were cauterized and capped with silicone to prevent regeneration. The SETS nerve transfer from the peroneal to the tibial nerve was then performed as described previously. The epineurial/perineurial window was created in the side of the tibial nerve approximately 5 mm distal to the capped end of the distal stump.

In all animals, muscle and skin were reapproximated, anesthesia was reversed, and the animals recovered on a warming pad and were monitored for postoperative complications.

At a 5-week end point, animals (n = 6/group) were reanesthetized and the incision site was opened. Neurolysis was performed to expose the sciatic and tibial nerves. A 10-mm section of the tibial nerve both proximal and distal to the SETS coaptation site was removed *en bloc.* An additional group of animals (n = 6/group) underwent muscle force analysis performed at 8 weeks after surgery.[27] Once muscle force testing was complete, the nerves were sharply excised in the same manner as the 5-week groups for histomorphometric and electron microscopic analysis. The gastrocnemius muscle from the operated side and the nonoperated side of the 8-week animals were removed for mass analysis to calculate specific force. The animals were then killed. An additional cohort of animals divided into the same 3 groups (n = 6/group) underwent retrograde labeling as described later.

Light microscopy and histomorphometry

Harvested tibial nerves from the Lewis rats were processed as previously described.[27,28] Cross-sections,