Convection-enhanced delivery in intact and lesioned peripheral nerve

JOHN K. RATLIFF, M.D., AND EDWARD H. OLDFIELD, M.D.

Surgical Neurology Branch, National Institute of Neurological Disorders and Stroke, National Institutes of Health, Bethesda, Maryland; and Department of Neurosurgery, Louisiana State University, New Orleans, Louisiana

Object. Although the use of multiple agents is efficacious in animal models of peripheral nerve injury, translation to clinical applications remains wanting. Previous agents used in trials in humans either engendered severe side effects or were ineffective. Because the blood–central nervous system barrier exists in nerves as it does in the brain, limited drug delivery poses a problem for translation of basic science advances into clinical applications. Convection-enhanced delivery (CED) is a promising adjunct to current therapies for peripheral nerve injury. In the present study the authors assessed the capacity of convection to ferry macromolecules across sites of nerve injury in rat and primate models, examined the functional effects of convection on the intact nerve, and investigated the possibility of delivering a macromolecule to the spinal cord via retrograde convection from a peripherally introduced catheter.

Methods. The authors developed a rodent model of convective delivery to lesioned sciatic nerves (injury due to crush or laceration in 76 nerves) and compared the results to a smaller series of five primates with similar injuries. In the intact nerve, convective delivery of vehicle generated only a transient neurapraxic deficit. Early after injury (postinjury Days 1, 3, 7, and 10), infusion failed to cross the site of injury in crushed or lacerated nerves. Fourteen days after crush injury, CED of radioactively-labeled albumin resulted in perfusion through the site of injury to distal growing neurites. In primates, successful convection through the site of crush injury occurred by postinjury Day 28. In contrast, in laceration models there was complete occlusion of the extracellular space to convective distribution at the site of laceration and repair, and convective distribution in the extracellular space crossed the site of injury only after there was histological evidence of completion of nerve regeneration. Finally, in two primates, retrograde infusion into the spinal cord through a peripheral nerve was achieved.

Conclusions. Convection provides a safe and effective means to deliver macromolecules to regenerating neurites in crush-injured peripheral nerves. Convection block in lacerated and suture-repaired nerves indicates a significant intraneural obstruction of the extracellular space, a disruption that suggests an anatomical obstruction to extracellular and, possibly, intraaxonal flow, which may impair nerve regeneration. Through peripheral retrograde infusion, convection can be used for delivery to spinal cord gray matter. Convection-enhanced delivery provides a promising approach to distribute therapeutic agents to targeted sites for treatment of disorders of the nerve and spinal cord.

KEY WORDS • peripheral nerve • neurotrophin • spinal cord • nerve regeneration • drug delivery • rat • Macaca mulatta

Research on nerve regeneration has yielded greater understanding of responses to injury in the peripheral and central nervous systems. A number of excellent reviews summarize advances in neurotrophin physiology and peripheral nerve regeneration. Numerous agents with possible therapeutic applications have been developed. Most of these agents are macromolecules, however, and effective translation of basic science insights into successful treatment methods has not occurred because results from human trials have shown that these agents engender either considerable systemic side effects or limited therapeutic efficacy.

Abbreviations used in this paper: BSA = bovine serum albumin; CED = convection-enhanced delivery; MR = magnetic resonance; NAP = nerve action potential; NIH = National Institutes of Health; PBS = phosphate-buffered saline; QAR = quantitative autoradiography; SFI = sciatic functional index; Vd = volume of distribution; Vi = volume of infusion.

The mechanisms underlying traumatic injuries of the brachial plexus and other nerves predispose a young, physically active group of individuals to such injuries. Because treatment for these nerve injuries is frequently elective, any new therapy must have minimal side effects. A desirable drug-delivery system would carry high concentrations of a therapeutic agent to the site of nerve injury while minimizing systemic exposure to detrimental effects.

An advance in the development of therapy for peripheral nerve injury may be CED. This approach provides for delivery of high concentrations of both large- and small-molecular-weight bioactive agents within the epineural sheath, with minimal systemic exposure. Furthermore, addition of an implantable pump, which has already been developed in a primate model for convective distribution within the brain, could provide long-term perfusion if needed.

Previously Lonser and colleagues described the CED distribution characteristics in the intact sciatic nerve and
spinal cord in primates. The behavior of the infusate in cases of damaged nerve, however, is unknown. Expanding on this CED model, we present a protocol for use of CED in the peripheral nerve of rodents and primates. We concentrated on delivery of albumin to lesioned nerves, examining the distribution characteristics of CED after nerve crush injury and after nerve section followed by microsurgical repair. We also performed similar infusions in intact animals, assessing the functional effects of interstitial infusion with intraoperative electrophysiology, light and electron microscopy, and SFI measurement. Finally, we report on the potential of using retrograde infusion into a peripheral nerve to reach and perfuse a region of the spinal cord.

Materials and Methods

Animal Preparation
This work was completed in accordance with NIH guidelines on the use of animals in research and was approved by the National Institute of Neurological Disorders and Stroke Animal Care and Use Committee. All guidelines provided by the NIH Radiation Safety Committee were closely followed. Seventy-six Sprague–Dawley rats of either sex, each weighing between 250 g and 350 g, were maintained on a 12-hour light/12-hour dark cycle and given free access to rodent food and water. Each experimental animal underwent surgical exposure and lesioning of the left sciatic nerve. Five adult primates (Macaca mulatta) were maintained in accordance with NIH animal care protocols. The primates underwent bilateral exposure and sectioning of peroneal and tibial divisions of the sciatic nerve.

Infusion Apparatus and Infusate
A gas-tight noncompliant delivery system that has no dead volume was previously described in a report on convective infusion to the peripheral nerves in primates. Briefly, two 250-μl gas-tight CX syringes were attached to either end of water-filled polyether-etherketone tubing, forming a hydraulic drive. One syringe was placed in a syringe pump that generates even, continuous pressure throughout the infusion. The plunger of the other CX syringe was attached directly to the plunger of a gas-tight, infusate-filled, glass Hamilton syringe. Pressure from the syringe pump was delivered to the Hamilton syringe and to the infusion cannula. The infusion cannula (inner diameter 100 μm, outer diameter 170 μm) was composed of fused silica and secured to Teflon Luer fittings with epoxy glue. The Luer fitting articulated directly with the infusate syringe. The same infusion apparatus was used in both rat and primate experiments. Albumin labeled with carbon-14 (0.024 mCi/mg N-C-labeled BSA [0.79 mg/ml] in stock 0.01 M sodium phosphate buffer [pH 7.2]) was diluted in 10 × PBS (9.8:1 vol/vol, yielding a final osmolality of 280–290 mOsm) and used as infusate. Evans blue dye was added to the infusate to aid gross and microscopic visualization (4 μL Evans blue dye [4.7 mg/ml in 1 × PBS] to each 100 μl of N-C-labeled albumin solution). For MR imaging examinations, we used gadolinium (Magnevist)-labeled BSA (0.1 mM).

Surgical Procedure

Surgery in Rodents. Anesthesia was induced in rats according to a standard protocol (0.5–5% isoflurane delivered through a face mask). One percent lidocaine was injected along the incision line to ensure adequate analgesia. No lidocaine was used in animals that were evaluated with the aid of intraoperative electrophysiology. The left hindquarter was prepared and shaved using a standard sterile surgical technique, and an incision approximately 1 cm in length was made along the midline. The sciatic nerve was identified and secured with a small vessel loop. In 22 of the experimental animals, the nerve was sharply divided using a fresh scalpel. After division, the ends of the nerve were surgically reattached using a No. 8–0 Prolene suture and a microsurgical procedure. The surgically created nerve communication was sealed using fibrin glue. In the remaining experimental animals (24 rats), the sciatic nerve was crushed twice (two separate but adjacent locations) for 30 seconds each by using a No. 7 jeweler’s forceps. The crush sites were marked by an epineural No. 10–0 Prolene suture. The wounds were closed via muscle approximation with No. 4–0 nylon sutures, and the skin was closed using sterile skin staples. Appropriate postoperative analgesia therapy was administered in accordance with an NIH protocol, with an intervention chart used to standardize treatment. Complete nerve injuries in both models were confirmed by intraoperative electrophysiological and postoperative functional assessment.

At 1, 3, 7, 10, 14, 21, 28, 35, and 42 days postinjury (and postoperatively), groups of chimeras (two to six rats/group) underwent repeated surgery following a similar protocol. At reoperation, the sciatic nerve was again identified. In cases in which significant scarring adhered the nerve to surrounding tissues, external neurolysis was performed. In random animals, we used electrophysiology to assess recovery of NAPs distal to the site of injury. At the time of reoperation, a silica infusion catheter was inserted into the center of the nerve proximal to the site of laceration or crush injury. The catheter was placed so that it entered the nerve approximately 10 mm proximal to the injury site, with the tip approximately 5 mm proximal to the site of injury. The cannula was secured to the epineurium by using fibrin glue or tissue adhesive. Four microliters of N-C-labeled albumin was infused (0.2 μL/minute for 20 minutes). To examine the effect of changing the infusion volume, in a separate group of two to six rats/random days, we doubled the infusion volume (0.2 μL/minute for 40 minutes). In 11 control animals, only infusion was performed, without prior nerve lesioning. Sciatic nerves were harvested, and the length and volume of distribution were assessed using QAR. After the procedure, the animals were killed and disposed of in accordance with NIH radiation and medical pathological waste protocols.

Surgery in Primates. The surgical procedures performed in primates were similar in character to those performed in rats, and only relevant differences will be reviewed. At all times standard sterile surgical technique was maintained. Anesthesia was induced in the animals by using the standard NIH protocol of ketamine (7 mg/kg) and xylazine (1 mg/kg) delivered intramuscularly, followed by intubation and maintenance of general anesthesia by administration of isoflurane (1.5–3%). Heart rate, body temperature, O₂ saturation, and PCO₂ were monitored at all times during the procedure. In primates, both lower extremities were used. The tibial and peroneal divisions of the sciatic nerve were separated by internal neurolysis at the midheight level. In four animals, we lesioned the divisions separately, alternating laceration and crush injuries; hence, in each animal, two crush and two laceration injuries were performed. Laceration injuries were repaired with four to six No. 7–0 Prolene sutures by again using microsurgical procedures. Crush injuries in primates were generated using an Ochsner clamp closed maximally on the nerve at two separate but adjacent sites for a total of 90 seconds. Wound closure was accomplished using No. 3–0 absorbable sutures for muscle layers and a running subcuticular No. 5–0 absorbable suture for the skin. At 28 (one animal) and 35 (two animals) days after nerve injury, the animals underwent repeated surgery and convective infusion was executed following the methodology described earlier. In primates, the infusion rate was 0.5 μL/minute for 100 minutes. The sciatic nerves were harvested, and the anatomical distribution and Vd were assessed using QAR. Immediately after infusion and nerve harvest were complete, the animal fossa were opened by an overlying incision and observed for markers that are visible on MR images were placed on the skin overlaying the lesions. Retrograde infusion was also performed (see following section). This animal was killed after MR imaging examination was completed.
In an additional animal, we performed control unilateral infusion of gadolinium-labeled albumin into the left tibial nerve and retrograde infusion into the left peroneal division. This animal recovered after surgery and was followed clinically to detect potential postinfusion deficits.

**Retrograde Infusion.** In two primates, the dissection on the left lower extremity extended proximally to the sciatic notch. Here, the silica catheter was introduced and directed retrogradely into the intrapelvic portion of the sciatic nerve. The catheter was advanced until resistance was met (80 mm in one animal and 135 mm in the other). The gadolinium-labeled albumin was infused (0.5 μL/minute for 120 minutes in one animal and 170 minutes in the other), and the animals were immediately taken to the MR imaging suite where sagittal and axial 5-mm T1- and T2-weighted images of the pelvis and lumbar spine were obtained. Fat suppression was used on axial images. One animal was killed after the MR imaging examination was completed. The other animal was permitted to recover from anesthesia and was followed clinically.

**Electrophysiological Examination and Functional Assessment**

In a separate group of rats, we performed a functional assessment of the effects of convective infusion. Intraoperative electrophysiological examination was conducted by measuring NAPs with the aid of small platinum bipolar electrodes. Short distances of exposed nerve complicated the NAP assessment. Often, the onset of the NAP was lost in the trailing edge of the stimulus artifact. Therefore, we measured NAP velocity from the wave peak, which yielded higher nerve velocities than those previously reported. The electromyographic apparatus used in this experiment had the following settings: time/division 0.2 msec, volts/division 10 to 100 mV, filter 20 Hz/10 kHz, duration of stimulus 0.05 msec, repetition rate 1 pulse/second, and intensity 0 to 50 V. We obtained baseline NAPs before initiating crush injury in a subset of animals subjected to infusion, and then repeated the measurements on postinjury Day 14. Functional assessment was also performed using walking track analysis, as described by de Medinaceli and associates. We constructed a walking track similar to the one reviewed by Brown, et al., and Hare and colleagues. We used the formula modifications reported by Bain, et al. This measure of functional assessment is reliable within and between observers. Two separate sets of control animals were used. In the initial set of five animals, the sciatic nerve was exposed and circumferentially dissected. This controlled for nerve injury resulting from dissection. Only baseline control NAPs were recorded in these animals. In a second group of four control animals (catheter group), the silica catheter was introduced and the nerve was left exposed for 20 minutes. With this group we controlled the trauma of catheter insertion and exposure of the nerve during the infusion process. In four experimental animals, we infused 290 mOsm PBS at 0.2 μL/minute for 20 minutes. The NAPs were recorded before and after insertion of the silica catheter with or without infusion. All animals received preoperative baseline walking track assessment, followed by serial examinations after surgery, which were performed on postoperative Days 1, 3, 7, and 14.

**Quantitative Autoradiography**

Sciatic nerve specimens from both rats and primates were immediately flash-frozen at −70°C after harvest (which should limit passive redistribution of the infusate) and then serially sectioned into 20-μm-thick portions by using a cryostat. These sections were mounted and freeze-dried overnight at −70°C. Every fifth section was exposed to autoradiographic film for 4 hours. Image analysis was performed using NIH imaging computer software on Macintosh-based computers. The Vd was calculated by summing derived values and multiplying these by 0.1 mm. In the crush-injured nerves and in the lacerated-and-repaired nerves, data from postinjury Days 1 and 3 were combined. Similarly, in crush-injured animals, data from postinjury Days 7 and 10 were combined. The distance from the catheter tip to the lesion site was measured at surgery and used to establish the point of infusion within the nerve when analyzing the results of QAR. Statistical analysis was performed using statistical software on Macintosh-based computers.

**Immunohistochemical Study**

Neurofilament immunohistochemical staining was conducted using a previously reported protocol. Rabbit anti–human neurofilament M (concentration 1:4000) was used. Biotinylated goat anti–rabbit immunoglobulin G secondary antibody and avidin–biotin complex were used to detect primary antibodies in accordance with supplier directions. Tissue sections were developed using diaminobenzidine and were counterstained with hematoxylin.

**Electron Microscopy Studies**

We used electron microscopy to assess the nerves for ultrastructural changes resulting from infusion. In rats destined for electron microscopy examination, CED was accomplished using PBS with a methylene blue marker. Perfusion fixation was completed with 2% glutaraldehyde/2% paraformaldehyde in a 0.1-M cacodylate buffer. The posterior thighs of the animals were injected with additional fixative and allowed to set for 2 hours. The nerves were then dissected free and immersed in fixative overnight. Nerve sections were removed from sites proximal, distal, and within the area of infusion. For controls, sections were excised from uninfused animals that had been subjected to similar fixation. Electron microscopy examinations were performed at various magnifications by using standard methods.

**Results**

**Infusion in Control Nerves**

Infusion into control sciatic nerves produced predictable Vds (9.5 ± 2.7 mm²), with a Vd/VI ratio of 2.5 ± 0.7. The distance traveled by the infusate (4 μl was infused) averaged 10.1 ± 2.2 mm. Convective infusate homogeneously filled the entire cross-sectional area of the nerve, with the epineurium providing a barrier to lateral distribution. The infusate coursed distally from the catheter tip, following the path of nerve fibers. Little or no infusate tracked backward along the course of the silica catheter. We detected no leakage of infusate at the site of catheter insertion. The appearance on QAR was similar to that previously presented in primates.

**Infusion in Crush and Laceration Injuries**

Soon after injury (postinjury Days 1, 3, 7, and 10), infusion failed to cross the site of injury in crushed or lacerated nerves. Results of QAR demonstrated homogeneous filling of the nerve, but no filling distal to the lesion site. As a
Fig. 1. Comparison of hematoxylin and eosin–stained tissue (upper view of each nerve specimen) and QAR film of the tissue (lower view of each nerve specimen) 14 days after injury in crush-injured and lacerated-and-repaired nerves. Convection-enhanced delivery homogeneously fills the entire nerve with 14C-labeled albumin in the crush-injured specimen, whereas no contrast agent reaches the portion of nerve distal to the injury in the lacerated-and-repaired nerve sample. Appearances were similar at later postinjury dates in both models. H&E, original magnification × 25. POD = postoperative (and postinjury) day.
result of the infusion, some nerves developed obvious dispersion in the region of infusion proximal to the injury site, particularly in specimens of lacerated nerves. Infusate tracked backward along the course of the catheter in some specimens. With visual inspection and manual overlay of QAR film and hematoxylin and eosin–stained specimens on postoperative Day 14, we confirmed that the infusate had crossed the site of injury and reached the distal aspects of the nerve in the crush-injury model. At the same time interval in lacerated-and-repaired nerves, however, the infusate stopped at the lesion site (Fig. 1). In the laceration model infusate did not cross the injury site until postoperative Day 35, and then did so with only limited filling of the distal portion of the lacerated nerve. In the laceration model, thorough filling of the distal portion of the nerve was not observed during the 42 days of observation. The Vd gradually increased with time postinjury in crushed nerves, but the differences over time did not reach statistical significance. Volumes of distribution in lacerated nerves ranged widely, from 5.7 ± 1.8 mm$^3$ in the group in which infusion was performed soon after injury to 22.3 ± 0.5 mm$^3$ on postoperative Day 35 (control 9.5 ± 2.7 mm$^3$). In lacerated nerve samples the Vd did not correlate with success or failure in crossing the site of nerve injury, perhaps as a result of the dispersion of the nerve at the site of infusion. Hence, in many specimens the Vd was high despite the limited length of perfusion of nerve and was not predictive of the outcome of infusion (Fig. 2). The distance traveled by the infusate provided a more accurate measure of the capacity of the infusate to cross the site of injury. Distances traversed in crush-injured nerves were low in specimens examined early and in those examined on postoperative Days 7 and 10 ($p < 0.05$ compared with controls). On postoperative Day 14 the distance traveled by the infusate increased toward control levels (control 10.2 ± 2.2 mm; crush-injured group 8.4 ± 1.7 mm). This persisted at postoperative Days 21 and 28. In contrast, distances never reached control levels in nerves subjected to laceration and suture (Fig. 2). By doubling the Vi in crush-injured animals examined on postoperative Day 14 the Vd markedly increased (Vd for control and with 4-μl infusion 9.5 ± 2.7 mm$^3$; Vd with 8-μl infusion 49.3 ± 11.5 mm$^3$), but the distance traveled by the infusate was essentially unchanged, despite the increase in infusion volume (Fig. 3).

**Immunohistochemical, Functional, and Electrophysiological Assessments in Crush and Laceration Models**

Staining for neurofilament showed that the distal aspects of crush-injured nerves had not completed regeneration by 14 days after injury (Fig. 4) and, hence, convective infusion reached regenerating neurites. Performing intraoperative electrophysiology at the time of infusion on postoperative Day 14 in crush-injured nerves, an NAP was recordable at and 1 mm distal to the site of nerve injury. We found no recordable NAPs 4 mm distal to the lesion, indicating incomplete regeneration (data not shown). Lacerated nerves on postoperative Day 42 showed recordable NAPs 8 mm distal to the site of injury. Serial functional testing showed that on postoperative Day 14 crush-injured animals still had complete injuries, with SFI values below −100 (Fig. 5). Crush-injured rats recovered function by postinjury Day 28 (data not shown), which was previously shown to occur in this model. In animals subjected to laceration injuries, functional assessment revealed significant return of nerve function at postoperative Day 42, indicating successful regeneration (data not shown).

**Primate Model**

By using direct overlay of QAR film and hematoxylin and eosin–stained nerve specimens, we found that the infusate had successfully crossed the site of injury in crushed nerves at postoperative Days 28 and 35 in primates, and that the distance traversed by the infusate approached historical controls (postoperative Day 28, 27 ± 4.6 mm; postoperative Day 35, 28.6 ± 1.3 mm). Nevertheless, infusion was not clearly successful in crossing the site of injury in lacerated-and-repaired nerves at any time during the 35 days of study. Distances traversed in lacerated nerves were considerably lower (postoperative Day 28, 16.8 ± 2.3 mm; postoperative Day 35, 19.8 ± 4 mm) than those in crushed nerves. Volumes of distribution varied widely. Marked nerve swelling in response to infusion was often evident, adding an obvious discrepancy when attempting to correlate the Vd with the distance of nerve perfused. The Vd again failed to correlate with success or failure of infusion to traverse the site of injury. Results of MR imaging examination demonstrated successful distribution of the macromolecule in both crush-injured nerves on postoperative Day 35 (Fig. 6). Filling occurred to the level of the popliteal fossa. Distances traversed by infusate on MR images appeared to be greater than those measured using QAR. Contraction of the nerve specimen during processing or incomplete harvest of the distal, perfused nerve may explain these findings.

**Convection-Enhanced Interstitial Infusion in Intact Nerve**

Electrophysiological assessment showed no statistical difference in nerve conduction velocity after nerve exposure and insertion of the silica catheter in control animals (102–104 m/second). Although the velocity slowed after vehicle infusion in experimental animals (97–90 m/second), the differences did not achieve statistical significance. Minor decreases in the SFI also occurred. In control nerves, no deficit developed after muscle dissection and circumferential nerve exposure. In the group in which there was no infusion, although the silica catheter was introduced and left in place for 20 minutes, a decrease in the SFI occurred on postoperative Day 1. This resolved by postoperative Day 3. Vehicle infusion generated a decrease in SFI values at 1 and 3 days after surgery, with return to control values after 3 days (Fig. 7). The decreases in the SFIs were mild; the animals had no obvious clinical deficits and behaved normally after surgery. Light microscopy revealed no changes after convective infusion. Electron microscopy assessment also showed no difference between control and experimental sections (immediately postinfusion and on postinfusion Days 3 and 7). We found no defects in myelination or ultrastructural changes in either small unmyelinated or large myelinated axons as a result of CED in nerves examined 3 and 7 days after infusion. The intraxonal contents, as measured using electron microscopy, were similar between groups.
Retrograde Infusion

After retrograde infusion into the sciatic nerve, gadolinium-labeled albumin was evident in the intrapelvic portion of the sciatic nerve and could be traced proximally within the nerve into the region of the anterior paraspinal musculature. Beginning posterior to the vertebral bodies at L-2 and L-3, filling of the spinal cord was evident. Sagittal images demonstrated that the intramedullary signal extended proximally to the thoracolumbar junction. On axial T1-weighted images obtained using fat suppression, we found complete filling of spinal cord gray matter (Fig. 8). The gadolinium-labeled albumin signal was present in left-sided nerve roots. The spinal cord did not appear to be enlarged. Images obtained from the lower thoracic segments of the spinal cord (T-9 and T-10) demonstrated no contrast enhancement.

Discussion

We sought to expand on the previous work of Lonser, et al., by investigating convected infusate in lesioned sciatic nerve of rats and, by extrapolation to a similar model, in a limited number of specimens in primates. We evaluated the effect of convection on nerve function by using electrophysiology, SFI assessment, and light and electron microscopy. Retrograde infusion into the spinal cord was attempted. The results indicate that CED via the interstitial space provides a promising approach to distribute macromolecules to peripheral nerve and spinal cord lesions.

Animal Models in the Investigation of Peripheral Nerve Injury

The physiology of peripheral nerve regeneration is highly species specific; significant differences exist between different animals and between nonhuman and human models. Kline and colleagues quantified these changes in different animal models and noted differences between species in response to laceration. The considerable capacity of peripheral nerves in lower animals to regenerate after nerve injury brings into question the clinical relevance of obser-
Convection-enhanced delivery in peripheral nerve

vations obtained solely from a rodent model. Without con-
firmation in primates, experimental findings may not nec-
essarily have clinical relevance. Therefore, we confirmed
rodent CED distribution characteristics in a primate model.

Neurotrophins and Other Therapeutic Agents for
Peripheral Nerve Injury

Clinical use of neurotrophins and other agents for nerve
regeneration has yielded limited success, in many instances
due to complications from dose-limiting systemic toxicity.
For instance, although ciliary neurotrophic factor produced
promising results in animal research,24,34,35,38,43 a cachet-
ic effect in rat skeletal muscle occurred in response to system-
ic treatment,34 a complication that correlated with occasion-
al severe reactions in humans enlisted in a trial in which
this agent was used as an experimental treatment for amyot-
rophic lateral sclerosis.7 Similarly, isaxonine initially ap-
peared promising for treatment of a variety of peripheral
neuropathies and for traumatic nerve injury,1,3,12,23,37 but the
drug was withdrawn from clinical trials early because hepa-
titis had developed in some patients.14,29 Furthermore, in
humans the time required for nerve regeneration extends
over months to years. The resulting long duration of de-
nervation and prolonged axotomy, which almost routine-
ly complicates human nerve regeneration, correlates with
poor functional recovery and requires an extended interval
of treatment.18,19 For instance, a neurotrophic agent used to
treat a sciatic nerve injury in a human may have to be deliv-
ered continuously or intermittently for months before distal
regenerating buds reach their targets.

Convective Delivery to Intact, Crushed, and Interrupted
Peripheral Nerves

In rodents, manual overlays of QAR film and hematox-
ylin and eosin–stained nerve sections demonstrated that
the signal of 14C-labeled albumin clearly crossed the site of
nerve-crush injury at postoperative Day 14, reaching far
distally with thorough filling of distal aspects of the injured
nerve. At this time postinjury, the nerves had not signif-
ically recovered electrical activity, with NAPs present
only 1 mm distal to the site of the crush injury. The animals
still had complete lesions functionally, with SFI scores
near −100; and neurofilament staining revealed incom-
plete regeneration, with normal-appearing neurofilaments
at the site of injury (presumably regenerating fibers), but no
visible staining in the distal aspects of the nerve. Thus,
CED of a macromolecule into the extracellular space of a
nerve reaches distal regenerating nerve fibers early after
crush injury and does so before evidence of regeneration
and before signs of returning function. Therapeutic inter-
vention with convective distribution of an agent that en-
hanced the pace or completeness of regeneration of the in-
jured nerve at this time could have functional significancen
In contrast, in animals whose nerves were lacerated and re-
ceived immediate microsurgical repair, the infusate did not
cross the site of surgically created nerve communication
until distal regeneration was nearly complete. After prox-
imal delivery by CED, 14C-albumin did not reach the nerve
distal to the laceration until postoperative Day 35. Even
then, only limited filling of the distal nerve segments oc-
curred, and it extended only a few millimeters beyond the
site of nerve transection. Functional and electrophysiologi-
cal assessment at postoperative Day 42 revealed active re-
nervation of target organs, with significant recovery of
SFI measurements. Concurrently we recorded NAPs 8 mm
distal to the laceration—considerably farther than the most
distant site reached by the convectively delivered 14C-la-
beled albumin. Hence, the success of convection in cross-
ing a surgically created nerve communication lagged con-
siderably behind regenerating neurites. Using similar injury
modeling in primates, successful delivery of infusate distal
to the site of crush injury occurred 28 days after lesioning.
Using manual overlay of QAR film and nerve specimens,
we found constriction of the flow of infusate at the site of
injury in some specimens; however, the labeled albumin
QAR signal clearly crossed the lesion and reached the dis-
tal degenerated nerve. Results of MR imaging confirmed
the QAR findings. In primates with lacerated and recon-
ected nerves, CED again was unsuccessful in crossing the
microsurgically created nerve communication.

Intraneural Fibrosis

External fibrosis and scar formation are known to devel-
op after nerve injury, and impaired recovery of function
may occur in these cases. External scarring may cause de-
lay in, or failure of, regeneration. Neurolysis in these cases
can improve function and encourage recovery,27 suggesting
that severe external scarring impedes nerve function. That

Fig. 3. Bar graphs showing a marked increase in the Vd (lower)
that occurred in response to increasing the V1 in crush-injured spec-
imens 14 days after injury, although the distance traversed by the
infusate did not increase above control levels (upper).

J. Neurosurg. / Volume 95 / December, 2001 1007
intraneural fibrosis blocks convective distribution in the extracellular space of the nerve at the site of laceration and microsurgical nerve repair, as reported here, suggests that internal fibrosis also interferes with regeneration. Reconstitution in the interstitial space that is adequate to carry molecules apparently occurs well after regenerating neurites have crossed the nerve coaptation. There was mild tension across the site of surgically created nerve communication in the rat model, which may yield greater scar formation. Although all surgically created nerve communications in primates were tension free, a similar pattern of conduction block occurred. Exuberant internal fibrosis may limit the pace of regeneration and this block may impart a limiting factor for nerve recovery after laceration or transection injury. The occlusion barring distribution of macromolecules into the extracellular space by bulk flow could impede the distribution of endogenous factors necessary for optimal recovery. Furthermore, this could occur from either direction, because regenerating axons may require signals from proximal and/or distal portions of the nerve for optimal stimulus of neurite outgrowth and guidance. The interstitial fibrosis could also interfere directly with axonal function in a variety of ways. Similar processes probably occur at the site of graft repair. Reduction of the internal scar may enhance nerve regeneration. Though CED was unsuccessful in the laceration model, modifications may allow success. The silica catheter could be used as a skeleton over which proximal and distal nerve ends are reconnected, similar to nerve repair through silicone conduits. Delivery of fibroblast inhibitory factors to remove the cause of the conduction block could decrease intraneural scar formation and allow more complete endogenous recovery or permit perfusion of the distal nerve. Furthermore, intermittent use of CED with a compound to provide trophic influence in the distal portion of a peripheral nerve might be useful for

---

**Fig. 4.** Photomicrographs showing a normal pattern of neurofilament (NF) staining immediately proximal to the site of crush injury in a specimen viewed on postoperative Day 14 (NF, proximal). At the site of the lesion greater cellularity is evident and there is more interfilamentous space (NF, at lesion). Immediately distal to the injury site, staining for neurofilament is nearly absent and this staining indicates the distal margin of nerve regeneration (NF, distal). No neurofilament staining is detected in the most distal aspects of the nerve reached by convective infusion of albumin (NF, far distal). Neurofilament immunohistochemical staining, original magnification × 25.

**Fig. 5.** Graph demonstrating that the SFI falls precipitously after crush injury in four rats (black circles). At the time of infusion on postoperative Day 14, the index remains less than −100, indicating a complete functional deficit. Five control animals underwent sham operation (open circles).
preservation of end organs until arrival of the regenerating nerve fibers, thus overcoming loss of these special structures because of protracted delay in distal regeneration.

**Functional Sequelae of Injections Into Peripheral Nerve**

In a study of previous models of nerve injury in which there was direct injection of materials into the nerve, extrafascicular infusion of various agents produced only minimal immediate sequelae and no clinical deficits.20 Nevertheless, SFI examination was not performed in that study.

Similarly, no clinically evident deficits developed in our study. By evaluating intraoperative NAPs and pre- and postoperative SFI measurements, however, we detected a neurapraxic injury. The velocity of NAPs declined in response to both silica catheter placement alone and infusion of vehicle. The SFI values decreased in both groups at postinjury Day 1, with a persistent deficit in infused animals observed at postinjury Day 3. By Day 7, all animals had returned to baseline. These functional deficits were mild and did not reach statistical significance. Thus, our findings indicate that use of CED for perfusion of the interstitial space of a nerve produces a mild, reversible injury.

**Retrograde CED**

Lonser, et al.,32 described CED in the spinal cord. They directly inserted a silica catheter into the dorsal columns through a laminectomy and obtained homogeneous regional filling of several spinal cord segments in rats and in nonhuman primates. Predictable VDs with varying Vis were achieved. Neurological deficits did not develop in infused animals. No anatomical barrier exists to the movement of materials retrogradely infused by CED into a nerve to reach the spinal cord. Interstitial spaces are likely to be preserved at the nerve root–spinal cord junction. Furthermore, anterior and posterior intramedullary fiber tracts may preferen-

---

**Fig. 6.** Coronal T1-weighted MR images of lower extremities in a primate after intraneural interstitial infusion of gadolinium-labeled albumin (0.5 μl/minute for 100 minutes) into each nerve (fat suppression applied around the popliteal fossa). The animal is prone. Upper: Skin fiducials (arrowheads) mark the sites of nerve lesioning. The high signal observed in the left thigh may indicate fat around the sciatic nerve. Center: In the animal’s left lower extremity, filling of the peroneal nerve is evident (arrow) with infusate tracking into the popliteal fossa. Lower: In the right lower extremity, the tibial division is also filled (arrow) distal to the site of nerve injury. Both nerves were subjected to crush injuries. The high signal on fat-suppression images could be tracked over serial sections across the site of injury to the distal region of delivery.

**Fig. 7.** Graph demonstrating the results of SFI assessment in the control group (control nerves, exposure only), catheter group (catheter nerves, nerve exposure followed by insertion of silica catheter and delay of 20 minutes), and experimental groups (infused nerves, infusion of sterile PBS at 0.2 μl/minute for 20 minutes). The catheter and experimental groups had decreased SFIs 1 day after surgery (catheter nerves $-33.3 \pm 19.8$; infused nerves $-16.8 \pm 15.7$). The deficit persisted in experimental animals at postinjury Day 3, but by postinjury Day 7 all groups returned to baseline (catheter nerves $-14.2 \pm 28.7$ [Day 3] and $-3.8 \pm 9.5$ [Day 7]; infused nerves $-20.5 \pm 20.9$ [Day 3] and $2 \pm 14.7$ [Day 7]).
gray matter is evident on the fat-suppression images. Disorders. It offers promising new avenues of inter-
ducible means to deliver macromolecules to regenerating
axonal materials, an obstruction that may be an important
cal obstruction of the movement of extracellular and intra-
peripheral nerves, suggesting the potential of physiologi-
lar space after laceration injury to and reconnection of
small catheter into the intrapelvic portion of the left sciatic nerve.
primate that underwent retrograde infusion with insertion of a
occurred (upper left and inferior cord segments (upper left and right). At higher segments,
更好的 filling of the gray and white components of the spinal cord
occurred (lower left and right) and near-complete casting of spinal
gray matter is evident on the fat-suppression images.

tially direct infuse into the gray matter of the spinal cord. Retrograde CED through a silica catheter peripherally
placed with its tip in a nerve provided successful intramed-
ullary infusion into the spinal cord for perfusion of gray and
white matter. Magnetic resonance imaging data revealed
thorough bilateral filling of dorsal and ventral horn gray
matter over multiple cord levels. The gadolinium-labeled
albumin distributed widely and homogeneously throughout
the cord with no evident cystic dilations. We observed one
animal over time to assess postoperative neurological func-
tion. He remains neurologically intact, with no evident se-
quela from the infusion.

Conclusions

We identified an intraneural obstruction of the extracel-
lar space after laceration injury to and reconnection of
peripheral nerves, suggesting the potential of physiologi-
ical obstruction of the movement of extracellular and intra-
xonal materials, an obstruction that may be an important
factor influencing regenerative potential after nerve inju-
ry. The CED method provides a safe, simple, and repro-
ducible means to deliver macromolecules to regenerating
peripheral nerves. It offers promising new avenues of inter-
vention in the treatment of peripheral nerve and spinal cord
disorders.

References

2. Bain JR, Mackinnon SE, Hunter DA: Functional evaluation of
complete sciatic, peroneal, and posterior tibial nerve lesions in
3. Barriere H: Essai clinique de l’isaxonine dans l’acropathie ulce-
ro-mutilante des alcooliques. Nouv Presse Med 11:1275–1277,
1982
server reliability of walking-track analysis to assess sciatic nerve
function in rats. Microsurgery 12:76–79, 1991
walking-track measurement using a Sciatic Function Index. Mi-
6. Carbonetto S, Muller KJ: Nerve fiber growth and the cellular re-
recombinant human ciliary neurotrophic factor (rhCNTF) in pa-
tients with amyotrophic lateral sclerosis. The ALS CNTF Treat-
ment Study (ACTS) Phase I-II Study Group. Clin Neurophar-
macol 18:515–532, 1995
8. Chen YS, Wang-Bennett LT, Coker NJ: Facial nerve regenera-
tion in the silicone chamber: the influence of nerve growth fac-
Neural 7:415–421, 1994
10. de Medinaceli L, Freed WJ, Wyatt RJ: An index of the functional
condition of rat sciatic nerve based on measurements made
from walking tracks. Exp Neurol 77:634–643, 1982
11. de Medinaceli L, Wyatt RJ: Neurobehavioral evaluation of func-
tion following experimental nerve damage. Neurobehav Toxic-
ol Teratol 6:415–417, 1984
12. Dehen H: Etude clinique et electrophysiologique de l’isaxonine
dans les paralysies faciales peripheriques. Nouv Presse Med
11:1262–1264, 1982
through artificial tubular implants. Prog Neurobiol 33:87–134,
1989
isaxonine hepatitis. II. Protective role of glutathione and toxico-
logical studies in mice. J Pharmacol Exp Ther 229:851–858,
1984
on the regeneration of motor nerve fibers in long nerve grafts: a
synopsis of experimental and clinical data. Microsurgery 17:
80–88, 1996
16. Frostick SP, Yin Q, Kemp GJ: Schwann cells, neurotrophic
factors, and peripheral nerve regeneration. Microsurgery 18:
397–405, 1998
17. Fu SY, Gordon T: The cellular and molecular basis of peripher-
18. Fu SY, Gordon T: Contributing factors to poor functional recovery
after delayed nerve repair: prolonged axotomy. J Neurosci
15:3876–3885, 1995
19. Fu SY, Gordon T: Contributing factors to poor functional recovery
after delayed nerve repair: prolonged denervation. J Neuro-
sci 15:3886–3895, 1995
Acad Sci 692:51–59, 1993
22. Hare GM, Evans PJ, Mackinnon SE, et al: Walking track analy-
sis: a long-term assessment of peripheral nerve regeneration. Plast
Reconstr Surg 89:251–258, 1992
23. Hugelin A, Leprasin Y, Bondoux-Jahan M: Nerve-growth pro-
moting action of isaxonine in rat. Experientia 35:626–627, 1979
24. Junger H, Junger WG: CNTF and GDNF, but not NT-4, support
corticospinal motor neuron growth via direct mechanisms. Neu-

Fig. 8. Axial T2-weighted MR images of the lumbar spine in a
primate that underwent retrograde infusion with insertion of a
small catheter into the intrapelvic portion of the left sciatic nerve.
Images with (upper and lower right) and without (upper and lower
left) fat suppression are presented. Incomplete filling occurred at
inferior cord segments (upper left and right). At higher segments,
better filling of the gray and white components of the spinal cord
occurred (lower left and right) and near-complete casting of spinal
gray matter is evident on the fat-suppression images.
Convection-enhanced delivery in peripheral nerve


Manuscript received December 4, 2000. Accepted in final form August 20, 2001.

Address reprint requests to: Edward H. Oldfield, M.D., Surgical Neurology Branch, National Institute of Neurological Disorders and Stroke, National Institutes of Health, Building 10, Room 5D37, Bethesda, Maryland 20892.