Reduction of Extraneural Scarring by ADCON-T/N after Surgical Intervention

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THE EFFECTS OF ADCON-T/N (Gliatech, Inc., Cleveland, OH), a carbohydrate polymer gel, on peripheral nerve scarring and regeneration were studied in rodents undergoing three types of surgical intervention. Procedure I involved external neurolysis of the sciatic nerve from surrounding tissues and separation of its tibial and peroneal components. Procedure II involved the addition of an abrasive injury. Procedure III involved transection and suture anastomosis of the tibial component. ADCON-T/N or a control gel was locally applied in a blind fashion. Additional animals received no gel, as a further control. Animals underwent second operations 4 weeks after Procedures I and II and 6 weeks after Procedure III. The surgical sites were evaluated using a numerical grading scheme to assess wound healing, sciatic nerve adherence to surrounding tissues, and separability of its tibial and peroneal components. Animals receiving ADCON-T/N demonstrated reduced nerve adherence to surrounding tissues and enhanced separability of the tibial and peroneal components, compared with animals receiving control gel or no gel. Quantitative histological analysis revealed a statistically significant reduction in the amount of dense scar tissue surrounding nerves treated with ADCON-T/N. No evidence of nerve toxicity caused by ADCON-T/N was noted. Counts of regenerating myelinated axons in animals undergoing nerve transection and suture repair did not statistically differ in treated and untreated animals. In conclusion, ADCON-T/N seems to be both safe and effective in reducing extraneural scar formation after peripheral nerve surgery and local trauma. (Neurosurgery 38:976–984, 1996)

Key words: Carbohydrate polymer, Neurolysis, Peripheral nerve, Regeneration, Scar inhibiton, Surgery, Trauma

Scar formation after tissue damage is an integral part of wound healing (7, 9). However, scar tissue can give rise to adhesions between important structures, which can result in compromised function with deleterious effects. In the peripheral nervous system, extraneural scarring may lead to tethering of nerves to adjacent tissues, which could interfere with the normal longitudinal sliding of nerves during limb movement. Studies in humans have demonstrated significant longitudinal excursion of nerves across joints during flexion and extension movements (5, 10, 15, 16, 21). It has been hypothesized that restriction of nerve mobility may result in nerve injury (1, 4, 11, 16, 21). For example, tethering of nerves can produce tension, which if severe and prolonged, can lead to ischemia and further nerve injury (12–14). Therefore, reducing extraneural scarring might improve the outcome after peripheral nerve surgery and other types of trauma. In addition, reducing scar formation around nerves would facilitate secondary operation and thereby decrease the risk of a complication.

Animal experiments have identified specific glycosaminoglycan chains of particular proteoglycans that can inhibit cellular motility in vitro (19) and in vivo (3, 20). Wujek et al. (22) demonstrated that a synthetic carbohydrate polymer could significantly reduce epidural scar formation after surgical trauma in a rat laminectomy model. Similar results with the use of a gel formulation containing this carbohydrate polymer have also been obtained in rabbit (18) and dog (8) laminectomy models as well as in an ongoing European clinical trial in patients undergoing unilateral removal of herniated discs (6).

In the present study, we have investigated the effects of a biodegradable gel formulation containing the carbohydrate

polymer ADCON-T/N (Gliatech, Inc., Cleveland, OH) on peripheral nerve scarring and axonal regeneration in rats after three types of surgical intervention. Our results demonstrate that ADCON-T/N can significantly reduce the formation of extraneural scar after the surgical manipulation of nerves. In addition, no toxic effects of ADCON-T/N upon nerves were noted. Finally, ADCON-T/N was not found to interfere with axonal regeneration after transection and suture repair of the sciatic nerve. Therefore, ADCON-T/N may prove useful in preventing nerve scarring after surgical intervention as well as in facilitating second operations when necessary.

MATERIALS AND METHODS

Gel preparation

ADCON-T/N, a proprietary gel as well as a control gel lacking a specific carbohydrate component, was prepared under sterile conditions by Gliatech, Inc. The gels were numerically coded before shipment to conceal their identity from the investigators performing the surgery and evaluations (JP, MM, MK).

Surgery

Lewis albino rats, weighing 250 to 300 g, were anesthetized with Equithesin and then the surgical site was cleaned with Betadine solution. With the use of sterile technique, the sciatic nerve was bilaterally exposed via a longitudinal incision along the posterior thigh and buttock. In each of the nine animals undergoing Procedure I, the nerve was isolated from the surrounding tissues and the tibial and peroneal components were separated back toward the sciatic foramen with the use of blunt dissection. After hemostasis, gel was applied in a blind manner, such that each side received 0.4 ml of either ADCON-T/N or control gel directly applied around the nerve. The muscle fascia was sutured using a continuous 4-0 Vicryl suture, and the skin was approximated with staples. An additional four animals did not receive gel on either side and served as controls for the surgical procedure.

Procedure II included 10 animals in which abrasive injuries were performed after isolation and separation of the sciatic nerve as described in Procedure I. Using a small piece of compressed steel wool, 20 consistent strokes were made on the upper and lower surfaces of the muscle cavity and sciatic nerve. This procedure was performed bilaterally and then 0.4 ml of either ADCON-T/N or control gel was applied around the sciatic nerve.

Procedure III included nine animals in which the tibial component of the left sciatic nerve was transected and reanastomosed. After isolation and separation of the nerve, 0.1 ml of lidocaine was applied to the nerve as a local anesthetic. The tibial component was sharply perpendicularly transected with microscissors ~10 mm from the sciatic foramen, and four interrupted 9-0 Prolene sutures were circumferentially placed in the epineurium to reanastomose the nerve. Either ADCON-T/N (n = 5) or control gel (n = 3) was randomly assigned for application to the nerve. Duration of all operative procedures was consistently kept between 15 and 20 minutes, and tissues were kept moist with normal saline irrigation. After recovery from anesthesia, animals were housed in cages and monitored daily.

Gross anatomic evaluation

All animals were evaluated and killed either 4 or 6 weeks after surgery. Scoring was performed by investigators (JP and MK) blinded to whether animals received ADCON-T/N, control gel, or no gel. Animal behavior was observed weekly to evaluate sciatic nerve function, including abnormal foot posture, diminished reflex withdrawal of the foot to pinching of the toes, toe spreading, and foot dorsiflexion and plantarflexion.

After anesthesia with sodium pentobarbital, the surgical sites were carefully reopened in stages and systematically evaluated. A numerical grading scheme was used to evaluate skin closure, muscle fascia closure, nerve adherence to the surrounding muscle cavity tissue, and separability of the tibial and peroneal nerve branches, as listed in *Table 1*. The skin and muscle fascia closure categories were designed to evaluate wound healing. Grade 1 reflected complete closure, Grade 2 reflected partial failure of the wound to close, and Grade 3 reflected no closure of the wound. Nerve adherence was evaluation of the tethering of the nerve to the surrounding muscle cavity. Nerve separability was evaluation of the adherence of the tibial and peroneal nerve components to each other. For these categories, Grade 1 indicated that the nerve was either free or required minimal blunt dissection to sepa-

TABLE 1. Numerical Grading Scheme for Gross Evaluation[a]

Tissue	Grade	Definition
Skin and muscle fascia	1	Skin or muscle fascia entirely closed
	2	Skin or muscle fascia partially open
	3	Skin or muscle fascia completely open
Nerve adherence and nerve separability	1	No dissection or mild blunt dissection
	2	Some vigorous blunt dissection required
	3	Sharp dissection required

[a] **Numerical grading scheme used for assessing animals at time of second surgery. See text for a detailed description.**

rate. Grade 2 reflected the need for moderate or vigorous blunt dissection for separation. Grade 3 indicated that sharp dissection with scissors was required for separation.

Electrophysiological evaluation

Nerve conduction velocities were determined during the second surgery. Bipolar stimulating and recording macroelectrodes were placed on the proximal sciatic nerve and distal tibial nerve, respectively. The two electrodes were separated by a distance of at least 20 mm, which required distal dissection and exposure of the tibial nerve within the calf muscles. A stimulus just above threshold was used, and the evoked compound action potential was recorded. We calculated nerve conduction velocities (meters per second) by dividing the latency of the compound action potential by the length of nerve between the stimulating and recording electrodes.

Histological processing

After the rats were killed with sodium pentobarbital, an intracardiac perfusion with 4% paraformaldehyde was performed. The sciatic nerve and the surrounding scar tissue were removed *en bloc* and stored in the fixative for at least 7 days. The mid portion of the sciatic nerve was then removed and processed further. Tissue from Procedure I was embedded in glycol methacrylate and cross-sectioned at 4 μm. Tissue from Procedure II was embedded in paraffin and cross-sectioned at 6 μm. Sections were stained with hematoxylin and eosin for cells, toluidine blue and Holmes' silver stain for axons, and van Gieson's stain for collagen. The sciatic nerves subjected to Procedure III were divided into three segments and transferred into 4% paraformaldehyde with 1% glutaraldehyde. The middle segment extended 1 mm proximal and 3 mm distal from the suture anastomosis site. This segment was dehydrated in alcohol, embedded in Epon, and cross-sectioned at 1 μm. These sections were then stained with toluidine blue.

Quantitative histological analysis

Quantitative evaluation of the scar surrounding the nerve was performed on van Gieson's-stained sections. The dense scar surrounding the nerve was distinguishable as an annular band of dark red-staining connective tissue. An image of the histological section was produced on a computer video screen at $\times$120 magnification and then analyzed with the use of the BioQuant IV program (R & M Biometrics Inc., Nashville, TN). A cross-sectional area of the nerve (NA) was measured by one of the investigators (JP), who was blinded to whether animals received ADCON-T/N, control gel, or no gel. A threshold of contrast was established on the computer screen to distinguish red-staining connective tissue from surrounding background. The area of connective tissue within the dense ring of scar surrounding the nerve (CTA) was then measured by circumscription of the outer edge of the ring and subtraction of the area of the nerve. This value was normalized by division by the cross-sectional area of the nerve ($CTA_{normalized}$ = CTA/NA). Three trials were run on each tissue section, and the average values were statistically analyzed.

Counts of degenerating axons

Counts of degenerating myelinated axons were performed on toluidine blue-stained sections from animals undergoing Procedure I. Darkly staining contracted axon profiles with a thin rim of myelin were identified under the microscope at $\times$400 magnification. The number of such profiles within a superimposed grid having a surface area of 0.062 mm^2 was determined in three adjacent sections per nerve.

Counts of regenerating axons

Counts of regenerating axons in animals undergoing Procedure III were performed on toluidine blue-stained sections 3 mm distal to the suture anastomotic site. A light photomicrographic montage of the entire sciatic nerve cross section was constructed at a magnification of $\times$550. Twenty regions with a surface area of 0.01 mm^2 each were randomly sampled, equaling approximately 10% of the total surface area of the sciatic nerve. All myelinated axons were counted within these regions, and these counts were normalized regarding surface area. Axon counts from three normal sciatic nerves were obtained in a manner similar to that for comparison.

Statistical analysis

Wilcoxon's test for paired data was used for internal comparison of the two sides in animals undergoing Procedures I and II. Comparison with external controls in Procedures I and II was performed with the use of the Mann-Whitney *U* test. Data from Procedure III were analyzed with the use of the Mann-Whitney *U* test.

RESULTS

In animals undergoing simple isolation and separation of sciatic nerves into their tibial and peroneal components (Procedure I), nerves treated with ADCON-T/N were easier to isolate from the surrounding muscle cavity than were nerves treated with either control gel or no gel. Nerves treated with ADCON-T/N were often detached from the muscle cavity or were surrounded by a very thin and lucent membrane (*Fig. 1A*). Nerves treated with either control gel or no gel were often encased in a tenacious and opaque sheath of scar tissue that tethered them to the surrounding muscle cavity (*Fig. 1B*). As a result, either vigorous blunt dissection or sharp dissection was required to re-isolate these nerves. Significantly lower scores in the nerve-tissue category were found in nerves treated with ADCON-T/N in comparison with nerves treated with control gel (*Table 2*) and no gel (*Table 3*). Residual ADCON-T/N or control gel could not be seen at 4 weeks.

The tibial and peroneal components of the sciatic nerve rejoined in all cases but one. Separation of the tibial and peroneal components required only mild blunt dissection in nerves treated with ADCON-T/N, as shown in *Table 2* under separability. In contrast, nerves receiving either control gel or no gel required vigorous blunt or sharp dissection with scissors (*Tables 2* and *3*). The difference in separability scores was statistically significant between nerves treated with ADCON-

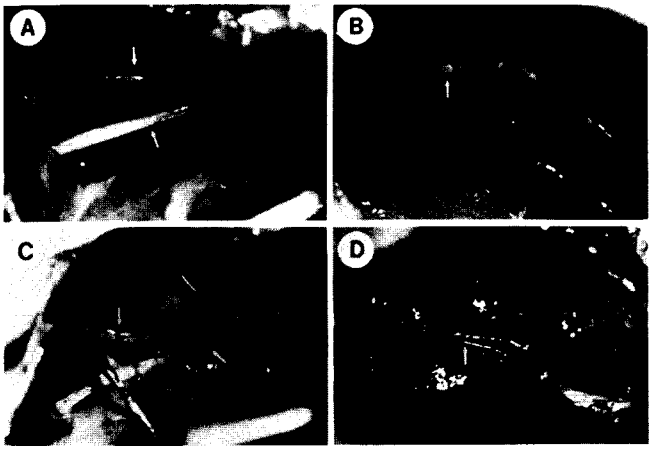

FIGURE 1. Photographs depicting bilateral comparisons of sciatic nerves reexposed in the thigh 4 weeks after the initial surgery. *Panels A* and *B* represent an animal undergoing Procedure I (see text). *Panels C* and *D* represent an animal undergoing Procedure II (see text). Nerves treated with ADCON-T/N (*A* and *C*) were easily dissected from the surrounding tissue, and the tibial (*lower arrow*) and peroneal (*upper arrow*) components were easily separated with blunt dissection. In contrast, contralateral nerves treated with control gel (*B* and *D*) were surrounded by scar and tethered to the surrounding tissue. In addition, the fused peroneal and tibial components (*single arrow*) could not be separated with blunt dissection. Overall, significantly less scarring and tethering to surrounding tissues occurred in nerves treated with ADCON-T/N.

T/N and nerves treated with either control gel (*Table 2*) or no gel (*Table 3*). In the one exception (*Table 2*, Animal NL-5), the tibial and peroneal components of an animal receiving control gel were tethered apart by extensive scarring to the surrounding muscle cavity.

Procedure II artificially produced a more severe injury by abrasion of the sciatic nerve and surrounding tissues. ADCON-T/N was also effective in reducing scarring in this model (*Fig. 1, C* and *D*). As shown in *Table 2*, nerves treated with ADCON-T/N demonstrated statistically significant lower scores in both nerve adherence and nerve separability categories, compared with nerves treated with control gel. In animals undergoing sciatic nerve transection (Procedure III), nerves treated with ADCON-T/N also demonstrated significantly lower scores in nerve adherence and nerve separability, compared with nerves treated with control gel.

In terms of wound healing, all animals demonstrated complete skin closure, as shown in *Tables 2* and *3*. The muscle and deep fascia layers failed to completely close in some animals treated with ADCON-T/N, as shown in the muscle fascia category of *Table 2*. However, this difference was not statistically significant in animals undergoing either Procedure I or II.

Animal behavior was observed to assess function of the sciatic nerve after surgery. All animals undergoing Procedure I demonstrated either no functional deficit or a transient reduction of dorsiflexion and plantarflexion with rapid recovery that was complete by 4 weeks. Animals undergoing the more severe abrasive injury in Procedure II often demonstrated deficits in dorsiflexing and/or plantarflexing their feet at evaluation after 4 weeks. Animals undergoing left tibial nerve transection (Procedure III) consistently initially had the expected deficit in plantarflexion of the left foot, which resolved over time. No consistent differences were noted in the recovery of sciatic nerve function between animals receiving and those not receiving ADCON-T/N.

Nerve conduction velocities demonstrated a great deal of variability among individual animals and in bilateral comparisons of animals undergoing Procedures I and II. No statistically significant differences were detected between nerves treated with ADCON-T/N, control gel, or no gel (*Tables 2* and *3*).

The effect of ADCON-T/N on scar formation was histologically evaluated in sections treated with van Gieson's stain, which specifically stains the collagenous connective tissue elements. A marked difference in the pattern of connective tissue staining surrounding sciatic nerves was noted between nerves treated with ADCON-T/N and nerves treated with either control gel or no gel. *Figure 2* shows van Gieson's-stained sections of a normal unoperated sciatic nerve (*Fig. 2A*) and of sciatic nerves 4 weeks after undergoing isolation and separation of their tibial and peroneal components (Procedure I; *Fig. 2, B, C,* and *D*). Sciatic nerves treated with no gel (*Fig. 2C*) or control gel (*Fig. 2D*) consistently demonstrated a thick band of dense epineurial connective tissue surrounding the nerve. In contrast, nerves treated with ADCON-T/N were surrounded by a few thin dark bands of connective tissue (*Fig. 2B*), which resembled the epineurium surrounding normal nonoperated sciatic nerves (*Fig. 2A*). Quantification of the dense connective tissue surrounding the nerves revealed a statistically significant reduction around nerves treated with ADCON-T/N, compared with nerves treated with control gel and nerves receiving no gel (*Table 4*). There was more dense connective tissue surrounding nerves treated with ADCON-T/N in comparison with normal nonoperated nerves (*Table 4*).

Sections stained with toluidine blue demonstrated only a few scattered degenerated axons in nerves treated with ADCON-T/N, control gel, and no gel in animals undergoing Procedure I. Counts of degenerated myelinated axons revealed no statistically significant difference among groups (*Table 5*). A greater number of degenerated axons was observed in nerves from animals undergoing Procedure II, compared with those of animals undergoing Procedure I, reflecting the greater degree of surgical trauma. After transection and suture repair of the tibial nerve (Procedure III), no statistically significant difference in counts of regenerating myelinated axons was noted between nerves treated with ADCON-T/N and those treated with control gel (*Table 6*), although counts in the former tended to be somewhat lower. These axon counts were approximately twice those obtained from normal control sciatic nerves, reflecting the tendency of regenerating nerve fibers to sprout (12). In addition, no evidence of residual ADCON-T/N or control gel could be seen in

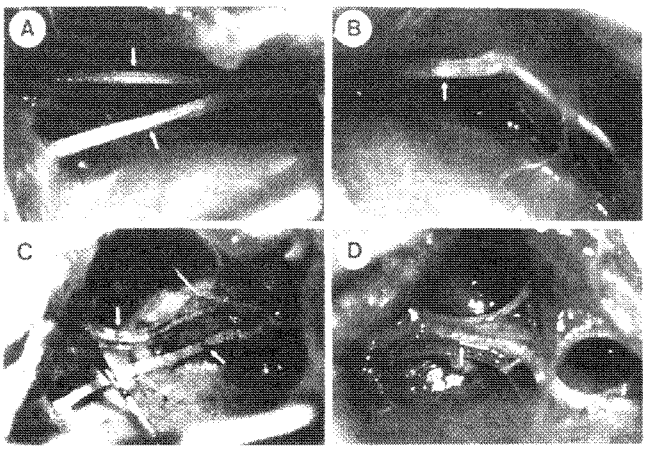

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TABLE 2. ADCON-T/N versus Control Gel: Gross Evaluation Grading[a]

Animal No.	Skin Closure	Muscle Fascia	Nerve Tissue	Separability	NCV (m/s)
Procedure I: External neurolysis with separation of tibial and peroneal components					
NL-1	1:1	2:1	1:2	2:3	ND:ND
NL-2	1:1	2:1	1:3	2:3	53:48
NL-3	1:1	2:1	1:3	2:3	56:27
NL-4	1:1	1:1	1:3	2:1	39:34
NL-5	1:1	3:1	1:2	1:3	31:28
NL-6	1:1	1:1	1:2	1:3	73:81.8
NL-7	1:1	2:1	1:2	1:3	ND:80
NL-8	1:1	1:1	1:2	2:3	73:ND
NL-9	1:1	1:1	1:3	1:2	88:76
P values	>0.05	>0.05	<0.05	<0.05	>0.05
Procedure II: Abrasive injury					
AI-1	1:1	1:1	1:3	1:3	45.7:56.7
AI-2	1:1	1:1	1:3	1:3	24.3:18.0
AI-3	1:1	1:1	1:3	1:3	22.0:44.0
AI-4	1:1	1:1	1:3	1:3	ND:60.0
AI-5	1:1	3:1	1:3	1:3	40.0:25.0
AI-6	1:1	1:1	1:3	1:3	29.0:24.0
AI-7	1:1	1:1	1:2	1:3	9.0:22.0
AI-8	1:1	2:1	1:3	1:3	23.0:19.0
AI-9	1:1	2:1	1:2	2:3	53.3:40.0
AI-10	1:1	1:1	1:3	1:3	52.5:26.6
P values	>0.05	>0.05	<0.05	<0.05	>0.05

[a] **Summary of scores and nerve conduction velocities obtained at evaluation during the second surgery in animals undergoing Procedure I and Procedure II (ADCON-T/N:control). A Wilcoxon's test for paired data, comparing the ADCON-T/N and control gel side of each animal, was applied to obtain *P* values. NCV, nerve conduction velocity; ND, not done.**

TABLE 3. No Gel: Gross Evaluation Grading—Procedure I: External Neurolysis with Separation of Tibial and Peroneal Components[a]

Animal No.	Skin Closure	Muscle Closure	Nerve Tissue	Separability	NCV (m/s)
NG-1	1	1	2	3	23
NG-2	1	1	2	3	27
NG-3	1	1	3	3	68
NG-4	1	1	3	3	70
NG-5	1	1	3	2	85
NG-6	1	1	2	2	100
NG-7	1	1	3	3	65
NG-8	1	1	3	3	70
P values[b]					
Versus ADCON-T/N	>0.05	>0.05	<0.05	<0.05	>0.05
Versus control gel	>0.05	>0.05	>0.05	>0.05	>0.05

[a] **Summary of scores and nerve conduction velocities in animals undergoing Procedure I that received no gel. A Mann-Whitney U test was applied to obtain *P* values comparing scores in animals receiving no gel with those in other animals receiving either ADCON-T/N or control gel (values listed in *Table 2*). NCV, nerve conduction velocity.**

[b] **Scores from *Table 2*.**

histological sections at either 4 or 6 weeks in any of the experimental groups of animals.

DISCUSSION

Our results demonstrate that ADCON-T/N significantly reduced extraneural scar formation after surgical manipulation of peripheral nerves. The gross evaluation data demonstrate that ADCON-T/N significantly decreased adherence of sciatic nerves to the surrounding tissue. In addition, ADCON-T/N was also shown to facilitate the separation of tibial and peroneal nerve components at secondary operation. Our model of abrasive injury (Procedure II) demonstrated the effectiveness of ADCON-T/N in an artificial setting designed to enhance scar

TABLE 4. Summary of Quantitative Histological Data of Extraneural Scar[a]

Animal No.	ADCON-T/N	Control Gel	Animal No.	No Gel	Animal No.	Normal
NL-1	0.414	0.714	NG-1	0.758	C-1	0.269
NL-2	0.448	0.639	NG-2	0.921	C-2	0.334
NL-3	0.443	0.847	NG-3	0.712	C-3	0.395
NL-4	0.469	0.713	NG-4	0.711	C-4	0.299
NL-5	0.582	0.729	NG-5	0.789		
NL-7	0.320	0.729	NG-6	0.569		
NL-8	0.381	0.618				
P values:						
Versus control gel	<0.05			>0.05		
Versus no gel	<0.05	>0.05				
Versus ADCON-T/N						<0.05

[a] **Quantification of collagenous scar tissue surrounding nerves in animals undergoing Procedure I and in normal control nerves. The area of densely staining scar was quantified and normalized by dividing by the surface area of the nerve. A Wilcoxon's test for paired data, comparing the ADCON-T/N and control gel sides of each animal, was applied to obtain *P* values. NL, external neurolysis with ADCON-T/N or control gel; NG, external neurolysis with no gel; C, unoperated control nerve.**

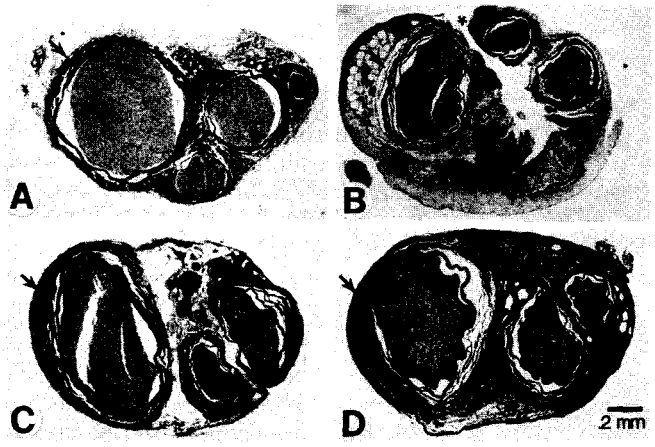

FIGURE 2. *Photomicrographs of cross sections of nerves and surrounding tissue. Sections were stained with van Gieson's connective tissue stain for collagen, which appears dark. A, van Gieson's connective tissue stain of a normal nerve. Note that the normal nerve has several thin layers of dark staining epineurial connective tissue (arrow) surrounding the nerve in an "onion peel" fashion. B, nerves treated with ADCON-T/N demonstrated several thin layers of darkly stained connective tissue (arrow) similar to the "onion peel" pattern observed in normal nerves. Quantitative analysis demonstrated significantly less connective tissue in the scar surrounding nerves treated with ADCON-T/N. *, within cleavage plane repeating nerve fascicles. C, nerves treated with no gel demonstrated a dense band of darkly stained connective tissue (arrow) surrounding the nerve and encasing the nerve bundles. D, nerves that received control gel demonstrated a dense band of darkly stained connective tissue (arrow) surrounding the nerve, similar to that of nerves treated with no gel.*

formation. The severe extraneural scarring with tethering found on the control sides of these animals contrasted with the mildly scarred and minimally adhered nerves treated with ADCON-T/N. Quantitative histological assessment of scar tissue using the van Gieson's stain confirmed the smaller surface area of the dense band of connective tissue surrounding nerves treated with ADCON-T/N. The nerve conduction studies and demyelinated axon counts demonstrated no evidence for toxicity of ADCON-T/N to nerves. In addition, myelinated axon counts in distal nerve stumps 6 weeks after transection suggested that ADCON-T/N does not interfere with axonal regeneration and sprouting.

Healing with complete closure of the skin occurred in all animals. However, our results suggest that ADCON-T/N may affect closure of the muscle cavity. It should be noted that we applied a constant and relatively large amount of gel directly to the nerve in each animal. This large volume of gel resulted in excess gel leaking directly onto the edges of the muscle cavity in some animals. Although the reduced closure of the muscle cavity found in some animals treated with ADCON-T/N was not statistically significant, this finding suggests that ADCON-T/N should be placed only in locations where tissue adherence is not desired. Application of smaller volumes (i.e., 0.2 ml) of ADCON-T/N has subsequently been shown to be equally efficacious in reducing extraneural scar formation while not interfering with closure of the muscle cavity (unpublished observations).

Animals in our study were examined 4 and 6 weeks after surgical intervention and the application of ADCON-T/N, which provided only two time points during the process for potential scar formation. However, reduced scar and adhesion formation has been demonstrated after 26 weeks in rat (2) and rabbit (17) laminectomy models and after 6 months in two case reports of human laminectomy (6). Therefore, the degree of scar formation, or lack thereof, established early on seems to be maintained at later time points despite the disappearance of the ADCON-T/N gel by 4 weeks after its application.

A certain amount of extraneural scar formation is an inevitable sequela of peripheral nerve surgery. The numerical grading scheme we used in the gross evaluation of surgical scarring was chosen to reflect three levels of severity of scar formation that could be reliably distinguished. These distinctions are clinically important during secondary operation, because extraneural scar that requires sharp dissection as opposed to no dissection or blunt dissection increases the risk

TABLE 4. Summary of Quantitative Histological Data of Extraneural Scar[a]

Animal No.	ADCON-T/N	Control Gel	Animal No.	No Gel	Animal No.	Normal
NL-1	0.414	0.714	NG-1	0.758	C-1	0.269
NL-2	0.448	0.639	NG-2	0.921	C-2	0.334
NL-3	0.443	0.847	NG-3	0.712	C-3	0.395
NL-4	0.469	0.713	NG-4	0.711	C-4	0.299
NL-5	0.582	0.729	NG-5	0.789		
NL-7	0.320	0.729	NG-6	0.569		
NL-8	0.381	0.618				
P values:						
Versus control gel	<0.05			>0.05		
Versus no gel	<0.05	>0.05				
Versus ADCON-T/N						<0.05

[a] **Quantification of collagenous scar tissue surrounding nerves in animals undergoing Procedure I and in normal control nerves. The area of densely staining scar was quantified and normalized by dividing by the surface area of the nerve. A Wilcoxon's test for paired data, comparing the ADCON-T/N and control gel sides of each animal, was applied to obtain *P* values. NL, external neurolysis with ADCON-T/N or control gel; NG, external neurolysis with no gel; C, unoperated control nerve.**

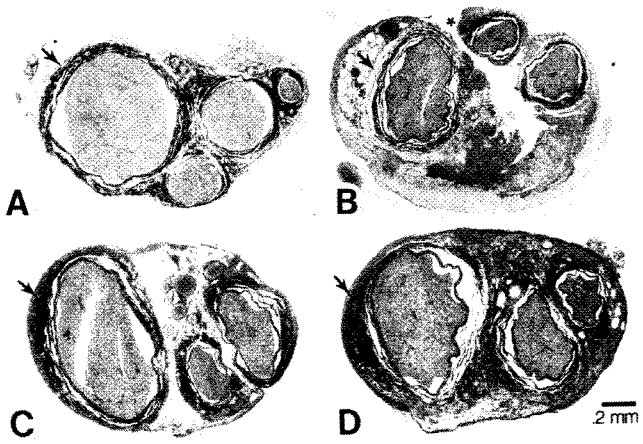

FIGURE 2. Photomicrographs of cross sections of nerves and surrounding tissue. Sections were stained with van Gieson's connective tissue stain for collagen, which appears dark. *A*, van Gieson's connective tissue stain of a normal nerve. Note that the normal nerve has several thin layers of dark staining epineurial connective tissue (*arrow*) surrounding the nerve in an "onion peel" fashion. *B*, nerves treated with ADCON-T/N demonstrated several thin layers of darkly stained connective tissue (*arrow*) similar to the "onion peel" pattern observed in normal nerves. Quantitative analysis demonstrated significantly less connective tissue in the scar surrounding nerves treated with ADCON-T/N. *, within cleavage plane repeating nerve fascicles. *C*, nerves treated with no gel demonstrated a dense band of darkly stained connective tissue (*arrow*) surrounding the nerve and encasing the nerve bundles. *D*, nerves that received control gel demonstrated a dense band of darkly stained connective tissue (*arrow*) surrounding the nerve, similar to that of nerves treated with no gel.

formation. The severe extraneural scarring with tethering found on the control sides of these animals contrasted with the mildly scarred and minimally adhered nerves treated with ADCON-T/N. Quantitative histological assessment of scar tissue using the van Gieson's stain confirmed the smaller surface area of the dense band of connective tissue surrounding nerves treated with ADCON-T/N. The nerve conduction studies and demyelinated axon counts demonstrated no evidence for toxicity of ADCON-T/N to nerves. In addition, myelinated axon counts in distal nerve stumps 6 weeks after transection suggested that ADCON-T/N does not interfere with axonal regeneration and sprouting.

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Animals in our study were examined 4 and 6 weeks after surgical intervention and the application of ADCON-T/N, which provided only two time points during the process for potential scar formation. However, reduced scar and adhesion formation has been demonstrated after 26 weeks in rat (2) and rabbit (17) laminectomy models and after 6 months in two case reports of human laminectomy (6). Therefore, the degree of scar formation, or lack thereof, established early on seems to be maintained at later time points despite the disappearance of the ADCON-T/N gel by 4 weeks after its application.

A certain amount of extraneural scar formation is an inevitable sequela of peripheral nerve surgery. The numerical grading scheme we used in the gross evaluation of surgical scarring was chosen to reflect three levels of severity of scar formation that could be reliably distinguished. These distinctions are clinically important during secondary operation, because extraneural scar that requires sharp dissection as opposed to no dissection or blunt dissection increases the risk

TABLE 5. Counts of Degenerating Myelinated Axons[a]

Animal No.	ADCON-T/N (n)	Control Gel (n)	Animal No.	No Gel (n)
NL-1	0	0	NG-1	17
NL-2	4	7	NG-2	30
NL-3	3	0	NG-3	7
NL-4	1	8	NG-4	34
NL-5	34	1	NG-5	0
NL-6	13	24	NG-6	0
NL-7	4	0	NG-7	19
NL-8	1	1	NG-8	15
NL-9	12	0		

[a] **Comparison of degenerating myelinated axon counts in animals undergoing Procedure I. Darkly staining contracted axon profiles with a thin rim of myelin were identified at ×400 magnification. The number within a superimposed grid having a surface area of 0.062 mm^2 was determined in three adjacent toluidine blue-stained sections per nerve. The mean number of degenerated axons was calculated and is listed. A Wilcoxon's test for paired data was used to obtain *P* values in comparing the ADCON-T/N and control gel sides of each animal. A Mann-Whitney *U* test was used to obtain *P* values in making comparisons across different groups of animals (i.e., ADCON-T/N versus no gel and control gel versus no gel). Statistical analysis is as follows: ADCON-T/N versus control gel, *P* value >0.05; ADCON-T/N versus no gel, *P* value >0.05; and control gel versus no gel, *P* value >0.05.**

TABLE 6. Procedure III Axon Counts[a]

Animal No.	Treatment	Axon Count ± Standard Deviation/mm^2
CT-1	ADCON-T/N	14816 ± 4144
CT-2	ADCON-T/N	25280 ± 6838
CT-3	ADCON-T/N	21861 ± 4973
CT-4	ADCON-T/N	18338 ± 3626
CT-5	ADCON-T/N	25176 ± 5284
CT-6	Control gel	24554 ± 3315
CT-7	Control gel	24451 ± 5905
CT-8	Control gel	25901 ± 6631

[a] **Comparison of regenerating myelinated axon counts after sciatic nerve transection and suture anastamosis (Procedure III). No significant difference was noted between nerves treated with ADCON-T/N and control gel, with the use of a Mann-Whitney *U* test. ADCON-T/N versus control gel, *P* value >0.05.**

of traumatizing peripheral nerves. Excessive scarring can also tether peripheral nerves to surrounding tissues (1, 5, 11, 14–16, 21). These factors acting either alone or in concert have the potential to generate inflammation, edema, and ischemia leading to further extraneural scarring (14). The possible contribution of extraneural scar in producing painful dysesthesia and reducing peripheral nerve function is supported by the efficacy of surgical neurolysis in treating particular peripheral nerve conditions (4, 11) and of early postoperative mobilization, when possible, to promote nerve gliding (11, 21).

The precise manner by which ADCON-T/N reduces scar formation in vivo remains unclear. It has been shown that the glycosaminoglycan moiety of certain proteoglycans can establish boundaries for cellular migration both in vitro (19) and in vivo (3, 20). ADCON-T/N contains a carbohydrate polymer that has been shown to block the migration of fibroblasts in vitro (unpublished data). It is therefore possible that ADCON-T/N acts in vivo by establishing an inhibitory boundary to invading fibroblasts, which results in less scarring of the nerve to surrounding tissues. Additional experiments observing earlier and serial time points after the application of ADCON-T/N will be necessary to further delineate its mechanism(s) of action.

CONCLUSION

ADCON-T/N has been shown to be effective in reducing extraneural scar formation after surgical procedures involving the neurolysis of peripheral nerves. In addition, no evidence for toxicity or interference with nerve regeneration was found. These findings suggest that ADCON-T/N may be clinically useful in the setting of peripheral nerve surgery. At the very least, this compound is likely to facilitate secondary operation when necessary. Further studies are necessary to demonstrate that ADCON-T/N can actually improve functional outcome by reducing scar formation.

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MK and JS are founding members of and have a commercial interest in Gliatech, Inc.

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COMMENTS

This article describes three experimental paradigms in rats, external neurolysis and divisional split, abrasive injury to the sciatic nerve, and severance and suture. Animals in each paradigm were then treated with either a carbohydrate polymer gel (ADCON-T/N; Gliatech, Inc., Cleveland, OH) or a control gel or not treated. Assessment of separability of nerve and of one division from another in the first two paradigms favored the ADCON-T/N-treated nerves. Connective tissue proliferation or scarring was also reduced in the treated nerves compared with the control nerves. There was no difference or trend in the nerve action potential studies or in the nerve fiber counts. Not surprisingly, there were no additional differences in severed and sutured nerves other than in the amount of surface or epineurial scar involved. These observations suggest that a material such as ADCON-T/N might be of value in situations in which maintaining as smooth and unscarred a neural surface as possible to permit the nerve to glide or move with joint motion, such as that of the wrist, the elbow, or the knee. Thus, the manufacturer already plans on a multicenter trial in patients undergoing carpal tunnel release for carpal tunnel syndrome. The outcome of such clinical studies, especially if a large number of patients are gathered and they are performed in a controlled fashion, should be interesting.

It does need to be emphasized and reemphasized that application of this or similar materials to the surface of nerve has no effect at all on intraneural scar. The latter is quite prevalent after most serious injuries to human nerve. Such connective tissue proliferation not only often thwarts functional axonal regeneration but also plays a role in the formation of painful neuromas. Other strategies need to be devised to control intraneural scarring.

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We all are interested to hear about a substance that may reduce postoperative scarring. This controlled and blinded experimental study deals with the inhibitory influence of a carbohydrate polymer gel (ADCON-T/N) on scar formation after external neurolysis (Procedure I, n = 9), abrasive injury (Procedure II, n = 10), and transection and epineurial suture of rat sciatic nerve (Procedure III, n = 9), compared with animal groups with either no gel or a control gel. After the application of ADCON-T/N, there was significantly less extraneural scarring than in the other two animal groups. The substance does not interfere with nerve regeneration after suture, does not damage nerve fibers, and is not toxic. However, it has the same disad-

vantages as any other substance that reduces scar formation; it may impair wound healing. Although skin closure was never affected, the muscle fascia was open in 8 of the 19 animals 4 weeks after Procedures I and II (nothing is said about the animals after suture). Whether this can be avoided with the use of smaller gel volumes, as assumed by the authors, has yet to be shown. The polymer is thought to form an inhibitory boundary to invading fibroblasts. It would be interesting to learn whether the application of ADCON-T/N will interfere with the revascularization after nerve grafting.

There remains the enduring skepticism of whether these results from rat sciatic nerve can be transferred to humans. This would be difficult to prove, because it would necessitate a reinspection of the surgical site and comparison with a similar group of patients without the gel applied. A helpful compromise may be a similar experimental study in primates. This is worthwhile, regarding the potential broad application of a substance which is said to prevent scar formation around nerve tissue. In the meantime, however, is it already justified to apply the gel, which, to the best of my knowledge, will be rather expensive?

Hans-Peter Richter
Ulm, Germany

ANNOUNCEMENT

Foundation for International Education in Neurosurgery Update and Request for Volunteers

The Foundation for International Education in Neurosurgery (FIENS) continues to develop activities that are primarily designed to foster education in developing areas of the world. These initiatives are coordinated with the activities of the World Federation of Neurosurgical Societies, which, through its Education Committee, has sponsored a number of regional educational programs for neurosurgeons in developing areas. The Foundation assists in providing faculty for these larger educational efforts. The major current activities of FIENS consist of programs in Africa. The Foundation has worked with neurosurgeons in Ghana in an attempt to help develop an indigenous neurosurgical training program there. This has involved sending senior level residents and faculty for varying periods of time, usually 3 to 6 months for residents and 2 to 4 weeks for faculty support. FIENS has supported a program in Zimbabwe to provide neurosurgical assistance to the program already in place in that country, and it has supported an African trainee, who currently is a resident at Yale, in the initial steps to develop a neuroscience initiative for Southern Africa. She has been a liaison with the Panafrican Neurosurgical Association, which strongly supports this concept, and we anticipate rapid development. Another new project involves a Senior Neurosurgical Interchange with Peru under the guidance of Dr. Anselmo Pineda and the Peruvian-American Neurosurgical Society. Opportunities exist for volunteer neurosurgeons to go to Peru and visit and work in a number of different medical centers around the country. FIENS maintains a roster of neurosurgeons interested in serving as volunteers. For volunteer experiences of at least 4 to 6 weeks, the Foundation will support the volunteer's air travel expenses. The host country is ordinarily able to underwrite most of the volunteer's expenses incurred at work. Further information and volunteer request forms can be obtained from the Office of the Secretary of the Foundation, Dr. David Fairholm: Division of Neurosurgery, Department of Surgery, Room 3100, 910 W. 10th Avenue, Vancouver, BC, Canada V5Z 4E3.