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Benefit of Chondroitinase ABC on Sensory Axon Regeneration in a Laceration Model of Spinal Cord Injury in the Rat

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Abstract

Background—Chondroitin sulfate proteoglycans (CSPGs) are upregulated in the spinal cord following spinal cord injury (SCI) creating a molecular barrier inhibitory to axon growth. Chondroitinase ABC (ChABC) degrades CSPGs *in vitro* and *in vivo*.

Methods—We studied whether intrathecal (IT) ChABC promotes axonal regeneration in a laceration model of SCI. Three groups of Sprague Dawley rats were used: control and rats treated with low dose and high dose IT ChABC. CSPG breakdown products were measured by 2-B-6 expression, and intact CSPGs by CS-56 expression. Sensory axonal regeneration was traced following CTB injection into the median, ulnar, and sciatic nerves.

Results—CS-56 expression was downregulated and 2-B-6 expression was increased in the groups treated with IT ChABC but not in the control. Laminin and GFAP immunoreactivity was unaltered in the ChABC groups. The number of axons growing into the scar was 3.1 times greater ($p < 0.01$) in the high dose ChABC group and 2.1 times greater ($p < 0.01$) in the low dose group compared to the controls. The length of axonal growth following high and low dose ChABC was 9.9 ($p < 0.01$) and 8.3 ($p < 0.01$) times greater, respectively, than in the control group. Axons extended across the lesion gap and into the distal spinal cord stump in 2/8 (low dose) and in 3/9 (high dose) rats compared to none in the control group.

Conclusions—IT ChABC administration caused a slight decrease in CSPGs in the scar following a laceration SCI with a minimal increase in sensory axonal regeneration into and across the laceration gap.

Keywords

Chondroitinase; Spinal cord injury; Spinal cord laceration; Spinal cord regeneration; Axonal regeneration; Glial scar; Chondroitin sulfate proteoglycan; Extracellular matrix; Vibraknife; Sensory axon

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1. Introduction

Spinal cord axons are incapable of regeneration following spinal cord injury (SCI) in adult mammals, thus, axon regeneration is one strategy currently being studied to treat SCI. Failure of axon regeneration is attributable in part to the intrinsic incompetence of mature neurons to grow as well as to the unfavorable molecular and physical environment encountered by axon growth cones. Growth cones of severed axons grow in response to inhibitory and permissive signals in the nervous system, respectively [28]. Following injury, the spinal cord scar consists of a mixture of growth-promoting molecules such as laminin [10,39], cell adhesion molecules [57], and growth factors in combination with growth-inhibitory molecules. Unfortunately, inhibitory molecules dominate, resulting in a net inhibition of axon growth.

Several inhibitory molecules have been identified including myelin derived Nogo [18,19,21, 52], oligodendrocyte myelin glycoprotein (OMgp) [34,58], myelin-associated glycoprotein (MAG) [13,38,41,56], and membrane-bound semaphorins (Sema) [20,26]. The family of chondroitin sulfate proteoglycans (CSPGs) serves as another major inhibitory molecule. Following the atraumatic micro-transplantation of dorsal root ganglia (DRG) neurons into the adult rat CNS [11], CSPGs significantly inhibit their neurite outgrowth, to an even greater extent than components of CNS white matter. Abortive attempts of neurite spouting from transplanted DRG neurons correlate with increased proteoglycan levels within the extracellular matrix at the transplant interface [14]. Sensory neuron implantation into adult CNS white matter also leads to inhibition of axon regeneration at CSPG-rich sites [2]. Members of the CSPG family inhibit neurite outgrowth *in vitro* [15,48] and undergo altered expression following spinal cord and brain injuries *in vivo* [16,36,37,44]. Certain CSPGs, specifically, NG2, neurocan, brevican, and versican, are upregulated in the glial scar following SCI [9,12,28, 31]. CSPG upregulation adjacent to the CNS injury inhibits axon regeneration, axon sprouting, and growth [45,50,62].

CSPG receptors have not been identified on growth cones [47] and, therefore, ChABC degradation of several CSPG components may provide a strategy for axon regeneration. Such treatment has promoted neurite outgrowth *in vitro* [39,40] as well as CNS axonal regeneration *in vivo* [42], including improved locomotor function and proprioception in rats [5].

Experimental SCI in rats is complicated by the formation of post-traumatic spinal cord cysts that create a physical barrier to axonal regeneration [63]. Post-traumatic cysts occur following intramedullary hemorrhage, ischemia, and cell death. Our model of SCI was created by a precisely controlled laceration using the Vibraknife that contributed to the reduction in size of the post-traumatic cyst [64]. In this study, we have evaluated the effect of IT administration of ChABC on the digestion of CSPG and sensory axon regeneration.

The study of a laceration SCI is important as this type of injury represents a major problem clinically. Twenty-five percent of all SCI are caused by lacerations (knife, gunshot wound, sharp bone fragments), thus our model is clinically significant [6]. We have created a dorsal spinal cord hemisection and, therefore, only sensory axon regeneration will be evaluated.

2. Materials and Methods

Surgical procedures and animal care adhered to the guidelines outlined by the Care and Use of Laboratory Animals (Institute of Laboratory Animal Resources, National Research Council, 1996) and the University of Louisville Institutional Animal Care and Use Committee (IACUC).

2.1. Surgical model

Twenty-two adult female Sprague-Dawley rats (200–225 g) were anesthetized with sodium pentobarbital (50 mg/kg i.p.) and treated with prophylactic gentamicin (0.03 mg/kg IM). A C3-5 laminectomy was performed, the dura opened, and the C3 vertebra immobilized by stainless steel fixation clamps attached to the Vibraknife base. A C3 dorsal hemisection using the Vibraknife was made with a 3.6 mm blade which covered the width of the spinal cord. The laceration was created by elevating the rat spinal cord to the vibrating blade. This produced a laceration 1.5 mm deep [51,64] that extended across the central canal, effectively transecting all fibers in the dorsal funiculus. Lubrication at the blade-spinal cord interface was maintained using sterile Hank's Balanced Salt Solution (HBSS).

A polyethylene (PE-10) catheter (Becton Dickinson, Sparks, MD) was placed through a dural opening at C4-5 (Fig. 1A) and threaded rostrally in the subarachnoid space to reach the C3 spinal cord hemisection (Fig. 1B). The PE-10 tubing was heated and tapered to obtain a 100 μ m outer diameter (O.D.) at its tip. The entire catheter (80 mm long) contained a volume of 3 μ l. The tubing was heparinized prior to placement into the subarachnoid space to prevent occlusion by blood. The extradural end of the tube was coiled in the submuscular area and stabilized with three 6-0 sutures (Ethicon, Somerville, NJ) tied to the overlying muscle and passed transcutaneously through a separate skin incision on the back of the animal. Dural closure at C3 was performed using a 10-0 monofilament nylon suture (Ethicon, Somerville, NJ). The dural flap was curved to prevent the dural closure at C3 from being superimposed over the spinal cord laceration gap (Fig. 1C). The dural repair at C3 and the dural exit of the PE-10 tubing at C4-5 were sealed with fibrin glue (Baxter, Glendale, CA). The tip of the tubing was visualized transdurally at the laceration site. Muscle and skin were subsequently closed. The exposed end of the PE-10 tubing was occluded by a stainless steel plug and opened only for injections into the intrathecal space.

2.2. Treatment

Rats were anesthetized with 2.5% halothane gas administered by a custom-made rat anesthesia mask. On the day of surgery and alternate days for a total of 5 injections (day 0, 2, 4, 6, and 8), each animal received an intrathecal injection through the catheter. Six μ l of the test solution or control solution was injected IT followed by 3 μ l saline were injected into the tubing using a Hamilton syringe. Penicillinase (30 μ g/ml) was used as the control (n=5). Chondroitinase ABC was obtained from Sigma-Aldrich (St. Louis, MO) (C3667) and was pure, protease free and supplied in vials (0.5 mg = 5.15 units). Each injection of ChABC was freshly prepared on the day of administration. Saline (167 μ l) was added to each vial, creating a solution of 0.18 units/ 6 μ l (high dose). The low dose solution was formulated by diluting this to .06 units/ 6 μ l. High dose ChABC (0.18 units/treatment; n=9) and low dose ChABC (0.06 units/treatment; n=8) were injected. During the first treatment (day 0), the dura bulged slightly while undergoing the injection without transdural extravasation or blockage of the PE-10 tubing. Rats were housed for 5 weeks following surgery.

2.3. Sensory axon tracing with Cholera Toxin B (CTB)

One week prior to being sacrificed, rats were anesthetized with pentobarbital (50 mg/kg i.p.). Both median and ulnar nerves close to the brachial plexus and sciatic nerves were exposed, and CTB (1% in distilled water; List Biological Laboratories, Campbell, CA) was injected slowly via a 30 gauge needle into both sciatic (5 μ l each), median (2 μ l each), and ulnar nerves (2 μ l each). The needle was kept in the nerve for 2 minutes following CTB injection to prevent extravasation prior to removal. The skin was then closed.

2.4. Spinal cord processing

Five weeks after laceration of the spinal cord, rats were deeply anesthetized with sodium pentobarbital (100 mg/kg i.p.), exsanguinated, and transcardially perfused with 100 ml of 0.1 M phosphate buffered solution (PBS) followed by 500 ml of 4% paraformaldehyde in 0.1 M PBS, pH 7.4. Spinal cords were removed and post-fixed for 2–3 hours in the same solution followed by cryoprotection in 30% sucrose at 4°C for 48 hours. The spinal cords were embedded in a TBS mounting medium (Triangle Biomedical Sciences, Durham, NC) and cut into 20 µm thick sagittal sections.

2.5. Immunohistochemistry and Immunoperoxidase stains

Sections were placed on a slide warmer at 33°C for 10 minutes and washed in 0.01M PBS three times for 10 minutes each. Sections were then blocked in 10% donkey serum in 0.01M PBS containing 0.3% Triton X-100 for 30 minutes. After a 10 minute rinse with PBS, slides were incubated overnight in a humidified tray at room temperature with the following primary antibodies: monoclonal SMI 31 antibody (Sternberger Monoclonals Incorporated, Lutherville, MD 1:1000) to identify the 210 kDa neurofilament protein; monoclonal anti-chondroitin sulfate antibody produced in the mouse (clone CS-56) (Sigma-Aldrich, St. Louis, MO, 1:200) to recognize the glycosaminoglycan (GAG) portion of intact chondroitin sulfate proteoglycan (CSPG); rabbit anti-NG2 chondroitin sulfate proteoglycan (Chemicon, Temecula, CA 1:200) to identify the NG2 proteoglycan; monoclonal anti-proteoglycan clone 2-B-6 (Seikagaku Corporation, Tokyo, Japan 1:200) to recognize 4-sulfated and 6-sulfated chondroitin and dermatan sulfate, products of ChABC enzymatic digestion; polyclonal rabbit anti-glial fibrillary acidic protein (GFAP; Biomedical Technologies, Stoughton, MA, 1:100) and polyclonal rabbit anti-laminin antibody (Sigma-Aldrich, St. Louis, MO, 1:200) to identify other possible scar components following digestion of the scar after ChABC treatment; and goat anti-cholera toxin B subunit (List Biological Laboratories Inc., Campbell, CA, 1:1000) to identify the CTB subunit following axonal tracing.

Slides were subsequently washed with 0.01M PBS three times for 10 minutes each at room temperature and incubated in secondary antibodies (all secondary antibodies were purchased from Jackson ImmunoResearch Laboratories, Inc., West Grove, PA) in a light protected tray on a shaker for 1½ hours at room temperature. The following secondary antibodies were used: Texas Red dye-conjugated AffinPure F(ab')₂ Fragment Donkey Anti-Mouse IgG(H+L), Fluorescein (FITC)-conjugated AffinPure F(ab')₂ Fragment Donkey Anti-Mouse IgG(H+L), Fluorescein (FITC)-conjugated AffinPure F(ab')₂ Fragment Donkey Anti-Goat IgG(H+L), Rhodamine RedTM-X-conjugated AffinPure F(ab')₂ Fragment Donkey Anti-Rabbit IgG(H+L). All secondary antibodies were diluted with 5% donkey serum in 0.01M PBST. Sections were washed 6 times with 0.01M PBS for 5 minutes each at room temperature and coverslipped with anti-fade mount medium (Biomedica Corp, Foster City, CA). All slides were stored at 4°C prior to image analysis.

Immunoperoxidase staining was superimposed on CTB tracing as a sensitive method to identify sensory fibers as described [1,57]. After incubation with a goat anti-CTB antibody overnight, sections were developed with an anti-goat ABC (Vector Laboratories, Burlingame, CA) according to the manufacturer's recommendations. Following three washes for 10 minutes each at room temperature with 0.01M PBS, sections were incubated for 5–10 minutes (depending on the speed of color development) with 0.05% DAB and 0.003% H₂O₂ in 0.01M PBS. Slides were further washed three times for 10 minutes each with 0.01M PBS, dehydrated, and coverslipped.

Evidence of axonal regeneration in the scar and across the lesion gap was recorded and quantified using the Neurolucida system (MicroBrightField, Colchester, VT) that was attached

to an Olympus BX60 microscope. CTB-labeled axons within the lesion gap were counted using the grid eyepiece. The number of CTB-labeled fibers encountered within the 0.2 mm segment of the scar parallel to the lesion interface line was counted (Fig. 2: top left). The lengths of regenerated CTB-labeled fibers were measured using an average of the 5 longest regeneration fibers from the caudal spinal cord-interface line on each section (Fig. 2: top right). A total of 5 sagittal sections (#1, 4, 7, 10, and 13 out of 14 sections) within a 0.3 mm thick region in the middle of the dorsal column site was measured.

2.6. Statistical analysis

Comparison among the control, low and high dose groups in the number and length of regenerated axons was performed using one-way ANOVA followed by Tukey HSD *post hoc* tests [21]. All values are expressed as mean $\pm$ SD.

3. Results

All dorsal hemisections of the spinal cord at C3 by the Vibraknife were precise, reproducible, and without evidence of contusion. Rats were beginning to walk and support their body weight two days post-operatively with subsequent rapid improvement of locomotion. Their gait appeared normal 5 weeks postoperatively, however, formal locomotor testing was not performed. One animal in the low dose ChABC group was excluded due to self-mutilation of the forepaw. The laceration injury using the Vibraknife combined with careful hemostasis and dural repair resulted in a narrow scar and clean spinal cord-laceration interfaces. There were no significant cystic cavitations observed at the lesion site which permitted straightforward evaluation of axonal regrowth.

3.1. Immunohistochemistry

Five weeks after SCI and 3½ weeks after completion of IT infusion, control animals demonstrated abundant expression of NG-2 and CS-56 (Fig. 3A,C). 2-B-6 immunoreactivity was absent in the control group (Fig. 3E). Following IT administration of ChABC, CSPG expression was decreased in the margins of the laceration, particularly with the administration of high dose ChABC. NG-2 (Fig. 3B) and CS-56 (Fig. 3D) were both mildly downregulated in the ChABC-treated groups. 2-B-6 immunoreactivity was apparent in both ChABC treated groups, reflecting the presence of CSPG cleaved products (Fig. 3F). IT ChABC infusion did not influence GFAP-IR in either reactive astrocytes or laminin-IR in the scar (Fig. 4). GFAP-IR staining was observed primarily in the spinal cord in proximity to the gap, while laminin-IR was present in the lesion gap and surrounding vascular structures.

3.2. Sensory axonal regeneration after ChABC treatment

There was an increase in the number of axons growing into the scar in the ChABC treated groups measured at a line 0.2 mm distal to the caudal spinal cord-laceration interface. The number of regenerating axons was 2.1-fold greater in the low dose ChABC-treated group and 3.1-fold greater in the high dose ChABC-treated group compared to the control group ($p < 0.01$) (Fig. 2: lower left). There was no statistical difference in the length of axons between the treated groups. The average length of most axons measured from the caudal spinal cord-laceration interface was 8.3 fold longer using low dose ChABC and 9.9 fold longer with the high dose compared to that of the control group (Fig. 2: lower right). Two animals (2/8) from the low dose ChABC group and three animals (3/9) from the high dose ChABC group demonstrated axon growth across the lesion gap that reached the rostral spinal cord-laceration interface (Fig. 5 B,D). This finding was not observed in any of the control animals (Fig. 5 A,C).

4. Discussion

CSPGs are a family of extracellular matrix molecules whose upregulation is observed in the spinal cord for several months following SCI [28,31,54,62]. Of the proteoglycans, NG2, neurocan, versican, and brevican reach peak levels 2 weeks following SCI, whereas phosphacan increases by 4 weeks and reaches a peak at 2 months [28]. CSPGs are major inhibitors of neurite outgrowth *in vitro* [15] and inhibit axonal regeneration following CNS injury *in vivo* [30]. Current strategies to promote axonal regeneration following SCI have focused on the suppression of the inhibitory influence within the scar [35,60]. ChABC has been shown to promote axonal regrowth in the CNS by removing a GAG side chain of the CSPGs, thereby attenuating their inhibitory nature [42,65]. Bradbury and colleagues reported an improvement in locomotor and electrophysiological function following the administration of intrathecal ChABC in the rat that may have been due to sensory and motor axonal regeneration [5]. They attributed the beneficial effects of ChABC to the reduction of CSPGs and to an increase of GAP-43 in the dorsal root ganglia.

The present study used a similar intrathecal ChABC administration design as that utilized by the Bradbury group although the spinal cord lesion differed. They used fine watchmaker's forceps to crush and sever axons in the dorsal columns that preserved the midline dorsal blood vessel [4,5]. We transected all the axons by a precise dorsal hemisection lesion using the Vibraknife [51,64] to ensure that no axons were preserved in the dorsal column. The Vibraknife represents an ideal model for the evaluation of axonal regeneration due to its ability to create a very narrow scar with clean spinal cord interfaces. Furthermore, the Vibraknife makes a predictable dorsal hemisection that reduces the variability of the lesion [51,64]. The blood-spinal cord barrier is disrupted following a laceration SCI, and scar tissue between the two spinal cord-laceration interfaces produces a CSPG gradient with the highest concentration at the epicenter and a decreasing gradient in the injury penumbra [50]. For this reason, we placed the tip of the ChABC catheter directly over the spinal cord laceration gap and performed several manipulations to ensure that the catheter tip remained at that site throughout the experiment. During each injection, there was no resistance to the test solution or saline being injected into the subarachnoid space (SAS), thus we were assured that the solutions were reaching the lesion gap.

This study does not closely reproduce clinically significant spinal cord lesions because laceration SCI are often complex lesions that include tearing the spinal cord by a sharp object (bullet, knife, or bone), intramedullary hemorrhage, contusion, and possible extrinsic pressure against the damaged spinal cord. The model we have described created a simple lesion which removed confounding pathological effects that would greatly increase the difficulty in assessing the effects of ChABC. The lesion produced by the Vibraknife simplifies our ability to evaluate the efficacy of therapies that have minimal therapeutic effects.

In both a previous study without the use of ChABC [64] and in the present study, we demonstrated that sensory axons may grow into the scar, but few traverse it. These findings were also reported by others [27]. Cafferty and colleagues demonstrated transgenic evidence that CSPGs restrict axonal growth in the CNS after traumatic injury [7]. They noted a significant encroachment of corticospinal axons into the glial scar, however, did not detect axon regeneration across the lesion or a return of motor function. In the current study, a small number of sensory axons extended across the scar into the distal spinal cord on a small scale (micrometers to millimeters length) in 3/9 instances (high dose ChABC) and in 2/8 occasions (low dose ChABC). Nonetheless, we failed to observe the robust axonal regeneration noted by Bradbury and colleagues [5] exhibited by bundles of axons that grew 2 mm through the lesion and several fibers that extended 4 mm beyond the lesion. IT ChABC administration following SCI in this study played a positive, albeit limited, role in sensory axonal regeneration into the

scar. In spite of a reproducible spinal cord laceration, axon-crossing was unpredictable and only detected in a minority of animals. Although the optimal dose and frequency of intrathecal administration of ChABC is unknown, the ability of axons to cross the gap was likely not related to the dose of ChABC. There was no significant difference in the extent of axonal growth using low dose or high dose ChABC in this study despite treating with a 3-fold greater dose than that used by the Bradbury group. Our findings are similar to those described by Steinmetz and colleagues [53] who observed only minimal regeneration when ChABC was used alone. However, they were able to induce exuberant axonal growth using a combination of IT ChABC along with stimulation of the intrinsic growth properties of the sensory neuron. They accomplished axon growth using an inflammatory induced preconditioning lesion by injecting zymozan into sensory ganglia [53].

Behavioral or electrophysiological studies were not performed following SCI since the extent of axonal regrowth was minimal, and furthermore, there were virtually no locomotor deficits recognized after 48 hours using our precise dorsal hemisection lesion. Thus, there would be no benefit in performing behavior studies. Regrowth of sensory fibers was confined within a few millimeters at or adjacent to the caudal spinal cord-laceration interface. The administration of intrathecal ChABC alone was unable to stimulate a major degree of axonal regeneration despite slightly decreased CS-56 and NG-2 levels and a minimal increase in 2-B-6 levels. The study by Bradbury and colleagues [5] demonstrated significant 2-B-6 immunoreactivity in rats treated with ChABC surrounding the lesion site and in white matter radiating 4 mm rostrally and caudally, reflecting the presence of digested CSPGs. 2-B-6 immunoreactivity was not observed in the control animals in that study.

Failure of robust axonal regeneration following intrathecal ChABC administration may also be related to the incomplete digestion of CSPGs. The ability of ChABC to remove the GAG side chain from CSPGs, while failing to completely cleave the GAG from the protein core may explain the limited role that ChABC plays in axonal regeneration [8]. Furthermore, the residual core (protein stub) also has an inhibitory effect on axon regeneration [50]. Digestion of the GAG side chain glycoprotein may reduce the size of the scar, thus, promoting axonal regeneration and functional recovery [25]. A DNA enzyme that is capable of further reduction of the GAG side chains stimulates axonal growth following SCI to a greater degree than by using ChABC alone [22].

In addition to utilizing ChABC to decrease the inhibition of CSPGs, studies have focused on blocking specific myelin-based proteins that are inhibitory to regeneration, including NoGo [49], myelin associated glycoprotein (MAG) [41], and oligodendrocyte-myelin glycoprotein (Omgp) [59]. The exogenous delivery of the neurotrophic factor neurotrophic 3 (NT3) encouraged the appearance of extensive sprouting within and beyond the lesion epicenter [4]. ChABC has been used in combination with brain-derived neurotrophic factor (BDNF) to stimulate sprouting of the retinal ganglion cell body [55]. Combinatorial therapies using ChABC in conjunction with NT3, cAMP, gene therapy, or cellular transplantation [17,29,33, 43,46] may suppress the inhibitory environment after SCI and enhance regeneration.

The glial scar with its complex interactions of growth-inhibiting components poses a formidable physical barrier to regeneration after SCI. The CSPGs significantly contribute to the molecular inhibitory effects and, thus the physical and molecular barriers serve as targets for modulation. Barritt and colleagues demonstrated that degrading CSPGs after SCI promotes sprouting of intact (serotonergic and primary afferent) and injured (corticospinal) systems in the spinal cord [3]. Furthermore, treatment with ChABC induced plasticity of these fibers in both degenerating and denervated areas that may serve as a mechanism for functional recovery. Houle and colleagues developed an autologous peripheral nerve “bridge” in association with application of ChABC in the extracellular matrix following a cervical hemisection lesion

[24]. They confirmed significant improvement in behavioral studies of forelimb function in the PN-bridged/ChABC-treated group of rats compared to the controls. In addition, transection of the PN graft decreased the functional advancements, suggesting that the regenerating axons are valuable elements in promoting functional activity.

In the present study, IT administration of ChABC played a positive, though limited, role in favor of neurite growth in the dorsal column. The impact of ChABC may be enhanced by combinatorial strategies that synergistically will promote regeneration. Several hurdles have yet to be overcome, including robust axonal growth across the scar, elongation of growth cones to the target, and the creation of synaptic contacts. Functional recovery following a SCI serves as the ultimate goal and requires a greater understanding of the mechanical and biochemical mechanisms that interplay after such an injury. In addition, the development of more refined tools and novel animal models are required to attain this objective.

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Abbreviations

CSPGs, Chondroitin Sulfate Proteoglycans
 SCI, Spinal Cord Injury
 ChABC, Chondroitinase ABC
 IT, Intrathecal
 OMgp, Oligodendrocyte Myelin Glycoprotein
 MAG, Myelin-Associated Glycoprotein
 DRG, Dorsal Root Ganglia
 IACUC, Institutional Animal Care and Use Committee
 HBSS, Hank's Balanced Salt Solution
 OD, Outer Diameter
 CTB, Cholera Toxin B
 PBS, Phosphate Buffered Solution
 GAG, Glycosaminoglycan
 GFAP, Glial Fibrillary Acidic Protein
 NT3, Neurotrophic 3
 BDNF, Brain-Derived Neurotrophic Factor
 SAS, Subarachnoid Space

Figure 1. Surgical procedure: (A) Dura was opened at C3 and a catheter (dotted line) threaded in the subarachnoid space from C5 to C3. (B) Dorsal hemisection using the Vibraknife with removal of the blood clot, and (C) Closure of dura. Arrowheads demarcate the dural margins and arrows point to the catheter.

Figure 2.

Sensory axonal regeneration in a 0.3 mm wide spinal cord section in the midline of the dorsal column composed of 14 mid-sagittal slices each 20 μ m thick. Regenerating axons extending from the caudal spinal cord-laceration interface are demonstrated within the triangles. The number of regenerating axons was counted in the highlighted line located 0.2 mm from the caudal spinal cord-laceration interface (top left). The length of the regenerating axons in the lesion gap was measured as the average distance of the axons from the caudal spinal cord-laceration interface (top right). The graphs demonstrate the difference in axon numbers (lower left) and axon length (lower right) in the control, low, and high dose ChABC groups (** indicates $p < 0.01$).

Figure 3.

Expression of CSPG and products of digestion in the lesion cavity 5 weeks after treatment. Without treatment with ChABC, (A) NG-2 proteoglycan, and (C) CS-56 antibody are abundant. Treatment with ChABC resulted in a mild decrease in expression of both (B) NG-2 and (D) CS-56 at the site of injury. (In C and D, arrows indicate the thickness of the CS-56 immunolabelled region). 2-B-6 immunoreactivity was not observed in the (E) control animals, however, (F) 2-B-6 was mildly upregulated at the lesion site following ChABC treatment. (gap outlined by dotted lines)

Figure 4. Laminin and GFAP immunoreactivity was not noticeably different in the control (A, B), low (C, D), or high (E, F) dose ChABC-treated animals.

Figure 5.

Regenerating sensory axons at the spinal cord-laceration interface. (A) No fibers crossed the lesion gap in control rats. (B) Long distance regenerating fibers crossed the lesion gap in the rats treated with high dose IT ChABC (arrowheads). (C) DAB stained axons were not observed across the lesion gap in the control group. (D) DAB stained axons regenerated more than 2 mm beyond the rostral spinal cord-laceration interface.