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An injectable, biodegradable hydrogel for trophic factor delivery enhances axonal rewiring and improves performance after spinal cord injury

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Abstract

The failure of long descending pathways to regenerate after spinal cord injury (SCI) is generally attributed to inhibitory proteins associated with the glial scar and myelin, or to the loss of neurons' intrinsic capacity to grow, or both. Here, we describe the use of hydrogels as a novel way to deliver molecules that promote axon growth in the injured CNS of adult rats. This method utilizes an injectable liquid polymer solution that crosslinks into a biodegradable, water-swollen hydrogel when photoactivated under visible light. Neurotrophin-3 (NT-3), a trophic factor known to act on corticospinal tract (CST) projection neurons, was used as a prototypic pro-regenerative molecule. Hydrogel release properties were established in vitro to ensure long-term, sustained NT-3 release over a 2-week period; this avoided the need for multiple injections or minipump implantation. Hydrogel/NT-3-treated animals showed improved recovery in the open-field BBB test and in a horizontal ladder walk test compared to controls implanted with hydrogel alone. At the anatomical level, hydrogel/NT-3-treated animals showed far greater axon growth than controls in two major descending pathways for motor control, the CST and the raphespinal tract. In the case of the CST, much of the NT-3-induced growth represented collateral branching from undamaged ventral CST fibers. These studies demonstrate the effectiveness of hydrogel technology as a clinically feasible delivery system to promote regeneration and enhance functional outcome after spinal cord injury.

Keywords: Axon regeneration; Spinal cord injury; Hydrogels; Plasticity; Neurotrophins; NT-3; Corticospinal; Raphespinal; Functional recovery

Introduction

The failure of injured axons to regenerate in the mature central nervous system (CNS) has devastating consequences for victims of spinal cord injury (SCI). This failure is generally attributed to inhibitory proteins associated with myelin (Schwab et al., 1993) and the glial scar (Silver and Miller, 2004) and to a loss of neurons' intrinsic growth capacity (Ramer et al., 2000). In this study, we have explored a novel approach of using injectable polymer networks as a platform for the controlled delivery of pro-regenerative molecules.

Methods for trophic factor delivery in the injured spinal cord have generally included direct injection (Schnell et al., 1994), genetically modified cells that produce the factors of interest (Cao et al., 2005; Grill et al., 1997; Liu et al., 2000; Tuszynski et al., 2003), adenoviral vectors (Zhou et al., 2003), osmotic minipumps (Coumans et al., 2001; Giehl and Tetzlaff, 1996; Ramer et al., 2000), and fibrin glue (Cheng et al., 1998). Because of the limitations inherent to these approaches, particularly for clinical application, we sought to design a delivery system that: (1) provides constant and tailorable delivery of one or more growth factors to the precise site where needed; (2) reduces the possibility of host–graft rejection, as may occur with the use of live tissue or cell preparations; (3) does not involve viral vectors, which may induce an inflammatory response and which may require prolonged delays in achieving high levels of gene expression; and (4) does not involve the use of devices that can malfunction or

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cause infections (e.g., pumps or catheters). To this end, we have designed an injectable hydrogel delivery system for sustained release of neurotrophin-3 (NT-3), a prototypic growth factor. Hydrogels are water-swollen insoluble polymer networks that can be made degradable by introducing the appropriate linkages into the polymer's backbone; when properly designed, these can deliver molecules with desired release kinetics over long periods. Although hydrogels have recently begun to be used as scaffolds for axon regeneration (Tsai et al., 2004), their use in delivering molecules to promote regenerative growth in injured CNS has not been explored. Our results show that a hydrogel designed for sustained NT-3 release to the area of injury enhances axonal rewiring and functional outcome after SCI.

Materials and methods

Hydrogel synthesis and characterization

The degradable macromer (acrylated PLA-b-PEG-b-PLA) was synthesized in a two-step reaction as described (West and Hubbell, 1995). Briefly, degradable lactic acid units were added to the hydroxyl end groups of poly (ethylene glycol) (PEG, MW ~4000 Da, Polysciences) via a ring opening polymerization of D,L-lactide (Polysciences) in the presence of stannous 2-ethyl hexanoate (Sigma). After dissolving in methylene chloride and adding triethylamine, acryloyl chloride (1:10 in methylene chloride) was added dropwise and the reaction continued for 36 h. The final product was precipitated in cold ethyl ether, filtered, and vacuum-dried. The macromer structure was characterized with ^{1}H NMR and showed nearly 100% acrylation and ~2.7 lactic acid units per side. All materials were obtained from Aldrich unless noted otherwise. For polymerization, the macromer was dissolved in PBS containing 0.02% eosin and 200 mM triethanolamine. NT-3 (25 μg/ml in PBS with BSA: R&D Systems, Minneapolis, MN) was added to a solution of photoinitiator and 10 wt.% macromer solution prior to photopolymerization. For in vitro release analysis, 50 μl of the solution was pipetted into a 5 mm diameter cylindrical mold and exposed to light for 60 s. Polymerized hydrogels (n = 3 per composition) were placed in Eppendorf tubes containing 1 ml PBS and placed on an orbital shaker. PBS containing released NT-3 and degradation products was removed at various time points, and NT-3 concentration was determined using DuoSet ELISA development kits (R&D Systems). Results are reported as cumulative % release as a function of time.

Spinal cord hemisection, BDA anterograde tracing, and NT-3 administration

Male Sprague–Dawley rats (n = 23, 250–300 g, Charles River Laboratories) were weighed, anesthetized by isoflorane inhalation followed by i.p. ketamine (75 mg/kg) and medetomidine (Domitor, 0.5 mg/kg), and then placed in a stereotaxic frame. A craniotomy was performed to expose the right sensorimotor cortex, and BDA (Molecular Probes: 350 nl/injection, 10% wt/vol in sterile saline) was injected into 18 pre-determined points in the sensorimotor cortex using a Nanoject apparatus (Drummond Scientific, Broomal, PA) under stereotaxic guidance (Benowitz et al., 1999) at depths of 0.5, 1.0, and 2.0 mm from the cortical surface. The craniotomy was covered with Gelfoam and the skin sutured. Rats then underwent a laminectomy at the T8 level, and the dorsal spinal cord was transected at a depth of 1.5 mm (using a Beaver Blade: BD Ophthalmic Systems, Waltham, MA), severing the dorsal and dorsolateral aspects of the CST. Ten microliters of prepolymer solution (i.e., macromer dissolved in PBS containing photoinitiator), with or without NT-3 (1 μg), was injected into the lesion cavity and immediately exposed to light (Curing Light 2500, 3M Dental Products) for 60 s to polymerize the hydrogel. The rapid gelation time prevents significant dispersion of the solution into the intrathecal space. The lesion cavity was covered with Gelfoam and sutured closed. All procedures were approved by the Children's Hospital Institutional Animal Care and Use Committee.

Behavioral assessment

Open-field locomotion

The Basso–Beattie–Bresnahan (BBB) locomotor score was used to evaluate open-field behavior (Basso et al., 1995). Rats were scored at day one and then weekly for 6 weeks by two blinded observers. One animal with a BBB score ≥8 at day one was excluded from the study, and in this case, histology confirmed incomplete CST transection. Several other rats were excluded from the study on the basis of post-mortem histology (n = 1, see below) or as a result of autophagia (n = 3).

Grid walk

Animals were tested 1 day post-surgery and once a week thereafter for 6 weeks on a ladder 1 m long elevated 30 cm above the ground, with the home cage at the far end (Metz and Whishaw, 2002). The spacing between rungs was irregular to prevent animals from learning a repetitive motoric pattern. A rectangular mirror was placed on one side of the ladder at a 45° angle to ensure that both sides of the animal could be seen simultaneously. All trials were videotaped (Canon ZR 90) and scored by a blinded observer using frame-by-frame analysis. The total number of steps that an animal used to cross the ladder was counted, and each step was categorized as an error or a correct step. An error represents a total miss of the rung or any kind of foot slip and was scored using the rating scale described previously (Lu et al., 2004). Coordination between hindlimbs and forelimbs was not evaluated. The number of correct steps was averaged for right and left hindlimbs, and the data are presented as percent correct steps over total steps.

Histology

Six weeks after the initial surgery, animals were anesthetized as above and perfused transcardially with heparinized saline (100 ml, 100,000 units/l) followed by 10% formalin (300 ml). The brain and spinal cord were removed, postfixed for 24 h, and impregnated with 30% sucrose. A 15-mm segment of spinal cord (n = 10 hydrogel/PBS, n = 10 hydrogel/NT-3) extending 10 mm rostral and 5 mm caudal to

the lesion epicenter was removed, frozen in OCT, and sectioned on a cryostat in the parasagittal plane at 40 μm. BDA was visualized within CST axons using the avidin–biotin–peroxidase complex kit (Vector Laboratories) on every second free-floating section. Sections were mounted (Superfrost Plus slides, Fisher Scientific), air-dried, dehydrated, coverslipped (Permount, Fisher Scientific), and examined by light microscopy under ×200 magnification. To determine lesion size, sections were analyzed under phase microscopy. The dorsoventral extent of the lesion was measured and represented as a percentage of the total dorso-ventral distance. One animal was excluded from the study for having a lesion that compromised 90% of the spinal cord depth. Additional analyses were done on 40 μm free-floating coronal sections taken 5–10 mm distal to the lesion site. To visualize serotonergic fibers, coronal sections (n = 9 hydrogel/PBS, n = 8 hydrogel/NT-3) were preincubated in 10% normal goat serum/0.3% Triton-X/PBS followed by incubation with polyclonal rabbit anti-5-HT (1:50,000, Immunostar) overnight and then a goat anti-rabbit IgG-HRP-conjugated antibody (1:500, Bio-Rad).

Axon counting

BDA-positive axons distal to the lesion site were visualized by light microscopy (×200) in parasagittal or coronal sections, and processes >40 μm were counted. To quantify serotonergic axon growth, immunopositive processes >10 μm were counted in the intermediolateral column and the ventral horn in coronal sections 5–10 mm distal to the lesion site. Data were analyzed using a two-tailed Student's t test.

Hydrogel release kinetics in vivo and further characterization of the lesion site

To determine the release profile of NT-3 in vivo, a separate group of rats underwent dorsal hemisections followed immediately by injections of hydrogels containing NT-3. Animals were euthanized after 3 h or at 6 or 14 days (n = 3 per time point). Seven segments of spinal cord 3 mm long, centered at the lesion site, were dissected, frozen at −80°C until use, and homogenized in buffer containing NP-40 and protease inhibitors (Promega Technical Bulletin TB243). The concentration of NT-3 was determined in triplicate by an enzyme-linked immunosorbent assay (ELISA: Emax, Promega, Madison, WI) and normalized by the weight of each sample. Baseline values were obtained from animals with no treatment euthanized 3 h after dorsal hemisection.

To characterize the glial response, we prepared an additional set of animals with dorsal hemisections, of which 3 received hydrogel implants and 3 did not. Animals were euthanized after 10 days and prepared for histology. Parasagittal sections were stained with a rabbit antibody to GFAP (1:100, Sigma, St. Louis, MO) followed by a fluorescently tagged goat anti-rabbit IgG antibody (1:200, Invitrogen/Molecular Probes, Eugene, OR). The gliotic response, as reflected in the intensity of GFAP immunofluorescence, was measured using NIH Image software, maintaining constant illumination for all samples.

Results

Degradable poly(ethylene glycol) hydrogels are formed from the radical polymerization of acrylated poly(lactic acid)-b–poly (ethylene glycol)-b–(poly lactic acid) (PLA-b–PEG-b–PLA) macromers (Burdick et al., 2002; West and Hubbell, 1995) and

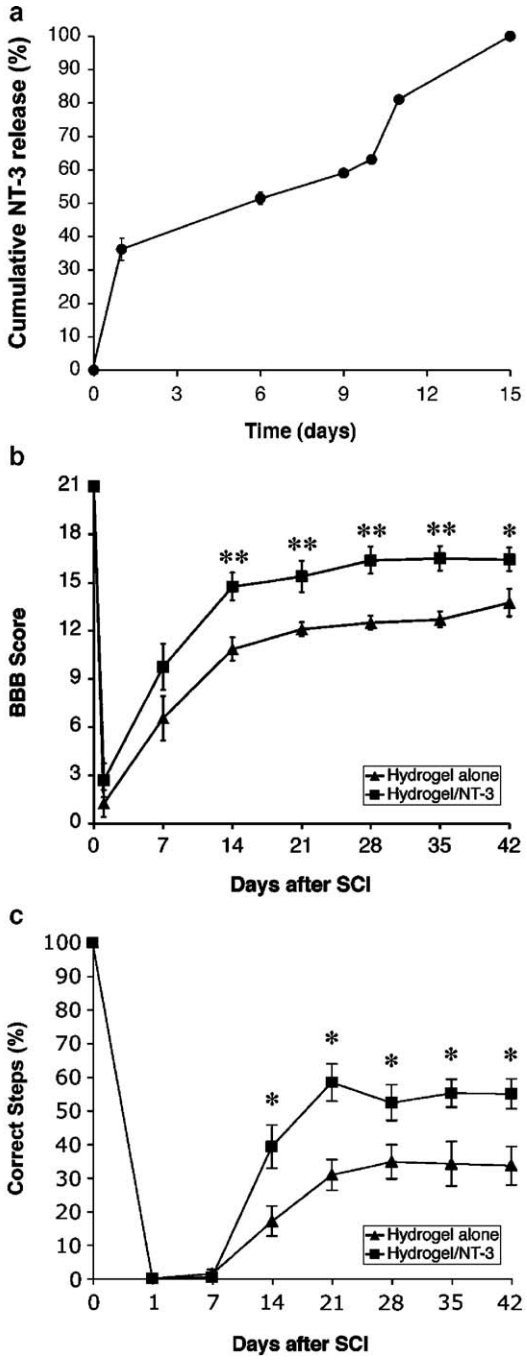

Fig. 1. Hydrogel release characteristics and behavioral recovery after dorsal hemisection of the spinal cord. (a) In vitro release profile of NT-3 encapsulated in a 10% wt/wt PEG hydrogel. y axis shows cumulative percentage of NT-3 released. (b) BBB scores of rats following dorsal hemisection at T8 and treatment with hydrogel releasing either PBS alone (n = 10) or NT-3 (n = 8). (c) Horizontal ladder walk performance of the same rats (percentage of correct steps relative to total steps). Values are reported as the mean ± SEM. *, **difference from controls significant at $P < 0.05$, $P < 0.01$, respectively.

degrade via ester hydrolysis in the network crosslinks. We controlled network degradation and subsequent molecular release properties by altering the network chemistry of these injectable hydrogels (e.g., number of degradable units) and network crosslinking density (e.g., macromer concentration). As a prototypic molecule to investigate the efficacy of degradable PEG hydrogels for spinal cord repair, we selected NT-3, a trophic factor which has shown promising effects in other studies of SCI (Coumans et al., 2001; Giehl and Tetzlaff, 1996; Grill et al., 1997; Lu et al., 2004; Schnell et al., 1994; Tobias et al., 2003; Xu et al., 1995; Zhou et al., 2003).

Preliminary studies were carried out to evaluate release kinetics in vitro. As shown in Fig. 1a, we observed a burst of NT-3 release over the first 24 h (ca. 40%) followed by

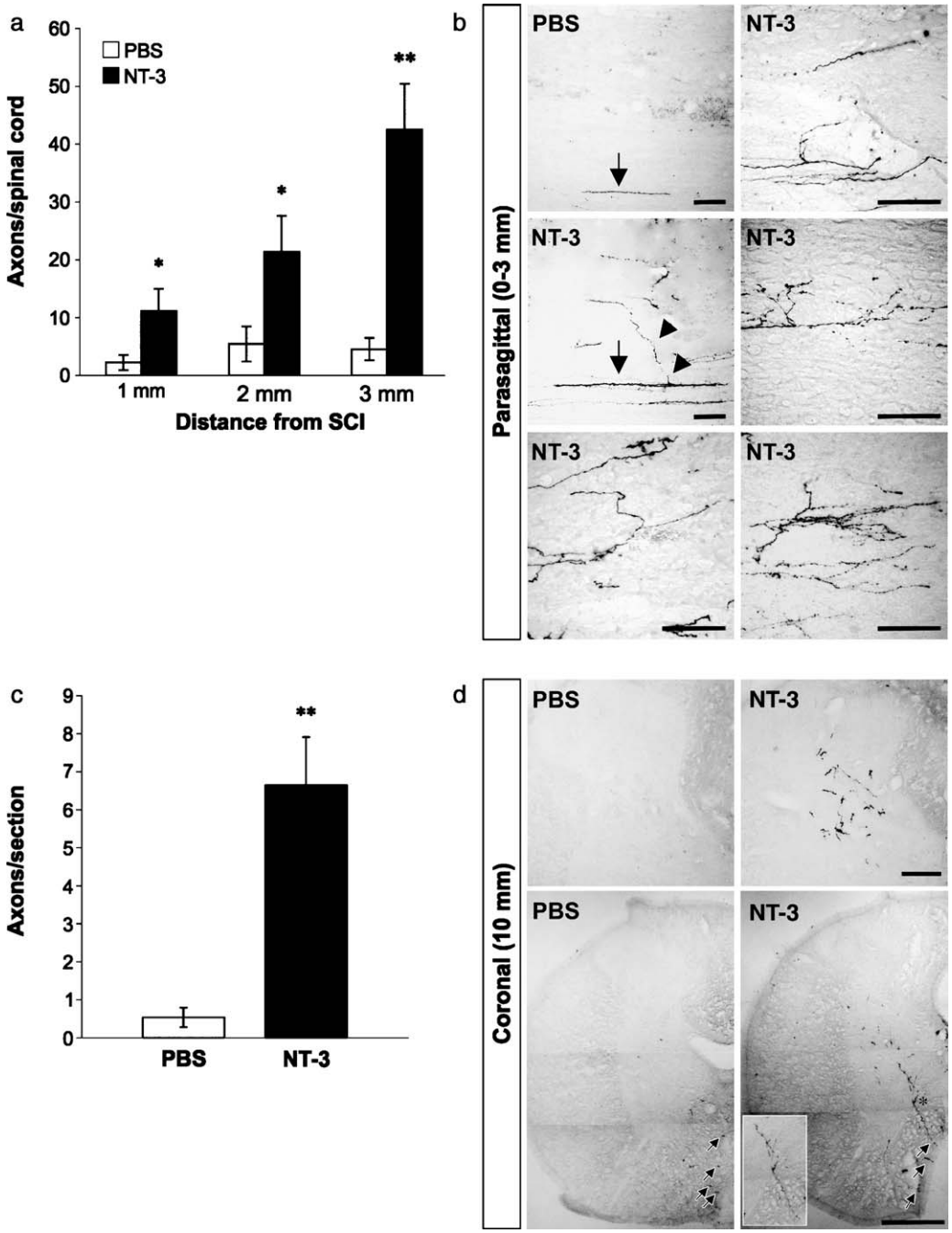

Fig. 2. Hydrogels releasing NT-3 promote CST reorganization. (a–b) BDA-labeled CST fibers in rats treated with hydrogels releasing PBS (n = 10) or NT-3 (n = 10). (a) Quantitation: Cumulative number of fibers ≥ 40 μm counted in every second parasagittal section at the indicated distances from the lesion site (mean ± SEM). (b) Examples of BDA-labeled axons in parasagittal sections from PBS- and NT-3-treated cases, as indicated. Arrows indicate vCST. Arrowheads indicate BDA-labeled axons branching from the vCST. (c–d) BDA-labeled CST axons in coronal sections at the level of the lumbar enlargement. (c) Average number of BDA-labeled fibers per section. (d) Examples of BDA-labeled fibers in PBS- and NT-3-treated animals. The bottom panels show coronal sections through the spinal cord of the two groups. Arrows point to labeled axons in the vCST. Asterisk shows a fiber branching from the vCST and reaching the gray matter. *P < 0.05; **P < 0.01. Scale bars: panel b = 20 μm; panel d = 200 μm (top), 500 μm (bottom).

sustained release over the next 2 weeks. For in vivo application, hydrogel precursor solutions containing the macromer, photoinitiator and NT-3 (or no trophic factor as a control) dissolved in PBS were applied directly to the lesioned cord and polymerized with rapid exposure to blue light.

Behavior

Two measures were used to investigate the functional consequences of hydrogel/NT-3 treatment after SCI: the BBB test, a well-validated, 21-point scale which evaluates open-field behavior (Basso et al., 1995), and the ladder grid walk, a test that requires cortical control of fine sensorimotor coordination (Metz and Whishaw, 2002). Rats were tested 24 h after spinal cord injury and then once weekly for 6 weeks.

In the open-field test, the performance of hydrogel/PBS controls improved noticeably over the first 2 weeks and more slowly over the next 4 weeks. Hydrogel/NT-3-treated animals showed significantly better performance than controls beginning at week 2 and maintained this superiority over the remainder of the study (Fig. 1b). At 6 weeks post-injury, the BBB score was 13.75 ± 0.72 for controls and 16.43 ± 0.86 for NT-3-treated animals (P 0.05). Functionally, this difference corresponds to the ability of 100% of the hydrogel/NT-3 group to achieve consistent forelimb–hindlimb coordination, compared to very few animals in the control group. NT-3-treated animals, but not controls, also achieved consistent plantar stepping with the predominant paw position being parallel to the body at initial contact with the surface. Although not statistically significant, the two groups showed hints of being different on the BBB score 1 day and 1 week post-lesion. On the ladder walk test, all animals showed severe impairments on day one and some degree of improvement over the course of testing. Measuring the ratio of correct foot placements to total steps, hydrogel/NT-3-treated animals consistently performed better than controls beginning at week 2 and continuing throughout the remainder of the study (Fig. 1c).

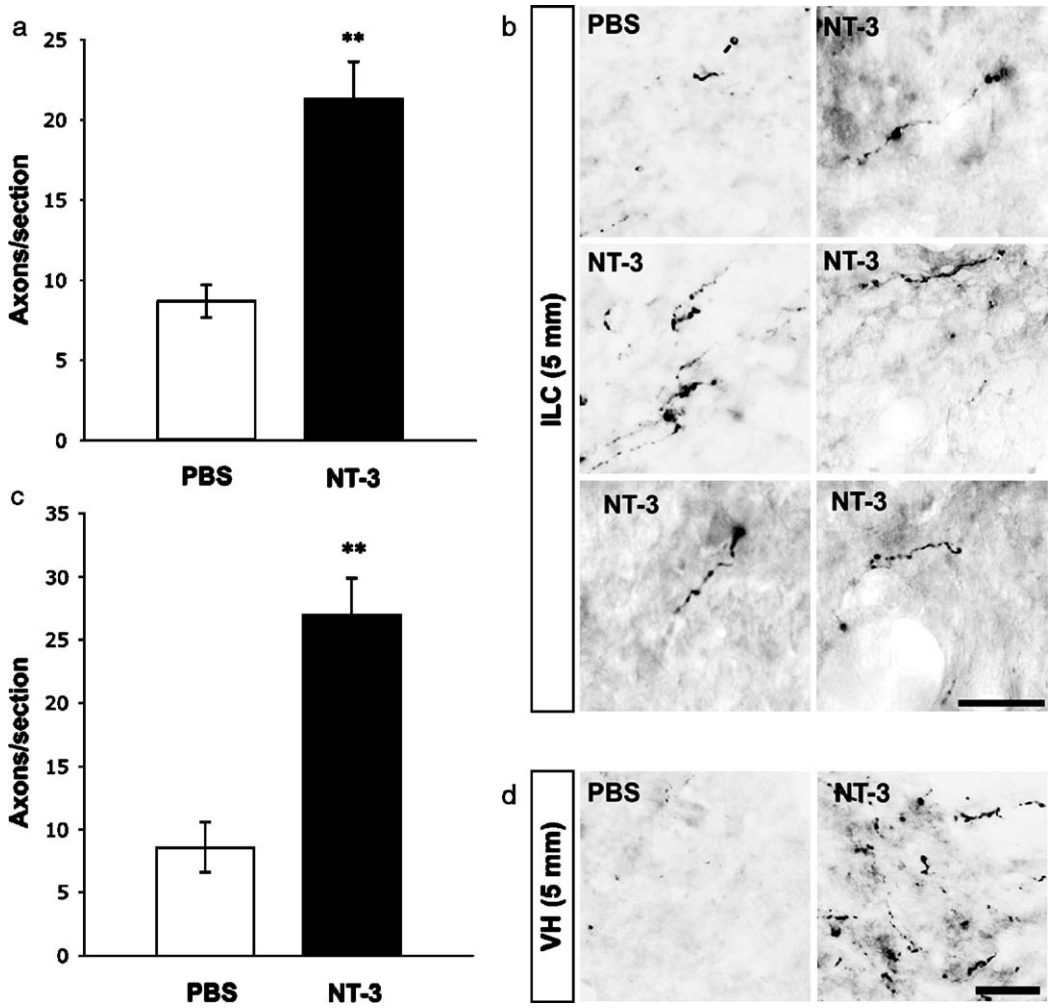

Fig. 3. Hydrogels releasing NT-3 promote axon growth in the raphespinal tract. 5-HT-immunoreactive fibers in the intermediolateral column (a–b) and ventral horn (c–d) of the spinal cord visualized in coronal sections 5 mm distal to the lesion site. (a, c) Quantitation of 5-HT$^+$ fibers per section in animals treated with hydrogels releasing PBS or NT-3 (mean ± SEM). (b, d) 5-HT-immunoreactive fibers in the intermediolateral column (b) and ventral horn (d). **$P < 0.01$. Scale bars: panel b = 50 μm; panel d = 20 μm.

Anatomical changes

We next investigated whether the behavioral differences found between hydrogel/NT-3 and control animals were associated with changes in anatomical reorganization. The hemisection model used here interrupts several pathways, including the dorsal (principal) and dorsolateral components of the corticospinal tract (CST) and a subset of raphespinal tract (RST) fibers. The CST originates in layer 5 pyramidal cells of the sensorimotor cortex and mediates volitional, skilled movements of the distal musculature. Changes in the organization of this fiber system were investigated by injecting the anterograde tracer biotinylated dextran amine (BDA) into multiple sites in the sensorimotor cortex. Within the first 3 mm caudal to the injury site, BDA-labeled fibers were quantified in parasagittal sections at 1 mm intervals, while further caudally, we quantified the number of BDA-labeled fibers in coronal sections.

In parasagittal sections through the lesion site, whereas hydrogel/PBS controls exhibited only a small number of BDA-labeled axons distal to the injury, hydrogel/NT-3-treated animals showed many such fibers (Figs. 2a, b). In many cases, these fibers could be traced back to their origin as branches of ventral CST (vCST) axons (Figs. 2b, d). The number of axonal processes continued to increase at greater distances from the lesion site in hydrogel/NT-3 animals (Fig. 2a). The difference between hydrogel/NT-3 and hydrogel/PBS groups was particularly clear in coronal sections through the lumbar enlargement, an area that normally receives a dense CST innervation (Figs. 2c, d). The extensive collateral growth seen at this level may reflect the large number of potential sites for synaptogenesis resulting from the massive surgical denervation of this area. Rostral to the injury site, the overall intensity of CST labeling was indistinguishable between groups (data not shown).

Raphespinal tract (RST) fibers originate in the raphe nuclei of the medulla and innervate layers 1 and 2a of the dorsal horn, layers 8 and 9 of the ventral horn, and the intermediolateral cell column of the thoracic cord. Because the RST contains the only serotonergic (5-HT) fibers in the spinal cord, its axons can be visualized using 5-HT immunohistochemistry. Growth was quantified as the number of 5-HT-positive fibers in the intermediolateral column and the

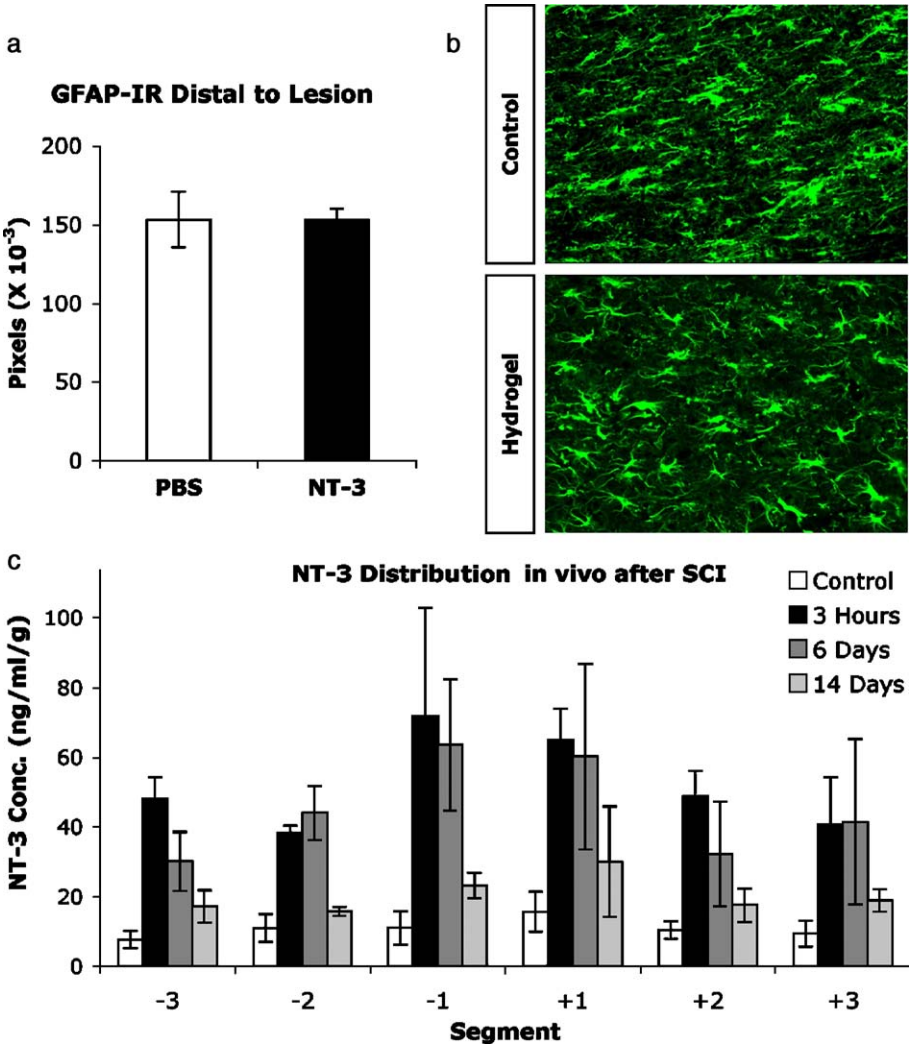

Fig. 4. Glial response to hydrogel and in vivo release profile of NT-3. (a–b) GFAP immunostaining and quantitative analysis in animals with and without hydrogel (n = 3 per group). (a) Intensity of immunofluorescence, (b) gray matter GFAP staining at the lesion site. (c) In vivo release profile of NT-3 analyzed by ELISA (n = 3 cases per time point; data represent mean concentration/g tissue ± SEM). Note that the central segment containing the hydrogel with NT-3 is omitted.

ventral horn in coronal sections 5 mm distal to the lesion site. In both of these areas, the number of serotonergic axons was 2–3 times greater in hydrogel/NT-3-treated animals than in hydrogel/PBS controls (Fig. 3). Thus, the improved behavioral performance seen in the hydrogel/NT-3 group correlates with increased axon innervation of both the CST and RST.

In terms of lesion depth, the mean depth of the lesions was 65.86% ± 1.83 (SEM) of the dorso-ventral extent of the spinal cord for the control group and 60.61% ± 2.37 (SEM) for the NT-3-treated animals ($P \sim 0.10$). All animals showed a complete interruption of the dorsal CST.

The astrocytic response was investigated by quantifying the intensity of GFAP immunoreactivity in the vicinity of the lesion site. As shown in Figs. 4a and b, hydrogels did not alter the astrocytic response compared to animals with lesions only.

NT-3 release profile in vivo

The release profile of NT-3, as determined using ELISA, showed a peak at the site of injury 3 h after hydrogel administration and high concentrations over a distance of at least 1 cm from that site on day 6 (Fig. 4c). Levels declined to approximately one-third maximum at 14 days. Over the first 6 days, NT-3 levels within the first few millimeters of the lesion site were in the range of 50 ng/ml/g of tissue when averaged over the entire tissue sample. However, since most of the NT-3 is presumably in the intracellular space and cerebrospinal fluid, the effective concentration available to cell surface receptors would be considerably higher than we calculate for the tissue as a whole. The ED_{50} for NT-3's effects upon embryonic cortical neurons in culture has been estimated to be ca. 25 ng/ml (Widmer et al., 1993), and injured nerve terminals of the CST and raphespinal tract are likely to have been exposed to this or higher concentrations over the first week in vivo.

Discussion

In recent years, a variety of approaches have resulted in some degree of axonal rewiring and functional improvement after SCI. These approaches have included molecular therapies to counteract inhibitory molecules present in myelin (Lee et al., 2003; Schwab et al., 1993; Z'Graggen et al., 1998) and the glial scar (Silver and Miller, 2004), activation of neurons' growth state (Benowitz et al., 1999; Cao et al., 2005; Schnell et al., 1994), elevation of intracellular cAMP (Filbin, 2003; Neumann et al., 2002), or introduction of embryonic tissue grafts (Nikulina et al., 2004) or specialized glial cells (Ramon-Cueto et al., 2000; Xu et al., 1995); more recently, the importance of using a combination of treatments has become apparent (Fouad et al., 2005; Lu et al., 2004; Pearse et al., 2004). The benefits of various treatments in turn point to the need for a nontoxic, easy-to-use delivery system that allows for the slow, continuous release of molecules at the site of injury. We show here that, by virtue of delivering therapeutic levels of a neurotrophic factor over a specified period of time, hydrogels provide an attractive alternative to either mechanical or biological delivery systems and provide a platform that can be readily adapted for the delivery of any number of therapeutic agents, alone or in combination. The synthetic hydrogel system does not require the fabrication of an implant that matches the size and shape of the SCI, does not introduce foreign tissue to the body, and cannot fail mechanically. The precise control over network degradation and molecule release properties allows for the controlled delivery of a wide range of molecules (e.g., neurotrophins, enzymes, antibodies, small drugs) and may allow for rapid translation of synthetic hydrogel technology for treatment of SCI clinically.

Much of the research in this field has focused on stimulating neurons to regenerate damaged axons back to their original targets. Although this goal has generally been difficult to achieve, several recent studies have demonstrated that uninjured fibers can sprout collaterals that contribute to the formation of new functional circuitry (Raineteau et al., 2001). Following lesions of the dorsal CST, the ventral CST spontaneously undergoes sprouting into denervated areas of the spinal cord (Weidner et al., 2001). In addition, several therapeutic agents, including NT-3 (Schnell et al., 1994), inosine (Benowitz et al., 1999), or interference with Nogo-A signaling (Lee et al., 2004; Z'Graggen et al., 1998), enhance compensatory sprouting from uninjured fibers of the dorsal CST. In our study, collateral sprouting appears to have dominated over long-distance regeneration. In many instances, BDA-labeled axons distal to the injury site could be traced back to the vCST, and the total number of axonal processes increased caudally as a result of continued branching. In the lumbar enlargement of the spinal cord, which lies far distal to the injury site, the number of labeled axonal processes greatly exceeded the number seen just beyond the injury site in the hydrogel/NT-3 group, which logically cannot be attributed to long-distance regeneration of severed axons (Steward et al., 2003). When delivered via hydrogel in the vicinity of injured nerve terminals, NT-3 is presumably transported retrogradely to cortical pyramidal cells (DiStefano et al., 1992) and promotes axonal branching (Schnell and Schwab, 1993; Tobias et al., 2003). Sprouting is less likely to occur in the vicinity of the lesion, where inhibitory molecules such as chondroitin sulfate proteoglycans (CSPGs) are likely to suppress outgrowth (Davies et al., 1999). At greater distances, axons from the ventral CST primed by NT-3 would be expected to encounter a less inhibitory environment and to continue forming branches and sub-branches.

Hydrogel delivery of NT-3 also promoted plasticity of the raphespinal tract, whose projections to the ventral and intermediolateral regions of the spinal cord modulate the central pattern generator, a reflex circuit located in the lumbar spinal cord that is essential for locomotion. Previous studies have indicated that the RST has considerably greater potential for plasticity after injury than the CST and can even regenerate to some extent after complete transection of the spinal cord in rats following NT-3 treatment (Coumans et al., 2001). Axons originating in the nuclei raphe obscurus and raphe pallidus descend in the ventral funiculus and would be spared following our dorsal hemisections, whereas fibers originating in the nucleus raphe magnus, which course in the dorsolateral funiculus (Tracey, 1985), would be interrupted. The number of 5-HT-positive axons found in the gray matter distal to the lesion was far greater in animals treated with hydrogels delivering NT-3 than in controls and could reflect either regenerative growth from

transected axons or local sprouting from the undamaged RST, as reported previously (Coumans et al., 2001; Tobias et al., 2003; Xu et al., 1995), or a combination of the two. The NT-3 receptor TrkC is present on multiple descending systems involved in locomotion (King et al., 1999). Previous studies have shown that NT-3 exerts a neuroprotective effect after spinal cord injury on the neurons that give rise to these fibers (Giehl and Tetzlaff, 1996), rescuing many from cell death (Tobias et al., 2003). Additionally, NT-3 promotes outgrowth of descending pathways involved in locomotor control other than those analyzed in this study, e.g., the vestibulospinal and rubrospinal tracts. Our results also show a trend for lesion size being slightly smaller in NT-3-treated vs. control animals. These factors could all contribute to the difference in BBB scores between NT-3-treated and control animals as early as 1 day and 1 week post-lesion.

Evidence accumulating over the past few years suggests that many patients who have suffered SCI, stroke or head trauma (Bracken et al., 1997) show a limited amount of functional improvement over time. The majority of spinal cord injuries that occur in humans are incomplete (Kakulas, 1999). If the same potential for compensatory sprouting and for establishing novel circuitry seen in rats also exists in humans, the delivery of therapeutic agents from biodegradable, nontoxic hydrogels could be an important tool for enhancing brain reorganization and improving functional outcome after SCI.

Acknowledgments

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