Controlled Release of GDNF Reduces Nerve Root-Mediated Behavioral Hypersensitivity

Raymond D. Hubbard, Joan J. Martínez, Jason A. Burdick, Beth A. Winkelstein

Department of Bioengineering, University of Pennsylvania, 240 Skirkanich Hall, 210 South 33rd Street, Philadelphia, PA 19104-6321

Received 2 January 2008; accepted 22 April 2008
Published online 16 July 2008 in Wiley InterScience (www.interscience.wiley.com). DOI 10.1002/jor.20710

ABSTRACT: Nerve root compression produces persistent behavioral sensitivity in models of painful neck injury. This study utilized degradable poly(ethylene glycol) hydrogels to deliver glial cell line-derived neurotrophic factor (GDNF) to an injured nerve root. Hydrogels delivered ~98% of encapsulated GDNF over 7 days in an in vitro release assay without the presence of neurons and produced enhanced outgrowth of processes in cortical neural cell primary cultures. The efficacy of a GDNF hydrogel placed on the root immediately after injury was assessed in a rat pain model of C7 dorsal root compression. Control groups included painful injury followed by: (1) vehicle hydrogel treatment (no GDNF), (2) a bolus injection of GDNF, or (3) no treatment. After injury, mechanical allodynia ($n = 6$/group) was significantly decreased with GDNF delivered by the hydrogel compared to the three injury control groups ($p < 0.03$). The bolus GDNF treatment did not reduce allodynia at any time point. The GDNF receptor (GFRα-1) decreased in small, nociceptive neurons of the affected dorsal root ganglion, suggesting a decrease in receptor expression following injury. GDNF receptor immunoreactivity was significantly greater in these neurons following GDNF hydrogel treatment relative to GDNF bolus treated and untreated rats ($p < 0.05$). These data suggest efficacy for degradable hydrogel delivery of GDNF and support this treatment approach for nerve root-mediated pain. J Orthop Res 27:120–127, 2009

Keywords: radiculopathy; neurotrophic factor; GDNF; hydrogel; allodynia

Chronic neck pain affects as many as 71% of adults at some point during their lives.[1,2] Painful cervical spine injuries can result from nonphysiologic loading of the neck as occurs in recreational accidents and contact sports,[3,4] when nerve roots can be compressed.[5] Nerve root compression induces persistent behavioral hypersensitivity in rat models of radiculopathy, in which painful responses are elicited in the affected dermatome by stimulation that does not normally provoke pain (mechanical allodynia).[6–9] Further, hypersensitivity to a stimulus has been used as a sensitive clinical indicator of pain.[10] Compression of primary afferent neurons also produces increased neuronal excitability, ectopic axonal firing, Wallerian degeneration, endoneurial edema, inflammatory responses, and decreased spinal substance P.[7,8,11–16]

Current treatments for neuropathic pain include opioids, nonsteroidal anti-inflammatories, antagonists to ion channels, neuropeptides, cytokines, and trophic factors to promote cell survival and regeneration.[17–23] Neurotrophic factors can prevent secondary neuronal degeneration and reduce spontaneous firing. In particular, glial cell line-derived neurotrophic factor (GDNF) has analgesic effects and modulates nociceptive signaling by altering sodium channel subtype expression and reducing aberrant A-fiber sprouting into the cord.[18,19,24–26] However, in neuropathic pain models, GDNF is decreased after injury which may initiate nocicieptive mechanisms.[19,27] GDNF also upregulates somatostatin, directly opposing the nociceptive action of substance P.[24,26,28] GDNF is a member of the TGF-β superfamily and binds the GDNF family receptor (GFR)α-1, initiating an intracellular MAP kinase cascade that enhances neuronal survival via inhibition of apoptosis proteins.[20] Continuous GDNF delivery prevents behavioral and electrophysiological abnormalities in neuropathic pain and partially reverses increased GFRα-1 in large dorsal root ganglion (DRG) neurons if administered by an osmotic minipump.[18,29] However, implantation of osmotic minipumps,[18,22] repeated injections,[30] or gene therapy[31] all have inherent clinical limitations.

The delivery of neurotrophic factors from degradable polymers, such as hydrogels, obviates clinical issues, and may provide significant analgesia compared to an equivalent dosing in a single injection treatment. A variety of studies have utilized hydrogel matrices for tissue engineering and drug delivery,[32–34] but few have applied trophic factor release from hydrogels in an in vivo model of neuronal injury.[35] Degradable hydrogels can be designed for a range of release profiles, based on crosslinking density, susceptibility to degradation, and hydrophilicity.[36,37] Degradable poly(ethylene glycol) (PEG) has been used to deliver neurotrophins and improve neurite outgrowth from retinal explants.[38] Trophic factor delivery in vivo to injured neural tissue significantly increased fiber sprouting and motor recovery for many hydrogels and trophic factor systems, including PEG.[35,39–41] However, no study has compared behavioral hypersensitivity following neural injury for controlled release of GDNF from a hydrogel system versus a single injection of an equivalent quantity of GDNF.

In our model of dorsal root compression, transient loading of the root produces behavioral hypersensitivity that persists for 7 days.[15,42] In other pain studies, neural compression reduces GDNF-immunoreactivity in the DRG,[19,27] induces axonal degeneration and macrophage infiltration in the dorsal root, and significantly decreases spinal neuropeptides.[42] To our knowledge, no study has

Correspondence to: Beth A. Winkelstein (T: 215-573-4589; F: 215-573-2071; E-mail: winkelst@seas.upenn.edu)

investigated controlled release of GDNF from degradable PEG hydrogels for reducing behavioral hypersensitivity and restoring GDNF-immunoreactivity in the DRG following painful dorsal root injury.

MATERIALS AND METHODS

Hydrogel Formulation and GDNF Bioassay

In vitro assays established the temporal release and bioactivity of degradable PEG-encapsulated GDNF prior to in vivo implantation. The hydrogel was formed from a macromer of acrylated polylactic acid and PEG (PLA-b-PEG-b-PLA, Polysciences, Warrington, PA).[35–37] The macromer was fabricated from 4kDa PEG (Sigma, St. Louis, MO) capped with ~2.7 lactic acid units per side and acrylated to ~100% efficiency, determined with ^{1}H NMR. For encapsulation, a 10 wt% macromer solution in PBS containing 0.05 wt% 2-methyl-1-[4-(hydroxyethoxy)phenyl]-2-methyl-1-propanone (Irgacure 2959, I2959, Ciba Specialty Chemicals, Basal, Switzerland) was prepared. For polymerization, GDNF was suspended in the polymer solution at the desired concentration (250 μg/ml for determination of release profile, 5 μg/ml). The solution (20 μl) was pipetted into a cylindrical mold and exposed to ultraviolet light for 5 min using a long-wave ultraviolet lamp (F8T5BLB, Topbulb, East Chicago, IN).

To characterize the release profile, hydrogels ($n = 3$) were ejected into Eppendorf tubes containing 1 ml PBS and placed in a 37°C incubator with gentle agitation. On days 1, 2, 4, 7, and 15, PBS containing released GDNF was sampled and stored at −20°C; tubes containing the hydrogels were refilled with fresh PBS, until complete gel degradation. GDNF content of each PBS sample was determined by ELISA (R&D Systems, Minneapolis, MN).

To establish the biological activity of the released GDNF, primary cultures of dissociated embryonic rat (E18) cortical cells were incubated with hydrogels containing GDNF. Cortical cells were plated at a density of 10,000 cells/well in neurobasal media containing Penicillin-Streptomycin (Invitrogen, Carlsbad, CA). At 48 h, media was exchanged, and hydrogels containing 100 ng of GDNF were suspended in triplicate wells. Control wells containing no hydrogels, hydrogels containing no GDNF, or 50 ng of free GDNF in the media were also analyzed in triplicate. At 72 h after adding GDNF, 200× images were taken of each well at five randomly spaced regions (334 × 422 μm) using an inverted light microscope. Images ($n = 15$/group) were analyzed using ImageJ to determine total cell counts and percentage of cells with processes. At 7 days, new images were analyzed to assess cell count, number of cells containing at least one process, average number of processes per cell, average process length, and longest process length.[43–45]

In Vivo Hydrogel Behavioral Studies

In vivo studies were performed using male Holtzman rats (250–350 g) (Harlan Sprague-Dawley, Indianapolis, IN). Rats were housed with a 12–12 h light–dark cycle and had free access to food and water. All surgical procedures were performed under isofluorane inhalation anesthesia and were approved by the Institutional Animal Care and Use Committee. A C6/C7 hemilaminectomy and facetectomy exposed the C7 nerve roots on the right side, and a 100-mN microvascular clip was applied to the dorsal root midway between the DRG and the dorsal root entry zone.[8,9] After compression for 15 min, one of the following treatments was applied directly to the injured root: (1) 20 μl hydrogel containing 5 μg GDNF (250 μg/ml *GDNF gel*; $n = 6$); (2) 20 μl vehicle hydrogel containing PBS (*vehicle gel*; $n = 6$); (3) 20 μl bolus of PBS containing 5 μg GDNF (250 μg/ml *GDNF bolus*; $n = 6$); or (4) no treatment (*injury*; $n = 6$). Additional rats received surgery to expose the root without compression and with a 5 μg GDNF hydrogel application (*sham*; $n = 6$). The incision was closed with sutures and surgical staples.

Rats were evaluated for bilateral forepaw mechanical allodynia on postoperative days 1, 3, 5, and 7.[8,9,46] Preoperatively, baseline measurements of paw withdrawal responses were recorded on consecutive days. A single blinded tester performed all allodynia testing. For each session, after 20 min of acclimation to the environment, rats were stimulated on the plantar surface of each forepaw using von Frey filaments (1.4, 2, 4 g) (Stoelting, Wood Dale, IL). Each session consisted of three rounds of 10 stimulations, 10 min apart. The number of withdrawal responses was recorded for each filament after each round. Allodynia, determined by an increase in withdrawals over baseline, was averaged by group on each day.

Assessment of GDNF and GFRα-1 in the DRG

Rats were euthanized on day 7 by an overdose of sodium pentobarbital (40 mg/kg) and transcardially perfused with 200 ml of PBS followed by 200 ml of 4% paraformaldehyde in PBS. The C7 ipsilateral and contralateral DRGs were harvested and placed in 4% paraformaldehyde for 1 h followed by 50% ethanol overnight. Tissue was dehydrated in graded ethanols and embedded in paraffin for longitudinal sectioning at 10 μm. DRG sections were labeled for GDNF and the GFRα-1 receptor by immunohistochemistry using polyclonal antibodies against GDNF (1:100; Santa Cruz, CA) and GFRα-1 (1:100; Neuromics, Bloomington, MN). Horse anti-goat or goat anti-rabbit secondary antibodies (Vector, Burlingame, CA) were used at a dilution of 1:200. All antibody dilutions were previously optimized. Sections were exposed to 3,3-diaminobenzidine for color development (Vector) and cover-slipped using a nonaqueous mounting medium. Immunostained sections were imaged at 200×. Neuron populations were identified by cross sectional area; small neurons (<600 μm^2) in the DRG were identified as a measure of nociceptive neurons, and large neurons (>600 μm^2) were taken as mechano/proprioceptive. The number of small and large neurons positive for each of GDNF or GFRα-1 was reported as a percentage of all small or large neurons in each DRG.

Data and Statistical Analyses

Cell counts and process length measurements from the primary neural cell cultures were averaged across the wells for each group and analyzed by one-way ANOVA with post-hoc Bonferroni correction. Paw withdrawal frequencies were compared using a two-way ANOVA with repeated measures to determine significant effects of treatment over time, followed by a one-way ANOVA with post-hoc Bonferroni correction for pairwise comparisons between experimental and control groups on each day. Differences in small and large GDNF- or GFRα-1-positive neurons among groups were compared by one-way ANOVA with post-hoc Bonferroni correction. Statistical analyses were performed using SYSTAT v10.2 (SYSTAT, Richmond, CA) with data presented as mean ± SD and significance at $p < 0.05$.

RESULTS

GDNF Release Profile and Bioassay

Over 15 days, degradation of the lactic acid units in the PLA-b-PEG-b-PLA macromer allowed the encapsulated GDNF to be released from the hydrogel, at which

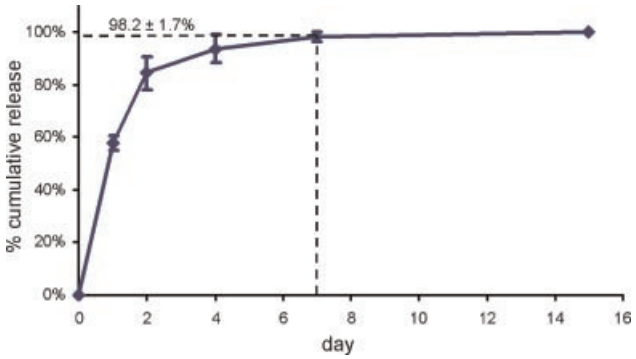

Figure 1. In vitro characterization of the cumulative GDNF release from degradable PEG hydrogels. Cumulative release is expressed as a percent of total GDNF released ±SD.

time the hydrogels were fully degraded (Fig. 1). The hydrolytic nature of degradation allowed for an assessment of GDNF release in a cell- and degradative enzyme-free system, thereby eliminating cell binding or enzymatic degradation as possible sources of measurement error. After 24 h, $57.8\% \pm 3.0\%$ of the GDNF was released. Hydrogels were nearly completely degraded within 7 days, releasing $98.2\% \pm 1.7\%$ of the GDNF (Fig. 1).

Addition of hydrogel-encapsulated GDNF to primary cortical cell cultures increased the cell count and outgrowth of processes at day 7 (Figs. 2 and 3A–C). Also, after 72 h, free GDNF and GDNF released from the hydrogel similarly increased the cell numbers and percentage of cells with processes over wells incubated without GDNF, indicating no significant loss of bioactivity from hydrogel polymerization (Fig. 3D). The number of viable neural cells, average process lengths, and lengths of the longest process were all significantly increased ($p < 0.043$) in cultures incubated with a GDNF hydrogel compared to those with no hydrogel (Fig. 3A–C). No significant increases occurred for wells containing gels with no GDNF (Fig. 3). Cultures incubated with the GDNF hydrogel averaged 15.1 ± 9.2 cortical cells per image (Fig. 3A), with 40.8% containing at least one process. Average process length was 74.8 ± 19.6 μm (Fig. 3B), the longest being 106.5 ± 74.4 μm (Fig. 3C). Cultures incubated without a hydrogel had 7.5 ± 5.0 cells, with only 25.8% containing at least one process. Processes averaged 40.8 ± 7.6 μm, the longest being 43.4 ± 37.2 μm. No significant differences were observed in the number of cells containing processes or the average number of processes per cell among any of the groups.

Behavioral Studies

Hydrogel-encapsulated GDNF treatment to the compressed root significantly reduced mechanical allodynia (Fig. 4). Qualitative trends in allodynia were similar and most robust for the 4-g filament; as such, responses to the 4-g filament are reported. For all groups receiving an injury except the *GDNF gel* group, allodynia in the ipsilateral forepaw was significantly greater than *sham* for 7 days ($p < 0.05$; Fig. 4A). Allodynia in the *GDNF gel* group was evident on day 1, but did not remain elevated above *sham* and significantly decreased relative to both *injury* and *vehicle gel* by day 5 ($p < 0.03$). Allodynia for the *GDNF gel* group was also significantly reduced relative to the *GDNF bolus* group by days 5 and 7 ($p < 0.03$; Fig. 4A).

Contralateral mechanical allodynia was produced by day 7 for all groups except the *GDNF gel* group (Fig. 4B). Contralateral allodynia in this latter group was significantly reduced relative to both the *injury* and *vehicle gel* groups ($p < 0.045$). GDNF treatment with the sham procedures did not alter ipsilateral or contralateral allodynia from baseline at any time point.

GDNF and GFRα-1 in the DRG

Root compression did not significantly affect the number or size distribution of DRG neurons. GDNF-immunoreactivity in ipsilateral DRG neurons was not significantly affected for any treatment group at day 7. *Sham* rats exhibited GDNF-immunoreactivity in $9.2\% \pm 3.3\%$ of the small and $25.9\% \pm 4.1\%$ of the large neurons, which were not significantly different than GDNF-immunoreactivity in normal rats (Table 1). Four tissue samples from the *injury* group were unusable for analysis; accordingly, additional matched samples were generated by the same injury procedure ($n = 4$ rats) to provide a complete data set. Those rats exhibited similar mechanical allodynia (data not shown) to those in the *injury* group. *Vehicle gel* and *injury* rats displayed

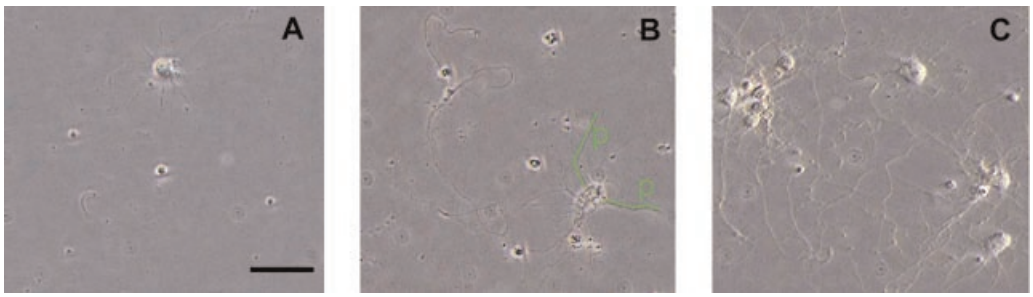

Figure 2. Representative images of cortical neural cells after 7 days of incubation with (A) no hydrogel, (B) a hydrogel containing no GDNF, and (C) a hydrogel containing 100 ng of GDNF. Cell count and length of processes for cultures incubated in the presence of a hydrogel containing 100 ng of GDNF were significantly increased ($p < 0.043$) over cultures without a hydrogel. (B) Representative length measurements by line segments for two processes (p) are depicted. In the event of process branching, the length of the longest branch of that process was recorded. Original magnification = 200×. Scale bar in (A) = 50 μm and applies to all.

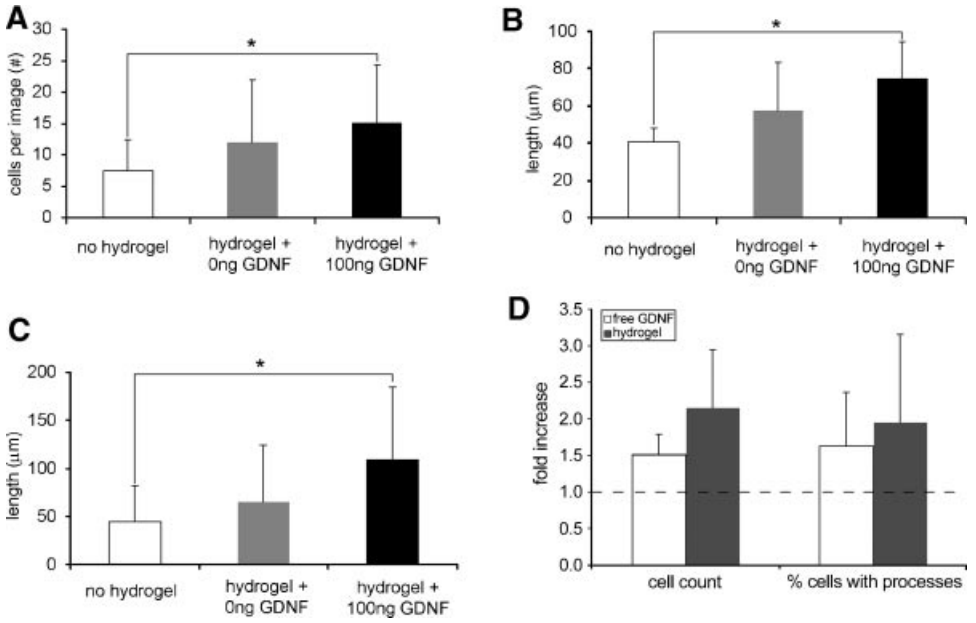

Figure 3. (A–C) Cell counts and length of processes (+SD) at day 7 for primary rat cortical cells cultured with no hydrogel, a hydrogel with no GDNF, and a hydrogel containing 100 ng of GDNF. (A) Cells per image (within a 0.08 mm^2 area), (B) average process length, and (C) length of the longest process all significantly ($^*p < 0.05$) increased after 7 days of incubation with a GDNF hydrogel. (D) Fold increase in cell numbers and the percentage of cells with processes at 72 h for primary cells cultured with either 50 ng of free GDNF in the media or a 100 ng GDNF hydrogel, normalized to cells cultured with no GDNF (dashed line). At this time, about 89 ng of GDNF had been released from the hydrogel. Hydrogel polymerization did not reduce the bioactivity of GDNF at 72 h based upon cell counts or process formation.

GDNF-immunoreactivity in $5.1\% \pm 1.0\%$ and $5.8\% \pm 1.8\%$ of the small neurons and $20.6\% \pm 5.5\%$ and $13.8\% \pm 6.7\%$ of large neurons, respectively, a moderate decrease relative to *sham*, *GDNF gel*, and *GDNF bolus* (Table 1). No differences in GDNF were detected in the contralateral DRG among any groups.

GFRα-1-immunoreactivity decreased significantly in small DRG neurons in the absence of a GDNF hydrogel (Fig. 5A). In small neurons of the ipsilateral DRG, GFRα-1-immunoreactivity was not significantly decreased for the *GDNF gel* group relative to *sham*. Yet, for *vehicle gel* rats, GFRα-1-immunoreactivity in small neurons ($8.14\% \pm 3.2\%$) was significantly decreased relative to the *GDNF gel* group ($16.0\% \pm 2.8\%$; $p < 0.006$). Additionally, for *injury* rats, GFRα-1-immunoreactivity in small neurons ($7.\ 4\% \pm 3.5\%$) was significantly decreased relative to the *GDNF gel* group ($p < 0.001$). The *GDNF bolus* group ($10.1\% \pm 3.8\%$) had significantly fewer GFRα-1-positive small neurons than the *GDNF gel* group ($p < 0.05$; Fig. 5A). Compression decreased GFRα-1-immunoreactivity in the contralateral DRG when untreated, but this trend was not significant (Fig. 5B).

GFRα-1 was moderately increased in large neurons of the ipsilateral DRG for the *injury* group ($21.7\% \pm 5.1\%$) compared to the *GDNF gel* ($16.4\% \pm 4.4\%$) group, and was significantly increased compared to *sham* ($13.5\% \pm 2.5\%$) (Fig. 5C). No trends in GFRα-1 were observed among groups in the contralateral DRG (Fig. 5D).

DISCUSSION

Controlled release of GDNF from degradable PEG hydrogels following compression of the cervical dorsal root reduced behavioral hypersensitivity and prevented decreases in GFRα-1 in small neurons of the ipsilateral DRG (Figs. 4 and 5). GDNF release is controlled by degradation and diffusion mechanisms that can be altered through hydrogel design (e.g., number of lactic acid units, macromer concentration). Osmotic minipump delivery rates as high as 12 μg/day did not produce toxic side effects,[18,22] so the release profile was optimized to deliver about half of the encapsulated GDNF (~3 μg) within the 1st day, corresponding to the largest decrease in GDNF in the DRG (unpublished data) and the most robust behavioral hypersensitivity.[15] Over

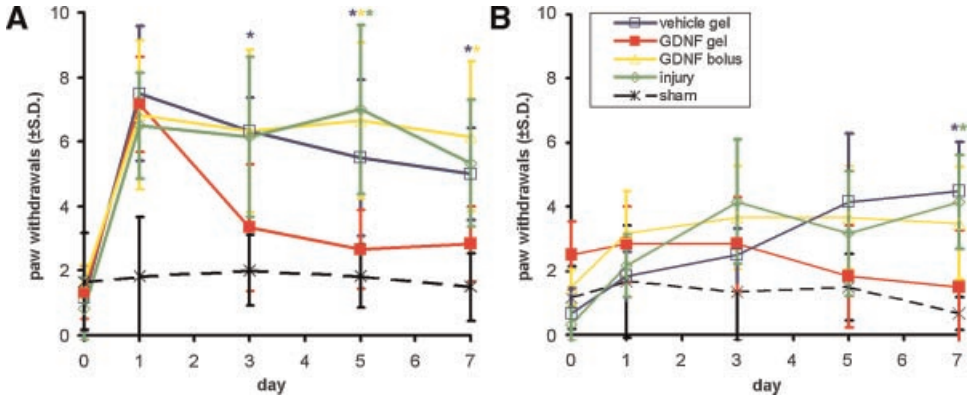

Figure 4. (A) Ipsilateral and (B) contralateral mechanical allodynia (±SD), reported as number of paw withdrawals. (A) All groups were significantly greater than *sham* on day 1. Only the *GDNF gel* group was reduced after day 1. (B) All injury control groups had significantly greater contralateral allodynia than *sham* at day 7. A significant increase relative to the *GDNF gel* group is indicated by (*).

Table 1. GDNF Immunoreactivity in the Ipsilateral DRG[a]

	Small Neurons (% GDNF-Positive)	Large Neurons (% GDNF-Positive)
Normal	8.0 ± 3.4	33.2 ± 9.7
Sham	9.2 ± 3.3	25.9 ± 4.1
GDNF gel	12.4 ± 7.5	29.3 ± 15.2
GDNF bolus	10.3 ± 3.9	27.6 ± 8.7
Vehicle gel	5.1 ± 1.0	20.6 ± 5.5
Injury	5.8 ± 1.8	13.8 ± 6.7

[a]Mean ± SD.

98% of encapsulated GDNF was released by day 7 in the in vitro assay, a time point relevant for chronic behavioral symptoms and degenerative pathology in this model.[8,9,42]

When treated with the 10 wt% degradable PEG hydrogel containing 5 μg GDNF, the allodynia normally observed following root compression was significantly attenuated and did not differ from *sham* after day 1. GDNF-immunoreactivity in the DRG was not significantly decreased following dorsal root compression (Table 1). Exogenous GDNF internalization by large and small DRG neurons may have increased slightly following GDNF application by bolus injection or hydrogel, yet this trend was not significant at day 7. GDNF-immunoreactivity was expected to increase in the DRG with application of exogenous GDNF;[47] however, the measured GDNF-immunoreactivitiy could not be specifically determined to be from cellular internalization or changes in endogenous expression. Also, after binding the GFRα-1 receptor, GDNF might have been metabolized and cleared from the DRG rather than becoming internalized by DRG neurons. In contrast, GFRα-1-immunoreactivity was significantly decreased in small DRG neurons and increased in large DRG neurons following injury, with no significant loss of small or large neurons. Continuous GDNF administration via the hydrogel maintained GFRα-1 expression at normal levels in the ipsilateral DRG (Fig. 5A, C). These results suggest that a decrease in GFRα-1 expression in small neurons and an increase in large neurons may facilitate aberrant neuronal behavior causing radicular pain, and that preventing these changes may decrease behavioral hypersensitivity.[18] A decrease in GFRα-1 expression in small, nociceptive neurons reduces the binding of GDNF-GFRα-1 to Ret, which subsequently decreases the action of the inhibitor of apoptosis proteins.[20] Therefore, aberrant neuronal firing in nociceptive neurons and increased pain sensitivity[18,24] may result from decreased cell viability due to decreased GFRα-1 expression. We also identified an increase in GFRα-1 expression in large DRG neurons that was prevented by continuous GDNF delivery, a phenomenon also observed after sciatic nerve transection.[29] Together, these studies suggest that continuous application of exogenous GDNF may reduce allodynia by promoting enhanced receptor expression and GDNF binding in small neurons that likely mediate nociception and by preventing increased expression in large, normally nonnociceptive neurons. Additional

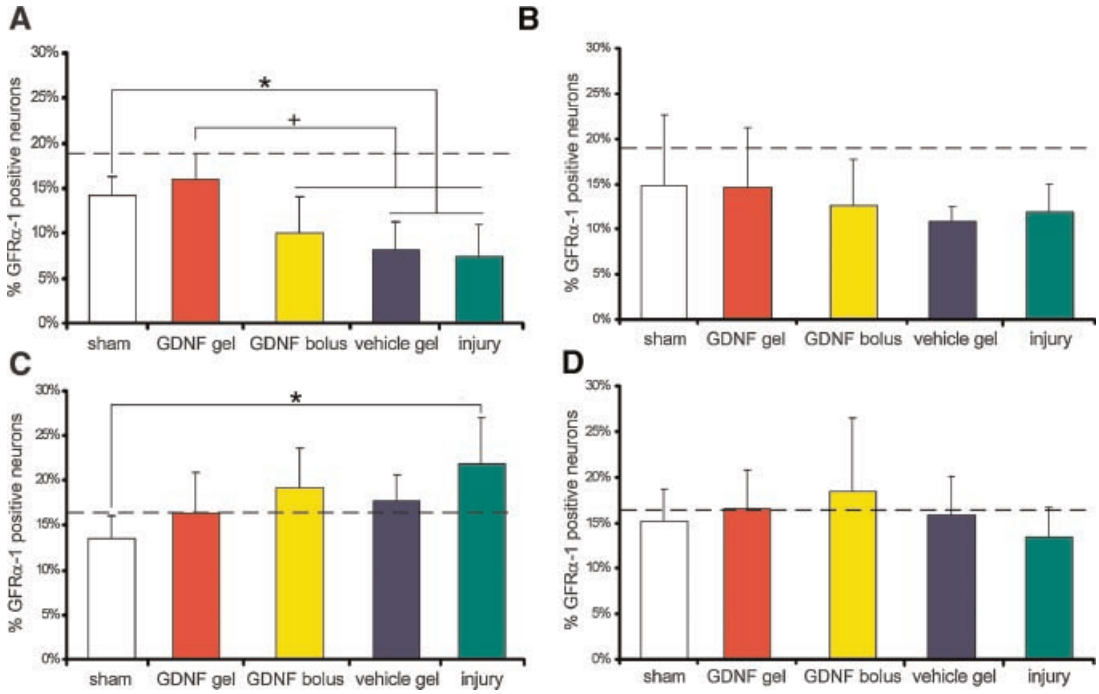

Figure 5. GFRα-1 immunohistochemistry in the (A,C) ipsilateral and (B,D) contralateral DRG, reported as a percent of total (A,B) small (<600 mm^2) or (C,D) large (>600 mm^2) neurons (+SD). The percent of immunopositive neurons in normal rat DRGs is indicated by the horizontal dashed line. (A) In the ipsilateral DRG, the percent of GFRα-1-positive small neurons was significantly reduced (+) for the *GDNF bolus*, *vehicle gel*, and *injury* groups relative to the *GDNF gel* group. GFRα-1-immunoreactivity small neurons of the *vehicle gel* and *injury* groups was also significantly decreased (*) relative to *sham*. (C) GFRα-1-immunoreactivity in large neurons significantly increased (*) for the *injury* group relative to *sham*.

quantitative analyses of GFRα-1 expression would provide additional insight into the mechanism by which exogenous GDNF-mediated increases in receptor expression prevent persistent pain symptoms.

Mechanical allodynia and GFRα-1-immunoreactivity for the *GDNF bolus* group was significantly different from the *GDNF gel* group in small neurons of the ipsilateral DRG, suggesting that continuous GDNF delivery is necessary to maintain its analgesic effect.[18] Given the short half-life of GDNF in vivo and rapid diffusion into adjacent tissues, hydrogel delivery allows GDNF to remain at the injury site during hydrogel degradation, whereas even high doses administered by bolus injection are quickly cleared or metabolized.[48] For example, while the burst release of ~3 μg of GDNF from a hydrogel over the 1st day provides continuous dosing, a 5 μg bolus injection provides twice the dosage within minutes. Furthermore, bolus injection poses the risk of toxic side effects such as focal cell loss, pia thickening, Schwann cell hyperplasia, and ingrowth of sympathetic fibers resulting from a high dose of local delivery.[38,48,49] Repeated administration of lower doses could replicate mechanical allodynia produced by the sustained delivery of a hydrogel or minipump. While repeated injections would more clearly define temporal profiles in vivo, the hydrogel delivery provides simplified treatment with alterable release kinetics eliminating multiple treatments or implantation of minipumps.[18,22,50,51] Hydrogels can be delivered with light-initiated photopolymerization or through two-component redox initiating systems, where gelation occurs in vivo after the two initiators are mixed, avoiding the need for surgical implantation.[35] This is important when light cannot reach the injury site. Degradable PEG hydrogels have been used to deliver neurotrophic factors to stimulate neurite growth and restore motor function in spinal cord injury models without toxic effects,[35,38–41] but, to our knowledge, this study is the first to use the controlled release of GDNF to alleviate nerve root-mediated pain.

Although allodynia was significantly reduced by GDNF, early allodynia was unaffected. Continuous GDNF delivery via osmotic minipumps reduces behavioral hypersensitivity as early as day 1.[18] This disparity may be explained by differences in delivery and dosing paradigms. In our study, 5 μg of GDNF was released over 7 days, with less than 3 μg delivered in the 1st day. Boucher et al. delivered 12 μg per day continuously at a rate of 0.5 μg/h.[18] This fourfold increase over the 1st day may have been sufficient to reduce early hypersensitivity, while the cumulative effect in our study did not reduce allodynia until the 3rd day. Cell numbers and process formation in cells incubated in free GDNF or hydrogel-encapsulated GDNF were comparable (Fig. 3D), indicating biological activity of encapsulated GDNF over 1 week. However, in vitro bioactivity evaluation must be interpreted as an estimated comparison because of the continuous release of GDNF from hydrogels over time. The release profile and biological activity of GDNF determined in vitro may not match in vivo release kinetics and activity. Nonetheless, hydrogel delivery substantially reduced bilateral behavioral sensitivity (Fig. 4). The improved analgesia following hydrogel delivery compared to a bolus injection further suggests efficacy of this method. Future studies involving repeated GDNF injections at different doses may indirectly provide insight into the in vivo release kinetics of GDNF.

In summary, controlled GDNF release from degradable hydrogels following transient compression of the cervical dorsal root alleviated bilateral behavioral hypersensitivity and prevented GFRα-1 receptor depletion in the DRG. Despite no evidence of altered GDNF-immunoreactivity in the DRG 7 days following injury or treatment, increased GFRα-1 expression in large neurons and decreased GFRα-1 expression in nociceptive primary afferents may have been responsible for the pain symptoms. This shift in receptor expression may have decreased the analgesic potential of GDNF, which was reversed by administration of excess exogenous GDNF from a degradable hydrogel. Controlled GDNF release from degradable PEG hydrogels in this radiculopathy model proved effective as a simple technique to deliver trophic factors continuously and directly to the injury site. These data suggest that hydrogel delivery provides significant trophic support to damaged primary afferents and is a promising treatment modality for nerve root compression-mediated pain.

ACKNOWLEDGMENTS

We thank Dr. Meaney and his lab for neuron cultures, and recognize funding from NIH (#AR047564), NSF, and the Sharpe and Ashton Foundations.

REFERENCES

1. Côté P, Cassidy JD, Carroll L. 2000. The factors associated with neck pain and its related disability in the Saskatchewan population. Spine 25:1109–1117.
2. Freeman MD, Croft AC, Rossignol AM, et al. 1999. A review and methodologic critique of the literature refuting whiplash syndrome. Spine 24:86–96.
3. Carter JW, Mirza SK, Tencer AF, et al. 2000. Canal geometry changes associated with axial compressive cervical spine fracture. Spine 25:46–54.
4. Torg JS, Guille JT, Jaffe S. 2002. Injuries to the cervical spine in American football players. J Bone Joint Surg [Am] 84:112–122.
5. Nuckley DJ, Konodi MA, Raynak GC, et al. 2002. Neural space integrity of the lower cervical spine: effect of normal range of motion. Spine 27:587–595.
6. Colburn RW, Rickman AJ, DeLeo JA. 1999. The effect of site and type of nerve injury on spinal glial activation and neuropathic pain behavior. Exp Neurol 157:289–304.
7. Winkelstein BA, Rutkowski MD, Sweitzer SM, et al. 2001. Nerve injury proximal or distal to the DRG induces similar spinal glial activation and selective cytokine expression but differential behavioral responses to pharmacologic treatment. J Comp Neurol 439:127–139.
8. Hubbard RD, Winkelstein BA. 2005. Transient cervical nerve root compression in the rat induces bilateral forepaw allodynia and spinal glial activation: mechanical factors in painful neck injuries. Spine 30:1924–1932.

9. Rothman SM, Kreider RA, Winkelstein BA. 2005. Spinal neuropeptide responses in persistent and transient pain following cervical nerve root injury. Spine 30:2491–2496.
10. Curatolo M, Petersen-Felix S, Arendt-Nielsen L, et al. 2001. Central hypersensitivity in chronic pain after whiplash injury. Clin J Pain 17:306–315.
11. Kobayashi S, Yoshizawa H, Hachiya Y, et al. 1993. Vasogenic edema induced by compression injury to the spinal nerve root. Distribution of intravenously injected protein tracers and gadolinium-enhanced magnetic resonance imaging. Spine 18:1410–1424.
12. Kobayashi S, Yoshizawa H. 2002. Effect of mechanical compression on the vascular permeability of the dorsal root ganglion. J Orthop Res 20:730–739.
13. Watkins LR, Maier SF, Goehler LE. 1995. Immune activation: the role of pro-inflammatory cytokines in inflammation, illness responses and pathological pain states. Pain 63:289–302.
14. Igarashi T, Yabuki S, Kikuchi S, et al. 2005. Effect of acute nerve root compression on endoneurial fluid pressure and blood flow in rat dorsal root ganglia. J Orthop Res 23:420–424.
15. Hubbard RD, Chen Z, Winkelstein BA. 2008. Transient cervical nerve root compression modulates pain: load thresholds for allodynia and sustained changes in spinal neuropeptide expression. J Biomech 41:677–685.
16. Ma C, LaMotte RH. 2007. Multiple sites for generation of ectopic spontaneous activity in neurons of the chronically compressed dorsal root ganglion. J Neurosci 27:14059–14068.
17. Wehling P, Cleveland SJ, Heininger K, et al. 1996. Neurophysiologic changes in lumbar nerve root inflammation in the rat after treatment with cytokine inhibitors. Evidence for a role of interleukin-1. Spine 21:931–935.
18. Boucher TJ, Okuse K, Bennett DL, et al. 2000. Potent analgesic effects of GDNF in neuropathic pain states. Science 290:124–127.
19. Boucher T, McMahon S. 2001. Neurotrophic factors & neuropathic pain. Curr Opin Pharmacol 1:66–72.
20. Airaksinen MS, Saarma M. 2002. The GDNF family: signalling, biological functions and therapeutic value. Nat Rev Neurosci 3:383–394.
21. Ma W, Eisenach JC. 2002. Morphological and pharmacological evidence for the role of peripheral prostaglandins in the pathogenesis of neuropathic pain. Eur J Neurosci 15:1037–1047.
22. Wang R, Guo W, Ossipov MH, et al. 2003. Glial cell line-derived neurotrophic factor normalizes neurochemical changes in injured dorsal root ganglion neurons and prevents the expression of experimental neuropathic pain. Neuroscience 121:815–824.
23. Jang J, Nam T, Paik K, et al. 2004. Involvement of peripherally released substance P and calcitonin gene-related peptide in mediating mechanical hyperalgesia in a traumatic neuropathy model of the rat. Neurosci Lett 360:129–132.
24. Bennett DL, Michael GJ, Ramachandran N, et al. 1998. A distinct subgroup of small DRG cells express GDNF receptor components and GDNF is protective for these neurons after nerve injury. J Neurosci 18:3059–3072.
25. Nakamura S, Myers RR. 1999. Myelinated afferents sprout into lamina II of L3-5dorsal horn following chronic constriction nerve injury in rats. Brain Res 818:285–290.
26. Malcangio M, Getting SJ, Grist J, et al. 2002. A novel control mechanism based on GDNF modulation of somatostatin release from sensory neurones. FASEB J 16:730–732.
27. Takahashi N, Nagano M, Suzuki H, et al. 2003. Expression changes of glial cell line-derived neurotrophic factor in a rat model of neuropathic pain. J Med Dent Sci 50:87–92.
28. ten Bokum AM, Hofland LJ, van Hagen PM. 2000. Somatostatin and somatostatin receptors in the immune system: a review. Eur Cytokine Netw 11:161–176.
29. Bennett DL, Boucher TJ, Armanini MP, et al. 2000. The glial cell line-derived neurotrophic factor family receptor components are differentially regulated within sensory neurons after nerve injury. J Neurosci 20:427–437.
30. Lu P, Yang H, Jones LL, et al. 2004. Combinatorial therapy with neurotrophins and cAMP promotes axonal regeneration beyond sites of spinal cord injury. J Neurosci 24:6402–6409.
31. Zhou L, Baumgartner BJ, Hill-Felberg SJ, et al. 2003. Neurotrophin-3 expressed in situ induces axonal plasticity in the adult injured spinal cord. J Neurosci 23:1424–1431.
32. Anseth KS, Metters AT, Bryant SJ, et al. 2002. In situ forming degradable networks and their application in tissue engineering and drug delivery. J Controlled Release 78:199–209.
33. Goraltchouk A, Scanga V, Morshead CM, et al. 2006. Incorporation of protein-eluting micospheres into biodegradable nerve guidance channels for controlled release. J Controlled Release 110:400–407.
34. Nomura H, Katayama Y, Shoichet MS, et al. 2006. Complete spinal cord transection treated by implantation of a reinforced synthetic hydrogel channel results in syringomyelia and caudal migration of the rostral stump. Neurosurgery 59:183–192.
35. Piantino J, Burdick JA, Goldberg D, et al. 2006. An injectable, biodegradable hydrogel for trophic factor delivery enhances axonal rewiring and improves performance after spinal cord injury. Exp Neurol 201:359–367.
36. Sawhney A, Pathak C, Hubbell J. 1993. Bioerodible hydrogels based on photopolymerized poly(ethylene glycol)-co-poly (alpha-hydroxy acid) diacrylate macromers. Macromolecules 26:581–587.
37. West JL, Hubbell JA. 1995. Photopolymerized hydrogel materials for drug-delivery applications. Reactive Polymers 25:139–147.
38. Burdick JA, Ward M, Liang E, et al. 2006. Stimulation of neurite outgrowth by neurotrophins delivered from degradable hydrogels. Biomaterials 27:452–459.
39. Lee AC, Yu VM, Lowe JB, et al. 2003. Controlled release of nerve growth factor enhances sciatic nerve regeneration. Exp Neurol 184:295–303.
40. Taylor S, McDonald J, Sakiyama-Elbert S. 2004. Controlled release of neurotrophin-3 from fibrin gels for spinal cord injury. J Control Release 98:281–294.
41. Taylor S, Rosenzweig E, McDonald J, et al. 2006. Delivery of neurotrophin-3 from fibrin enhances neuronal fiber sprouting after spinal cord injury. J Control Release 113:226–235.
42. Hubbard RD, Winkelstein BA. 2008. Nerve root compression produces axonal degeneration dependent on biomechanical thresholds for mechanical allodynia. Exp Neurol (in press).
43. Pong K, Xu RY, Beck KD, et al. 1997. Inhibition of glial cell line-derived neurotrophic factor induced intracellular activity by K-252b on dopaminergic neurons. J Neurochem 69:986–994.
44. Pozas E, Ibanez CF. 2005. GDNF and GFRalpha1 promote differentiation and tangential migration of cortical GABAergic neurons. Neuron 45:701–713.
45. Park S, Hong YW. 2006. Transcriptional regulation of artemin is related to neurite outgrowth and actin polymerization in mature DRG neurons. Neurosci Lett 404:61–66.
46. Ramer MS, Priestley JV, McMahon SB. 2000. Functional regeneration of sensory axons into the adult spinal cord. Nature 403:312–316.
47. Sajadi A, Bensadoun JC, Schneider BL, et al. 2006. Transient striatal delivery of GDNF via encapsulated cells leads to

sustained behavioral improvement in a bilateral model of Parkinson disease. Neurobiol Dis 22:119–129.
48. O'Malley BW Jr, Ledley FD. 1993. Somatic gene therapy. Methods for the present and future. Arch Otolaryngol Head Neck Surg 119:1100–1107.
49. Boyd RB, Hovland DN. 2004. Intraputamenal infusion of r-metHUGDNF for the treatment of Parkinson's disease. Gordon Research Conferences-Barriers of the CNS, Tilton, NH, June 27–July 2.
50. Xu XM, Guenard V, Kleitman N, et al. 1995. A combination of BDNF and NT-3 promotes supraspinal axonal regeneration into Schwann cell grafts in adult rat thoracic spinal cord. Exp Neurol 134:261–272.
51. Averill S, Michael GJ, Shortland PJ, et al. 2004. NGF and GDNF ameliorate the increase in ATF3 expression which occurs in dorsal root ganglion cells in response to peripheral nerve injury. Eur J Neurosci 19:1437–1445.