

Preliminary study of a genetically engineered spinal cord implant on urinary bladder after experimental spinal cord injury in rats

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Abstract—The objective of this study was to determine the effect of neurotrophin-secreting Schwann cell implants on the urinary bladder after spinal cord contusion. One hour after severe spinal cord contusion at the T8 to T11 level, carbon filaments containing nonsecreting Schwann cells, brain-derived neurotrophic factor (BDNF)-secreting Schwann cells, neurotrophin-3 (NT-3)-secreting Schwann cells, or Schwann cells secreting both BDNF and NT-3 were implanted into the spinal cord. Untreated spinal cord injured (SCI) rats and noncontused rats (C) were also studied. Two months after spinal cord injury, cystometry was performed and the bladders were studied using light microscopy. SCI rats had significantly increased bladder mass, thickness, and smooth muscle mass compared to C rats. Bladder capacity of SCI rats and rats with spinal cord implants were both significantly greater than that of C rats. This preliminary study suggests that neurotrophin-secreting Schwann cell implants may lead to improved bladder structure after spinal cord injury.

Key words: *bladder, capacity, compliance, nerve regeneration, rat, spinal cord injury, structure.*

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INTRODUCTION

Spinal cord injury (SCI) has an incidence of 23 to 55 injuries per million per year in the United States and is associated with significant urinary tract complications, which can lead to renal dysfunction and eventually renal failure [1,2]. Therefore, preservation of the urinary bladder is important in patients with SCI.

The central nervous system (CNS) has regenerative capacities under appropriate conditions. However, the necessary substances to initiate nerve regeneration are suppressed or absent in adult mammals [3]. Many studies have investigated the effects of neurotrophic and other factors on functional neuroregeneration [4–7], yet it remains to be determined if spinal cord regenerative treatments lead to improved bladder function after SCI.

We have had extensive experience studying a rat model of severe spinal cord contusion at the T8 to T11 level [8,9]. As with other models, untreated contused rats do not regain the ability to void to completion and need to have their bladders palpated to empty [10]. We obtained anecdotal data suggesting that contused rats treated with implanted carbon filaments embedded with neurotrophin-secreting Schwann cells regain some ability to void. This study was undertaken to determine the extent of changes in bladder structure after treatment with neurotrophin-secreting Schwann cells and carbon filament implantation following severe spinal cord contusion. We expected that the treatments would prevent some of the adverse effects of spinal cord contusion on the urinary bladder.

METHODS

Production of Implant

Retroviral vectors for brain-derived neurotrophic factor (BDNF) and neurotrophin-3 (NT-3) were constructed as previously described [11]. In brief, viruses were generated by transfection of PA 317 retroviral packaging cells with the plasmid forms of the retroviral vectors of BDNF and NT-3 [12]. The human Schwann cell line, NF-IT, was infected with the viruses released by the PA 317 retroviral packaging cells to produce Schwann cells that secrete either BDNF or NT-3 or both [11]. Immunocytochemical staining and ELISA results were used to demonstrate the secretion of neurotrophins by the Schwann cells [11]. Nonsecreting Schwann cells were used as a sham control group.

The carbon filament implants consisted of a bundle of approximately 10,000 5 μm to 7 μm in diameter, 5 mm long, carbon filaments (ThorneI™ Carbon Fibers, Amoco Performance Products, Parma, OH) [13]. Twenty-four hours before implantation, 400,000 neurotrophin-secreting Schwann cells contained in 1 μL serum-free media were cultured on the bundle of approximately 10,000 carbon filaments, which were attached to the bottom of a Petri dish (Figure 1).

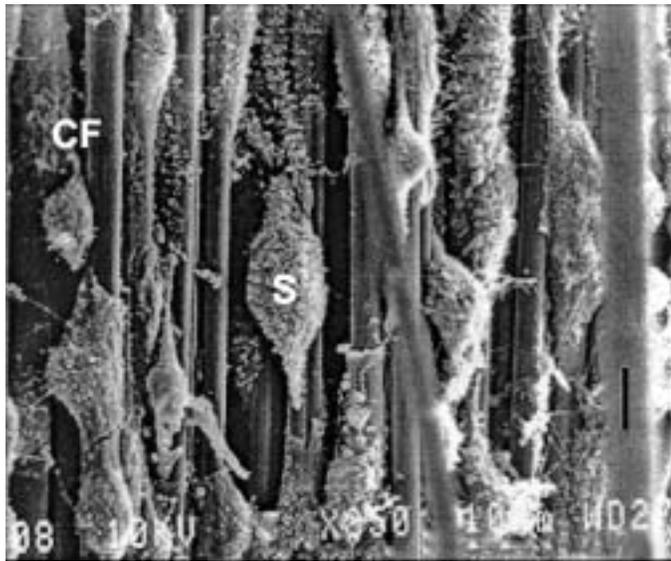


Figure 1. Scanning electron microscopy of Schwann cells (S) cultured on carbon filaments (CF) for 24 hours. Note that Schwann cells grow with same orientation as carbon filaments. Bar = 10 μm .

Creation of Severe Spinal Cord Contusion

We anesthetized the spinal cords of 24 adult female Sprague-Dawley rats (300 g) with an intramuscular injection of xylazine (8.5 mg/kg) and ketamine (120 mg/kg). Severe spinal cord contusion was created, as has been previously described [8]. In brief, using a posterior approach, we dissected the T8 to T11 vertebrae from the surrounding tissue. To assure that the spinal column was rigid, we suspended the animal by two adjustable vertebral clamps on the spinous process: rostral and caudal to the laminectomy site. A laminectomy was then performed at the T9 to T10 level. The Teflon impounder head of the contusion device, having a concave contour to match the contour of spinal cord, was placed directly in contact with the dura. To produce a severe contusion injury, we raised a 10-g weight to 18 cm above the impounder head and then released it. These weights and heights were selected to produce a severe contusion injury in rats based on previously published data [8]. Spinal cord contusion resulting from these methods is severe and reproducible: both rats and cats have been shown to be completely paraplegic, with no recovery of behavioral or electrophysiological function throughout the survival period [8].

One to two hours after contusion, we incised the dura mater longitudinally at the lesion site and performed a mid-dorsal myelotomy, as previously described [9]. Hemorrhagic and edematous tissue were removed by gentle aspiration or with a cotton tip applicator. A 0.5- to 1.0-cm cavity was formed after we removed all necrotic tissue from the lesion site. One carbon filament and Schwann-cell implant was placed into the lesion cavity, with the filaments oriented in parallel with the spinal cord.

The animals were implanted with carbon filaments containing either nonsecreting Schwann cells (NS; $n = 7$), BDNF-secreting Schwann cells (BDNF; $n = 4$), NT-3-secreting Schwann cells (NT-3; $n = 7$), or Schwann cells secreting both BDNF and NT-3 (BNT; $n = 3$). Three animals underwent severe spinal cord contusion but had no carbon filament implant (SCI). After implantation, we closed the dura using a 7 to 0 suture. Eight animals were used as age-matched noncontused controls (C). All animals received daily care in accordance with the Association for Assessment and Accreditation of Laboratory Animal Care (AAALAC) guidelines, and their bladders were palpated to empty twice a day.

Cystometry Testing

Two months after spinal cord contusion, we anesthetized the rats with urethane (1.2 g/kg) intraperitoneally (i.p.). Unlike ketamine/xylazine anesthesia, the animals retain voiding reflexes when anesthetized with urethane [14,15]. We catheterized the bladder through the urethra using PE-50 tubing. The catheter was attached, via a stopcock, to both a pressure transducer (Statham, P23XL) and a flow pump (KD Scientific, model 100). Pressure signals during bladder filling were amplified (World Precision Instruments, TBM4) and recorded on a computer at 10 samples/second (Labview, National Instruments: Micron Pentium, Windows 95). We drained the bladder and filled it with saline via the catheter at 5 mL/h for noncontused controls and 20 mL/h for all contused rats, since contused rats' bladders were expected to be significantly larger than the bladders of noncontused rats [10]. Results from each rat are a mean of 2 to 3 cystometrograms.

Bladder capacity was calculated as the volume filled when the rat leaked or voided saline around the catheter. If no bladder contraction or leakage occurred, the volume when bladder pressure reached twice plateau pressure was used as a measure of capacity [16,17]. If the bladder filled for 45 minutes (15 mL) with no void or leak and little pressure rise, we stopped the filling. Baseline bladder pressure (between spontaneous contractions) at 80 percent capacity was used to compare filling bladder pressures between groups. Bladder compliance at 80 percent capacity was calculated as bladder volume at 80 percent capacity divided by baseline bladder pressure at that volume [17].

Light Microscopy

After cystometry testing, we removed the bladders through a midline abdominal incision and emptied, blotted dry, and weighed them. The bladders were filled with saline (1 mL/100 mg) and immersion-fixed in phosphate-buffered paraformaldehyde (4 percent) and glutaraldehyde (2.5 percent). The volume of saline used was calculated to provide a standardized level of distension to all bladders [18]. The bladders were embedded in araldite, stained with toluidine blue, and sectioned with a glass knife (1 μ m) for light microscopy and bladder wall thickness measurement. We measured microscopically the total bladder wall thickness and smooth muscle thickness from cross sections of the bladder wall. Percentage of smooth muscle in the bladder wall was calculated as

$$100 \times \text{smooth muscle thickness} \div \text{total bladder wall thickness.}$$

Smooth muscle mass was calculated as bladder mass \times percentage of smooth muscle \div 100.

Data Analysis

All results are presented as mean \pm standard error. We used a one-way analysis of variance (ANOVA) followed by a Student-Newman-Keuls test to compare the different experimental groups. We also compared all groups with spinal contusion, treated with carbon filaments (NS, BDNF, NT-3, and BNT) with each other using a one-way ANOVA. If there was no significant difference, they were combined into one group of treated contused rats (TREATED) for further comparison to noncontused control (C) and untreated contused (SCI) groups. A statistically significant difference was determined by $p < 0.05$.

RESULTS

Untreated contused rats (SCI) had significantly greater bladder weights ($1,616 \pm 167$ mg) than noncontused rats (174 ± 26 mg). Although there was no significant difference in bladder weight between the treated groups, those rats treated with BDNF had the lowest bladder weight (701 ± 83 mg) of the treated rats. One rat in the NT-3 group had a high bladder weight (5,679 mg), increasing the mean and standard errors for that group ($1,354 \pm 727$ mg). When the treated rats were combined into one group (TREATED), they had bladder weights (997 ± 257 mg) between those of SCI and C rats and were not significantly different from either group (**Figure 2**).

SCI rats had greatly increased bladder capacity with little ability to generate bladder contractions (**Figure 3**). In general, the treated rats had large bladder capacities, similar to SCI rats, but were more likely to generate spontaneous bladder contractions (**Figure 3**), most of which were nonvoiding contractions.

Similar to bladder weight, bladder capacity of SCI rats was the greatest (11.1 ± 2.1 mL) and capacity of C rats was the least (1.6 ± 0.3 mL) (**Figure 4(a)**). Although there was no significant difference in bladder capacity between the treated groups, those rats treated with both BDNF and NT-3 (BNT) had the lowest bladder capacity (7.3 ± 1.6 mL) of the treated rats. Both SCI and all TREATED rats (8.9 ± 0.8 mL) had significantly greater bladder capacity than C rats.

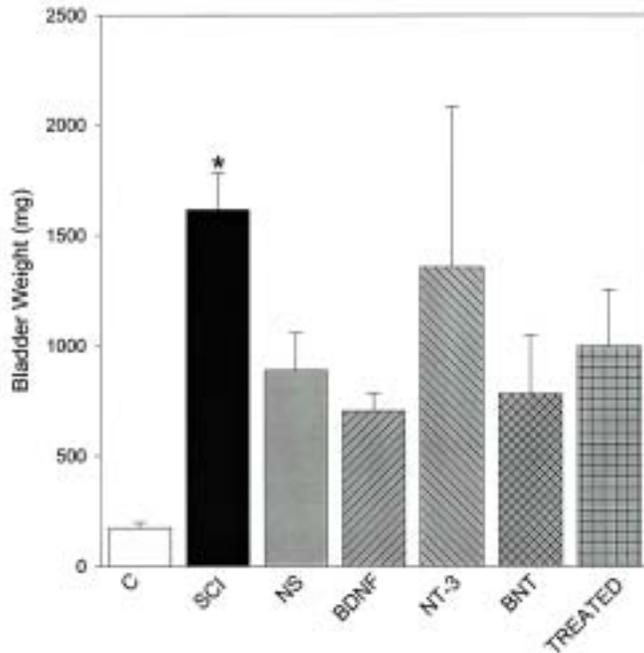


Figure 2.

Bladder weights of noncontused control rats (C), rats with untreated spinal contusion (SCI), contused rats with carbon filament implant containing nonsecreting Schwann cells (NS), contused rats with carbon filament implant containing BDNF-secreting Schwann cells (BDNF), contused rats with carbon filament implant containing NT-3-secreting Schwann cells (NT-3), contused rats with carbon filament implant containing BDNF and NT-3-secreting Schwann cells (BNT), and all contused rats treated with carbon filament implants in one group (TREATED). * indicates statistically significant difference compared to noncontused controls. Each bar represents mean \pm standard error on data from 3 to 21 rats.

C rats had significantly lower baseline bladder pressure at 80 percent capacity (12.2 ± 2.0 cm H₂O) than all other groups (**Figure 4(b)**). BNT rats had significantly greater baseline bladder pressure at 80 percent capacity (36.8 ± 2.9 cm H₂O) than both C rats and all other treated groups of rats, so this variable was not combined to form a group of all TREATED rats. There was no significant difference in pressure between BNT rats and SCI rats (28.5 ± 2.6 cm H₂O).

Since capacity increased to a greater extent than bladder pressure, SCI rats had increased compliance (**Figure 4(c)**). There was no significant difference in compliance between SCI and C rats. When combined into a single group, TREATED rats had significantly greater bladder compliance (0.39 ± 0.08 mL/cm H₂O) than C rats (0.13 ± 0.03 mL/cm H₂O). Although there was no significant dif-

ference in compliance between the treated groups, BNT rats had the lowest bladder compliance of the treated rats (0.16 ± 0.04 mL/cm H₂O), lower than that of SCI rats (0.31 ± 0.03 mL/cm H₂O). When individual treated groups were compared to the C group, the NS and BDNF groups had significantly greater compliance than the C group, as did the entire TREATED group.

Light microscopy demonstrated that abundant bundles of smooth muscle could be found in control bladders (**Figure 5(a)**). In these bladders, the urothelium is thin and distended. In contrast, SCI bladders undergo a large increase in bladder wall thickness, primarily from an increase in smooth muscle and urothelium (**Figure 5(b)**). The relative amount of smooth muscle appears to increase at the expense of the stroma. Bladders from rats in most of the different treatment groups were not visually different from each other (**Figure 5(c)** and **5(d)**), but they were distinguishable from the SCI bladders. Treated bladders had bladder wall thickness, urothelial thickness, and smooth muscle thickness greater than controls but not as great as SCI bladders (**Figure 5(c)** to **(e)**). The cross-sectional area of smooth muscle cells was noticeably smaller in treated bladders than in SCI bladders. The hypertrophy of the smooth muscle was not as pronounced in the BNT bladders (**Figure 5(e)**) as it was in the other treated bladders. The urothelium, however, increased to the same extent in all treated bladders.

The quantitative results of bladder wall thickness paralleled those of bladder weight. Bladders from SCI rats were significantly thicker than bladders from C rats (see **Table**). Although there was no significant difference in bladder wall thickness between the treated groups, those rats treated with BDNF had the lowest bladder wall thickness of the treated rats. When combined into a single group, TREATED rats had bladder wall thickness between that of SCI and C rats and were not significantly different from either group (see **Table**).

Although C rats had the smallest percentage smooth muscle thickness of any of the groups, there was no significant difference between any of the groups (see **Table**). SCI rats had significantly greater smooth muscle mass than C rats. Although there was no significant difference in smooth muscle mass between the treated groups, those rats treated with either BDNF or BDNF and NT-3 (BNT) had the lowest smooth muscle mass of the treated rats. When combined into a single group, TREATED rats had smooth muscle mass between those of SCI and C rats and were not significantly different from either group (see **Table**).

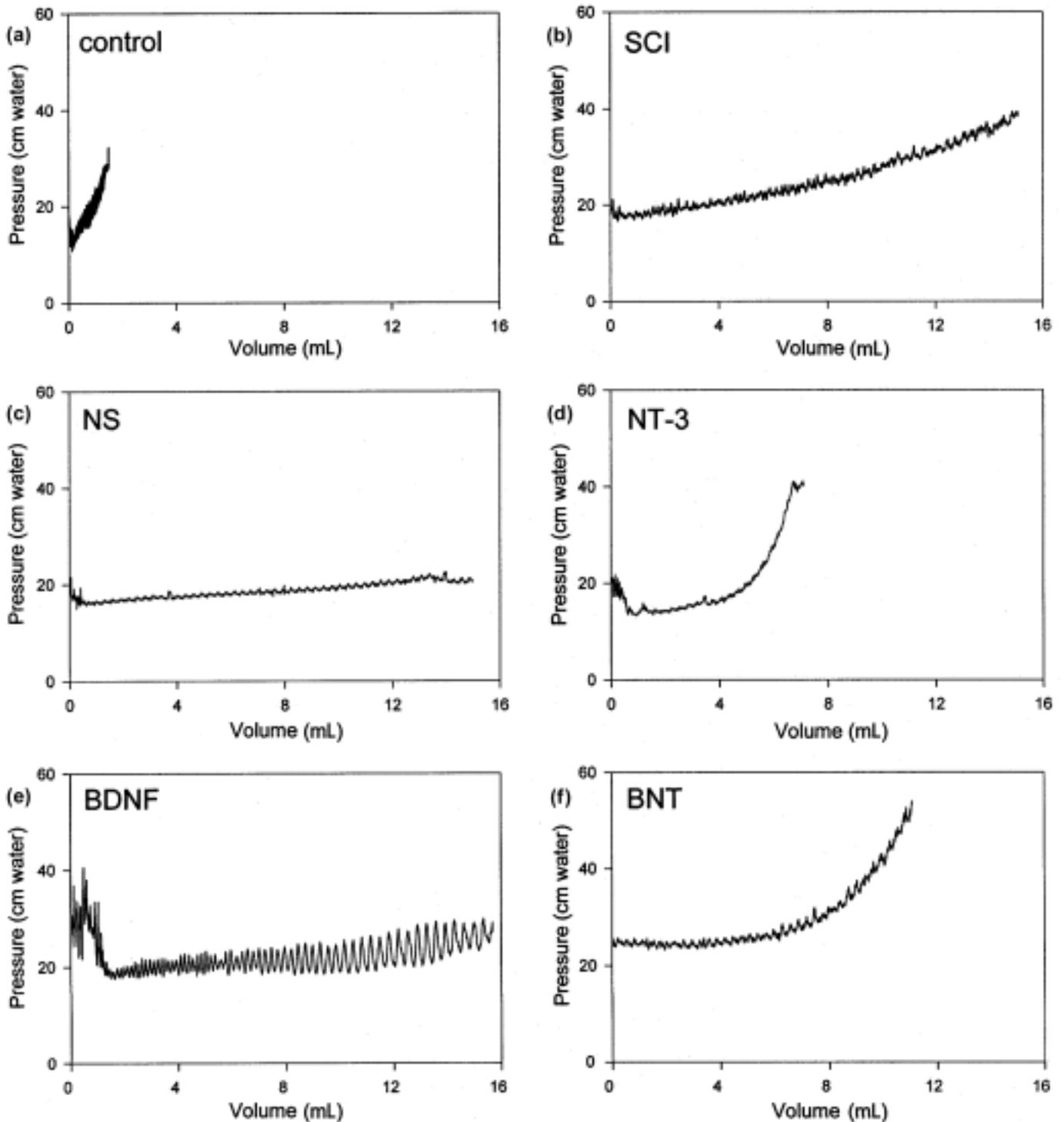


Figure 3.

Example cystometries of bladders from (a) control rat, (b) rat with untreated spinal contusion (SCI), (c) contused rat with carbon filament implant containing nonsecreting Schwann cells (NS), (d) contused rat with carbon filament implant containing NT-3-secreting Schwann cells (NT-3), (e) contused rat with carbon filament implant containing BDNF-secreting Schwann cells (BDNF), and (f) contused rat with carbon filament implant containing BDNF and NT-3-secreting Schwann cells (BNT).

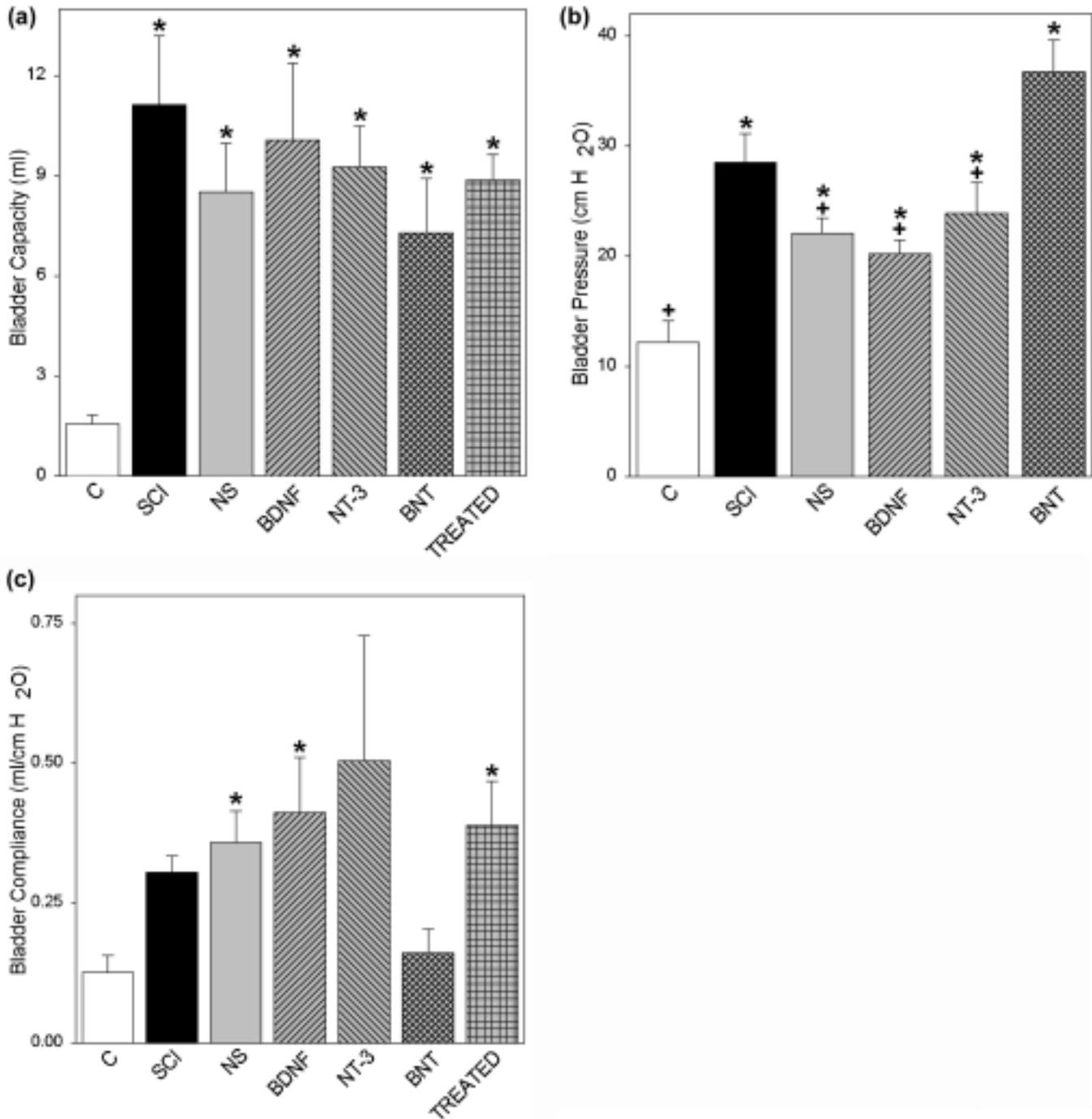


Figure 4.

(a) Bladder capacity, (b) bladder pressure at 80 percent capacity, and (c) bladder compliance at 80% capacity of noncontused control rats (C), rats with untreated spinal contusion (SCI), contused rats with carbon filament implant containing nonsecreting Schwann cells (NS), contused rats with carbon filament implant containing BDNF-secreting Schwann cells (BDNF), contused rats with carbon filament implant containing NT-3-secreting Schwann cells (NT-3), contused rats with carbon filament implant containing BDNF and NT-3-secreting Schwann cells (BNT), and all contused rats treated with carbon filament implants in one group (TREATED). * indicates statistically significant difference compared to noncontused controls. Each bar represents mean \pm standard error on data from 3 to 21 rats. + indicates statistically significant difference compared to BNT group. Bladder pressure from all treated groups was not combined because of statistically significant differences between different groups.

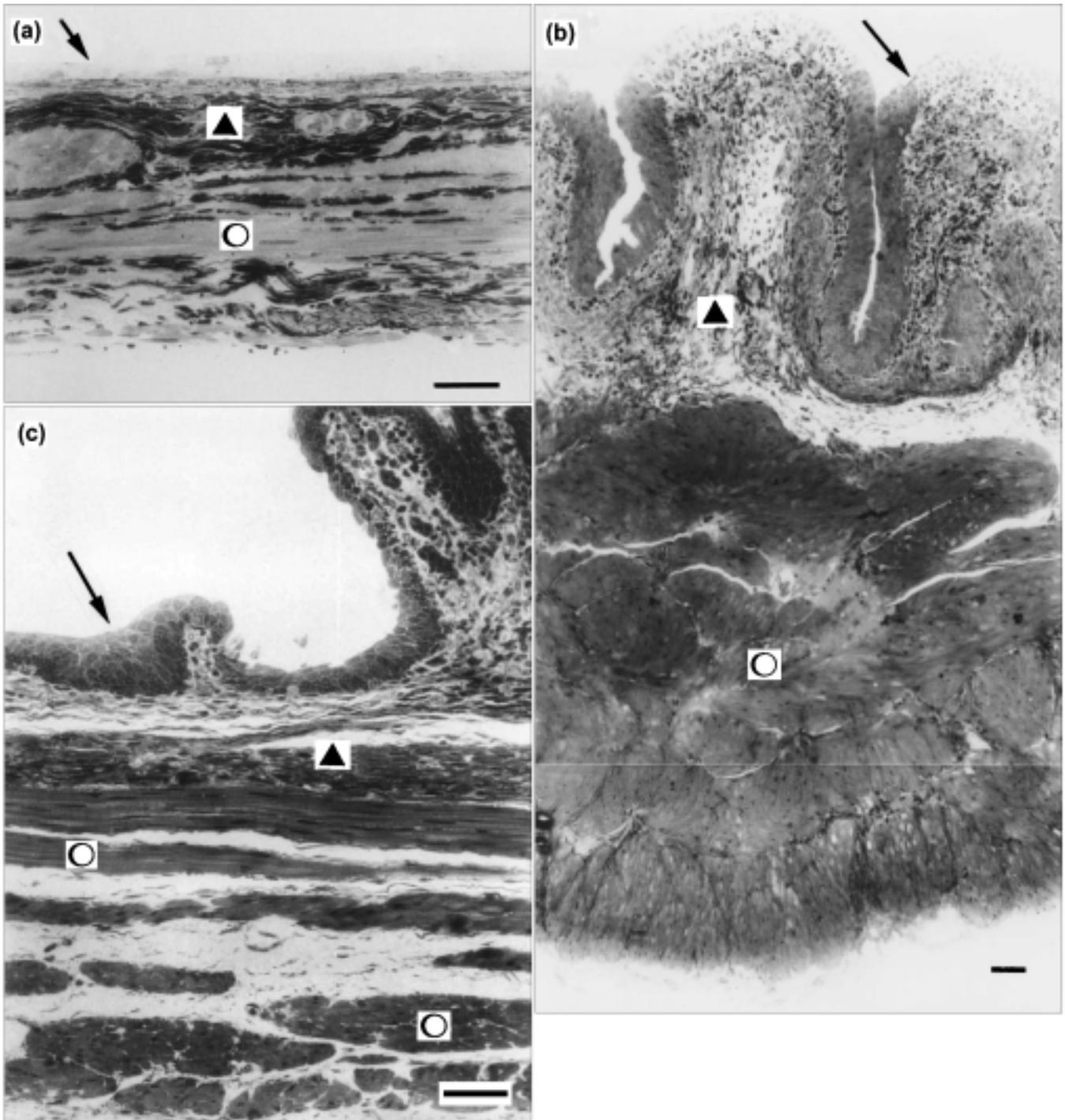


Figure 5.

Examples of light microscopy of bladders from (a) control rat, (b) rat with untreated spinal contusion, and (c) contused rat with carbon filament implant containing nonsecreting Schwann cells. → = urothelium; ▲ = submucosa; ○ = smooth muscle. Bar = 50 μm.

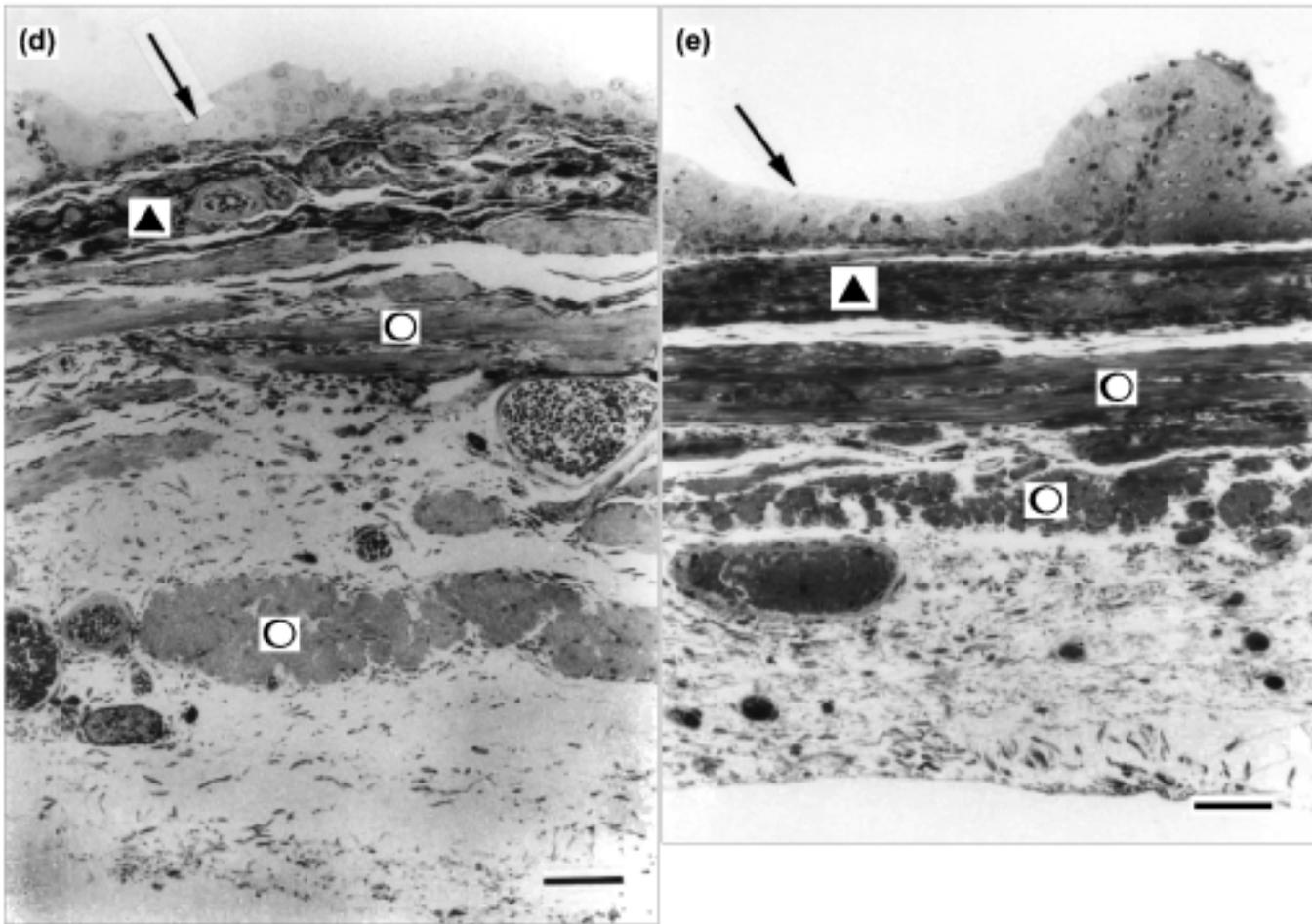


Figure 5. (Continued).

Examples of light microscopy of bladders from (d) contused rats with carbon filament implant containing BDNF-secreting Schwann cells and (e) contused rats with carbon filament implant containing BDNF and NT-3-secreting Schwann cells. → = urothelium; ▲ = submucosa; ○ = smooth muscle. Note: Increased thickness of bladder wall and smooth muscle layers in all contused bladders compared with noncontused control. Bar = 50 μ m.

Table.

Morphological properties of rat bladders with spinal cord contusion and neurotrophin-secreting carbon filaments.

	C	SCI	NS	BDNF	NT-3	BNT	TREATED
Bladder thickness (μ m)	140 \pm 20	627 \pm 286*	319 \pm 50	285 \pm 48	359 \pm 61	335 \pm 70	328 \pm 28
Percent smooth muscle in bladder wall	35 \pm 2	40 \pm 10	43 \pm 3	39 \pm 6	47 \pm 5	38 \pm 11	43 \pm 3
Smooth muscle mass in bladder (mg)	0.6 \pm 0.05	6.2 \pm 0.9*	3.6 \pm 0.4	2.6 \pm 0.1	4.9 \pm 2.0	2.3 \pm 0.1	3.7 \pm 0.7

Data is presented as mean \pm standard error of mean

C = noncontused controls (n = 8)

SCI = contused untreated rats (n = 3)

NS = contused rats treated with carbon filaments and nonsecreting Schwann cells (n = 7)

BDNF = contused rats treated with carbon filaments and BDNF secreting Schwann cells (n = 4)

NT-3 = contused rats treated with carbon filaments and NT-3 secreting Schwann cells (n = 7)

BNT = contused rats treated with carbon filaments and Schwann cells secreting both BDNF and NT-3 (n = 3)

TREATED = all treated groups taken together (n = 21)

*Indicates statistically significant difference compared to noncontused controls (C)

DISCUSSION

Because of the damage to motoneurons and reflex nerve activity, SCI patients often have bladder hyperreflexia (uncontrolled bladder contractions), detrusor-sphincter dyssynergia (uncoordinated contractions in the bladder and urethra), and an inability to voluntarily contract the bladder to empty [19–23]. This complex set of symptoms, known as neurogenic bladder, results in high bladder pressures because of the combination of a hyperreflexic bladder contracting against high urethral resistance [24]. Chronic exposure to high bladder pressures causes urine reflux, renal deterioration, and eventually renal failure [2,25]. Renal failure is a significant cause of death in patients with SCI, particularly among those who have had SCI for 20 years or more [26,27].

The CNS has regenerative capacities under appropriate conditions. However, growth inhibitory molecules are present [9,28,29], and the necessary substances to initiate nerve regeneration are suppressed or absent in adult mammals [3]. Neurotrophic factors, such as BDNF and NT-3, have been found to enhance neuroregeneration of injured axons after SCI and can lead to improvement of function [5,30,31]. In particular, motoneurons are responsive to BDNF *in vivo*, and BDNF may influence their survival [32–38]. NT-3 supports proprioceptive neurons projecting to skeletal muscle and somatosensory fibers [39]. Implantation of genetically engineered BDNF-producing fibroblasts into a spinal cord contusion injury site has been shown to enhance functional recovery, as measured by locomotor performance [4,5].

Other studies have shown that Schwann cells can enhance regeneration of axons in a lesioned adult spinal cord [6]. In addition, carbon filaments can provide a favorable adhesive and guiding surface to promote axonal growth across transected spinal cords in adult rats [13]. The purpose of this study was to determine the extent of prevention of adverse effects of spinal cord contusion on the urinary bladder after implantation with carbon filaments embedded with neurotrophin-secreting Schwann cells following severe spinal cord contusion. The untreated SCI bladders had a bladder weight nine times that of noncontused control bladders, a greater increase than observed previously [40–42]. Since bladder weight increases linearly with increasing time postinjury [42], this difference is probably because we observed the rats 2 months postinjury; whereas previous investigators observed rats 2 or 3 weeks [41,42] postin-

jury. TREATED bladders weighed less than six times controls, suggesting that the treatment had a beneficial effect but did not return the bladder to normal size.

Bladder wall thickness in SCI rats was 4.5 times that of control bladders, but smooth muscle mass in the bladder was 10 times that of control bladders. This difference suggests that bladder smooth muscle hypertrophied much more than the urothelium and serosa, as has been observed previously [43]. The TREATED group of bladders had thickness and smooth muscle mass two and six times that of control bladders, respectively, indicating that the smooth muscle hypertrophied only half as much as in the untreated SCI bladders. The BDNF and BNT groups had the smallest increase in bladder mass, bladder thickness, or smooth muscle mass of any of the treated bladders, suggesting that BDNF may provide an optimum of the tested treatments. However, the differences were not significant in this small sample size.

Bladder capacity increased seven times in the untreated SCI group, a similar observation to those by Pikov et al. and Yoshiyama et al. in rats with suprasacral SCI [42,44]. Capacity was 5.5 times control values in the TREATED group, suggesting that the spinal cord implants facilitated a small improvement in these large distended bladders. All bladders were palpated to empty twice a day, which may have strongly influenced capacity. Most likely, to maintain low capacity, both neuroregenerative treatments and acute rehabilitative treatments, such as clean intermittent catheterization, will be necessary to return bladder capacity to normal after clinical SCI.

Bladder pressure varied greatly during filling because of nonvoiding spontaneous contractions. Because of the variation and randomness of the spontaneous contractions, we determined that comparison of the spontaneous contractions between groups would not yield useful results. Therefore, we selected to use baseline bladder pressure at 80 percent capacity to compare baseline bladder pressure and compliance between the different groups. The SCI group had bladder pressure twice that of noncontused controls. The BNT group had the greatest bladder pressure of any group: three times that of controls, and significantly greater than all other treated groups.

The bladder pressure and capacity results were combined to calculate bladder compliance at 80 percent capacity. In contrast to humans with suprasacral SCI, all contused rats had increased compliance compared to controls [45]. This result has been observed previously in

rats with SCI and likely reflects differences in bladder emptying regimens and possibly differences in neural control systems between rats and humans [44]. The BNT group had the lowest compliance of the SCI animals, nearly identical to that of controls, suggesting that BNT may provide an optimal treatment.

We performed this study using anesthetized cystometries and light microscopy on a preliminary group of rats. We did not study voiding, since the urethral catheter creates an obstruction in rats, making resulting pressures inaccurate. Nonetheless, we were able to determine that the spinal cord implants improve bladder structure to some degree but do not return the bladder to normal structure. A larger study with the use of conscious urodynamics is needed to determine the effects of these treatments on urinary bladder function.

CONCLUSIONS

Neurotrophin-secreting Schwann cell spinal cord implants may help improve bladder structure after SCI as illustrated by reduced bladder weight, bladder wall thickness, and smooth muscle mass. A larger study including the use of conscious urodynamics is needed to determine the effects of these treatments on urinary bladder function.

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