Botulinum Toxin Type A for Neuropathic Pain in Patients with Spinal Cord Injury

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Objective: To evaluate the analgesic effect of botulinum toxin type A (BTX-A) on patients with spinal cord injury-associated neuropathic pain.

Methods: The effect of BTX-A on 40 patients with spinal cord injury-associated neuropathic pain was investigated using a randomized, double-blind, placebo-controlled design. A 1-time subcutaneous BTX-A (200U) injection was administered to the painful area. Visual analogue scale (VAS) scores (0–100mm), the Korean version of the short-form McGill Pain Questionnaire, and the World Health Organization WHOQOL-BREF quality of life assessment were evaluated prior to treatment and at 4 and 8 weeks after the injection.

Results: At 4 and 8 weeks after injection, the VAS score for pain was significantly reduced by 18.6 ± 16.8 and 21.3 ± 26.8, respectively, in the BTX-A group, whereas it was reduced by 2.6 ± 14.6 and 0.3 ± 19.5, respectively, in the placebo group. The pain relief was associated with preservation of motor or sensory function below the neurological level of injury. Among the responders in the BTX-A group, 55% and 45% reported pain relief of 20% or greater at 4 and 8 weeks, respectively, after the injection, whereas only 15% and 10% of the responders in the placebo group reported a similar level of pain relief. Improvements in the score for the physical health domain of the WHOQOL-BREF in the BTX-A group showed a marginal trend toward significance ($p = 0.0521$) at 4 weeks after the injection.

Interpretation: These results indicate that BTX-A may reduce intractable chronic neuropathic pain in patients with spinal cord injury.

Pain is one of the most common complications of spinal cord injury (SCI), and the prevalence rates of pain in patients with SCI ranges between 75 and 81%.[1–3] The most common subtype of pain in patients with SCI is neuropathic pain, which is defined as "pain caused by a lesion or disease of the somatosensory system."[4,5] Neuropathic pain is one of the most problematic complications, reducing quality of life and interfering with cognitive, emotional, and physical functioning following SCI.[6,7] In addition, neuropathic pain after SCI is generally severe, refractory to treatment, and persistent over time.[1]

Botulinum toxin (BTX) is commonly used to treat spasticity or dystonia. Recent studies have suggested that

BTX type A (BTX-A) is effective for the treatment of chronic pain conditions such as chronic migraine, postherpetic neuralgia, post-traumatic neuralgia, or diabetic neuropathy.[8–11] It has been suggested that BTX-A may inhibit neurogenic inflammation and the peripheral sensitization of nociceptive fibers by inhibiting the release of local neuropeptides such as substance P, calcitonin gene-related peptide, or glutamate, thereby reducing pain.[12,13] In addition, a retrograde effect of BTX-A on the spinal cord by axonal transport is proposed as the central mechanism of the antinociceptive effect of BTX-A.[14–16] We propose that BTX-A may be effective for the treatment of intractable neuropathic pain in patients with SCI due to these mechanisms. To the best of our knowledge, the

efficacy of BTX-A on neuropathic pain in patients with spinal cord lesions has been suggested in only a few case reports.[17,18]

This study is the first to evaluate the potential effects of BTX-A on neuropathic pain in patients with SCI using a double-blind, placebo-controlled, parallel group design.

Patients and Methods

This study was conducted at St Paul's Hospital, Catholic University of Korea in Seoul, South Korea and at National Health Insurance Service Ilsan Hospital, Gyeonggi-do, South Korea, and it was approved by the institutional review boards of St Paul's Hospital and National Health Insurance Service Ilsan Hospital. The patients were recruited between July 2012 and April 2013, and all patients provided written informed consent before inclusion.

Patients

Patients with SCI were recruited from the community and clinical settings and by referrals from clinicians. The inclusion criteria were (1) men or women > 19 years of age; (2) SCI of any level (American Spinal Injury Association [ASIA] impairment scale A–D); (3) at least 1 year after SCI with no change in the neurological status for at least 6 months; and (4) daily neuropathic pain for at least 3 months (a visual analog scale [VAS; 0–100mm] score ≥ 40 at baseline), with neuropathic pain being defined by the diagnostic criteria proposed by the International Association for the Study of Pain.[5,19] The exclusion criteria were (1) contraindication for BTX-A (eg, myasthenia gravis or other diseases of the neuromuscular junction), (2) hypersensitivity to the BTX-A formulation, (3) coagulation disorders, (4) any other painful condition, and (5) a history of previous BTX-A treatment. Concomitant analgesic medication was authorized, provided that the dose was stable for at least 1 month before enrollment and remained stable throughout the study.

At initial enrollment, we excluded all patients with pain other than those of neuropathic origin. Patients with musculoskeletal (nociceptive) pain in the injected area defined by the International Spinal Cord Injury Pain Basic Data Set, which includes cutaneously induced spasm-related pain, dull or aching pain related to movement, tenderness of musculoskeletal structures on palpation, response to anti-inflammatory medications, and evidence of skeletal pathology on imaging consistent with the pain presentation, were excluded.[20] Dull, aching, or cramping pain located in the thorax or abdomen was considered to be visceral pain and was also excluded. Furthermore, patients with neuropathic pain suspected to be unrelated to SCI (mononeuropathy, postherpetic neuralgia, central poststroke pain, etc) were also excluded during the enrollment process.

Protocol

We conducted an 8-week, randomized, double-blind, placebo-controlled, parallel group study. Figure 1 shows the study timeline. A total of 3 visits were scheduled over 8 weeks (at baseline, and 4 and 8 weeks after injection). Before the study was initiated,
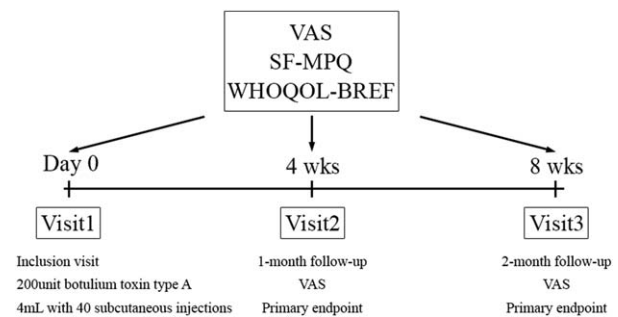

FIGURE 1: Study time line. Injections totaling 200U (in 4ml saline) of botulinum toxin type A were administered subcutaneously in a checkerboard pattern (40 injections) over the maximally affected area. The pain intensity according to the visual analogue scale (VAS), the Korean version of the short-form McGill Pain Questionnaire (SF-MPQ), and the Korean version of the WHOQOL-BREF were evaluated at baseline and 4 and 8 weeks after the injection.

an unmasked statistician who had no involvement with the remainder of the study developed a block randomization scheme (using SAS v9.1; SAS Institute, Cary, NC) to assign eligible patients at a 1:1 ratio to receive injections of BTX-A (Meditoxin [Medytox, Seoul, South Korea], 200U in 4ml) or saline (isotonic sodium chloride [Daihan Pharmaceutical Co, Seoul, South Korea], 4ml). Each device used for this study was identified by a serial number that corresponded to a patient's number in the randomization schedule. The dilution of BTX-A and preparation of syringes was performed by an independent nonblinded pharmacist. The placebo syringes were identical in appearance to the syringes containing BTX-A. The treatment allocation code was kept in a sealed envelope until the completion of the study. Medication was blindly injected subcutaneously. A total of 200U of BTX-A were reconstituted in 4ml of saline solution. BTX-A or saline was injected subcutaneously in a checkerboard pattern over the maximally affected area. When there was > 1 area of pain, the subjects were asked to choose the area with greater pain. The total injection area was limited to 20% or less of the total body surface area (eg, the injection area of one anterior and posterior leg was 18%).[21] When the total painful area to be injected was 20% or less of the total body surface, the whole affected area was injected. When the total painful area was >20% of the total body surface, injection area was limited to the 20% or less rule. Each patient received 40 injections in total, with a minimum distance of 1cm between injection sites. The placebo group received saline administered in the same manner. The patients, physicians who administered treatment, and assessors were all blind to the treatments throughout the study. The drugs were administered by a single physician in each institution.

Outcome Measures

The primary outcome measures included changes in the average pain intensity over the past 24 hours using the 100mm VAS (0 = no pain, 100 = unbearable pain) at 4 and 8 weeks after the injection.

The secondary outcome measures included changes in the pain quality and intensity at 4 and 8 weeks after the injection using the Korean version of the short-form McGill Pain
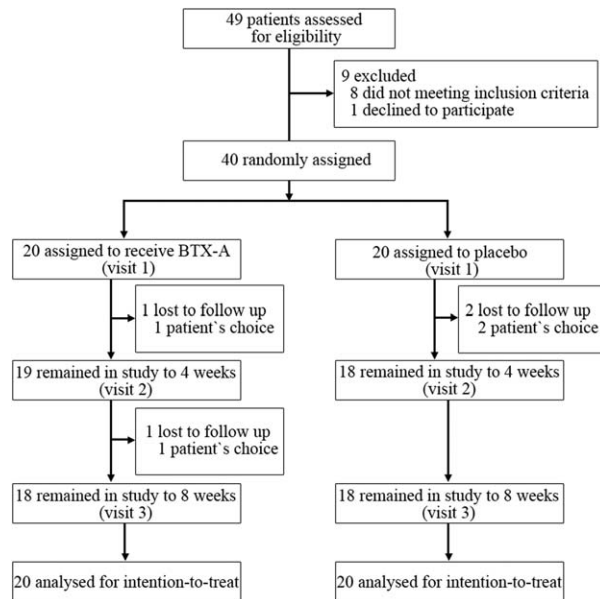
FIGURE 2: Study profile. BTX-A = botulinum toxin type A.

Questionnaire (SF-MPQ). Additionally, other secondary outcome measures included the proportion of responders who reported pain relief of 50% or greater, the proportion of responders who report pain relief of 30% or greater, and the proportion of responders who reported pain relief of 20% or greater at 4 and 8 weeks after the injection. Changes in quality of life using the Korean version of the World Health Organization WHOQOL-BREF quality of life assessment at 4 and 8 weeks after the injection were also evaluated.[22]

The adverse effects of BTX-A were evaluated throughout the study.

Statistical Analysis

Based on a similar study conducted in patients with diabetic neuropathic pain, mean difference between the study group and control group in change of VAS at week 4 from baseline was assumed to be 20mm with a standard deviation of difference of 20.4mm and 22.4mm. Considering a 5% level of significance, power of 80%, and a possible dropout rate of 10%, the total target number of subjects was calculated to be 20 for each group.[10] Reductions in the VAS scores of the BTX-A and the placebo groups at 4 and 8 weeks after the injection were assessed using Student t test or Wilcoxon rank sum test. The changes in the scores on the Korean version of the SF-MPQ and the Korean version of the WHOQOL-BREF between the BTX-A and the placebo groups were evaluated using Student t test or Wilcoxon rank sum test, depending on the normality of the data. The proportion of responders who reported pain relief of at least 50%, 30%, and 20% were assessed using Pearson chi-square test or Fisher exact test. We assessed all patients who received BTX-A injections (intention to treat population). Missing data were analyzed by the last observation carried forward method. A p value < 0.05 was considered statistically significant.

This study was registered with ClinicalTrial.gov, number NCT01579500.

Results

We screened 49 patients (Fig 2), and 40 subjects who fulfilled the inclusion criteria were randomly assigned to the BTX-A or placebo groups. The demographic variables, pain characteristics, and analgesic treatments did not differ between the two groups (Table 1 and Supplementary Table 1). Four patients withdrew from the study (2 from the BTX-A group and 2 from the placebo group) before reaching the 8-week follow-up without any given reason (see Fig 2).

Primary Outcome Measures

The reductions in the VAS score of the BTX-A group were 18.6 ± 16.8 at 4 weeks and 21.3 ± 26.8 at 8 weeks after the injection. The reductions in the VAS score of the placebo group were 2.6 ± 14.6 at 4 weeks and 0.3 ± 19.5 at 8 weeks after the injection (Fig 3A). Compared with the placebo group, the BTX-A group showed significant reductions of the VAS score at 4 ($p = 0.0027$) and 8 weeks ($p = 0.0053$) after the injection. The VAS score of the BTX-A group was 85.1 ± 13.6 at baseline, 66.5 ± 20.7 ($p < 0.0001$) at 4 weeks, and 63.8 ± 27.5 ($p = 0.0012$) at 8 weeks after the injection (see Fig 3B).

Secondary Outcome Measures

At 4 and 8 weeks after the injection, 10% and 20%, respectively, reported pain relief of 50% or greater in the BTX-A group, whereas the proportions were 5% and 10%, respectively, in the placebo group. Thirty percent of the patients reported pain relief of 30% or greater at both 4 and 8 weeks in the BTX-A group, whereas the proportions were 5% and 10%, respectively, in the placebo group. Fifty-five percent and 45% of the patients reported pain relief of 20% or greater at 4 and 8 weeks, respectively, after the injection in the BTX-A group, whereas the proportions were 15% and 10%, respectively, in the placebo group (see Fig 3C).

Table 2 shows sensory, affective, and total scores on the Korean version of the SF-MPQ. Compared with the placebo group, there were significant reductions in the sensory (5.1 ± 7.9, $p = 0.0033$), affective (1.0 ± 2.5, $p = 0.0131$), and total scores (6.1 ± 9.2, $p = 0.0008$) on the Korean version of the SF-MPQ in the BTX-A group at 4 weeks after the injection. In addition, there were significant reductions in the affective (0.5 ± 1.7, $p = 0.0086$) and total scores (3.6 ± 8.4, $p = 0.0197$) on the Korean version of the SF-MPQ in the BTX-A group at 8 weeks after the injection.

Quality of life was assessed by the Korean version of the WHOQOL-BREF. The changes in the scores for the physical health, psychological, social relationships, and environmental domains of the Korean version of the

TABLE 1. Baseline Demographic and Clinical Characteristics of the Patients (Intention-to-Treat Analysis)

| Demographic and Clinical Characteristics	BTX-A, n = 20	Placebo, n = 20
Age, mean ± SD (minimum–maximum)	53.1 ± 9.1 (34–71)	48.9 ± 14.2 (24–77)
Male sex, No. [%]	15 [75]	14 [70]
Pain duration, mo, mean ± SD (minimum–maximum)	46.0 ± 49.1 (3–151)	50.2 ± 46.1 (12–192)
Mean duration of spinal cord injury, yr, mean ± SD (minimum–maximum)	4.3 ± 5.2 (1–20)	4.7 ± 5.0 (1–20)
VAS baseline, mean ± SD	85.1 ± 13.6	77.1 ± 13.3
Etiology of spinal cord injury, No.		
Traumatic	16	18
Transverse myelitis	3	1
AVM rupture	1	1
ASIA impairment scale, No.		
A	9	6
B	2	5
C	4	6
D	5	3
Type of paralysis, No.		
Tetraplegia	14	12
Paraplegia	6	8
Type of pain, No.		
At-level pain	5	4
Below-level pain	15	14
Both	0	2

ASIA = American Spinal Injury Association; AVM = arteriovenous malformation; BTX-A = botulinum toxin type A; SD = standard deviation; VAS = visual analog scale.

WHOQOL-BREF questionnaire were compared between the two groups at 4 and 8 weeks after the injection. There were no significant differences or improvements found in these domains during the period of this study (see Table 2). However, improvements in the score for the physical health domain in the BTX-A group at 4 weeks after the injection (5.0 ± 9.4, $p = 0.0521$) showed a marginal trend toward significance.

Only 2 of 8 patients with complete SCI (ASIA impairment scale A) showed 20% or greater pain relief at 4 weeks after the BTX-A injection (Supplementary Table 2). However, 9 of 11 patients with incomplete SCI (ASIA impairment scale B–D) showed 20% or greater pain relief at 4 weeks after the BTX-A injection. The mean VAS score decreased by 27.7 in 11 patients with incomplete SCI compared with 7.6 in 8 patients with complete SCI at 4 weeks after the BTX-A injection.

In this study, there were 9 patients with at-level neuropathic pain, with 5 in the BTX-A group and 4 in the placebo group. Despite the small number included, the VAS scores were 85.6 ± 13.5, 80.6 ± 12.8, and 81.2 ± 16.2 at baseline, week 4, and week 8, respectively, in the BTX-A group, and analysis showed no statistically significant improvement in pain. The placebo group also showed similar results, with no statistically significant improvement between baseline (77.0 ± 15.9), 4-week (80.0 ± 11.5), and 8-week (79.7 ± 10.6) VAS scores. There were 29 patients with below-level neuropathic pain and among the 29, 15 were in the BTX-A group and 14 in the placebo group. In the placebo group, the VAS score was 77.2 ± 12.8 at baseline, 72.1 ± 17.4 at week 4, and 75.6 ± 23.7 at week 8, showing no statistical differences before and after the injections. However, the results for below-level neuropathic pain showed
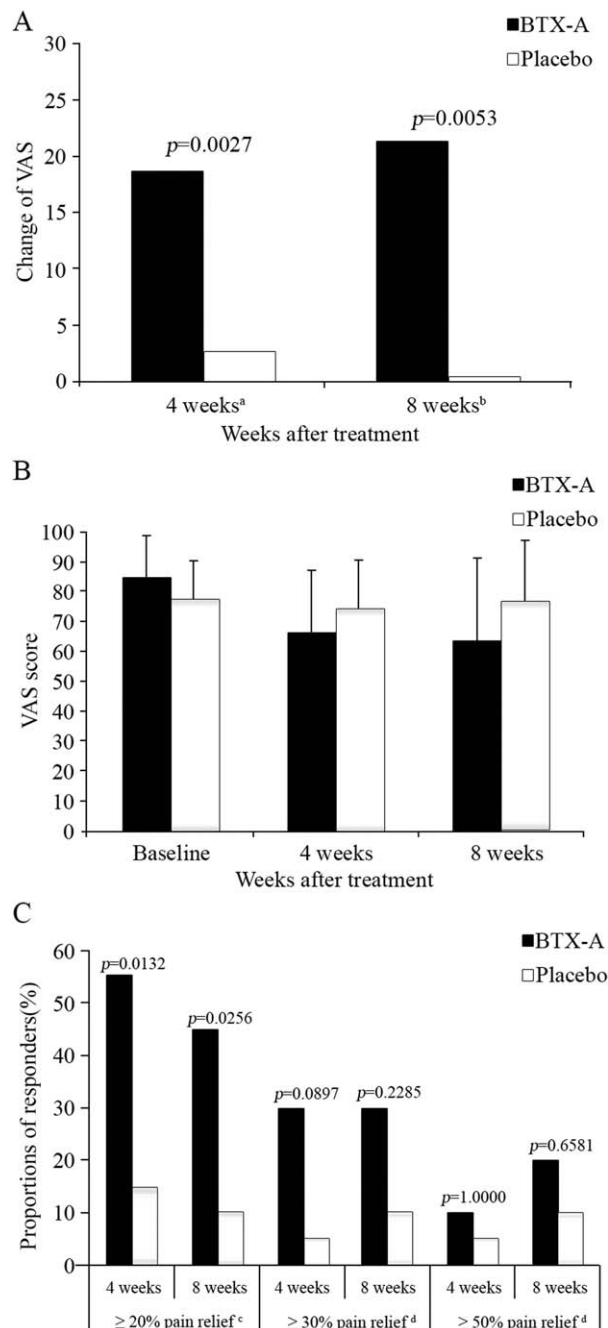
FIGURE 3: (A) Changes in the visual analogue scale (VAS) scores in the botulinum toxin type A (BTX-A) and the placebo groups at 4 and 8 weeks after the injection. (B) The VAS scores in the BTX-A and placebo groups at baseline and 4 and 8 weeks after the injection. (C) Proportions of responders who reported pain relief of 20%, 30%, and 50% or greater in response to BTX-A injections. [a]p value is based on Student t test. [b]p value is based on Wilcoxon rank sum test. [c]p values are based on Pearson chi-square test. [d]p values are based on Fisher exact test.

statistically significant improvements in VAS scores after injection with BTX-A. In the BTX-A group, VAS score was 85.0 ± 14.2 at baseline, 61.8 ± 21.0 at week 4, and 58.0 ± 28.4 at week 8, showing a significant reduction

in pain ($p < 0.0001$ and $p = 0.0009$, respectively) after BTX-A injection and also showing significant improvements compared to the placebo group ($p = 0.0049$ and $p = 0.0042$, respectively; see Table 2). In the BTX-A group, among the 5 with at-level neuropathic pain, only 1 showed $\geq 20\%$ pain relief at week 4 and week 8, whereas of the 15 with below-level pain, 10 at week 4 and 8 at week 8 showed $\geq 20\%$ pain relief (Table 3). Due to the small number of total subjects, these results were not statistically significant ($p = 0.1273$); however, the ratio of $\geq 20\%$ pain relief was much higher in below-level neuropathic pain.

Adverse Events

No allergic reactions occurred in either group. Some patients reported that the injections were painful or triggered spasticity, without any difference between the BTX-A (mild pain, 3; moderate pain, 2; severe pain, 0; spasticity, 3) and placebo (mild pain, 4; moderate pain, 2; severe pain, 0; spasticity, 4) groups. No other local or systemic side effects were reported during the injections or at any other time during the study.

Discussion

This study aimed to investigate the potential analgesic effects of subcutaneous BTX-A in the treatment of SCI-associated neuropathic pain using a randomized, double-blind, placebo-controlled design. BTX-A significantly improved the pain intensity according to VAS scores at 4 and 8 weeks after the injection. In addition, we observed that approximately 50% of patients with severe SCI-associated neuropathic pain showed 20% or greater pain relief for at least 8 weeks after the BTX-A injection. Our data suggest a new potential therapeutic indication for BTX-A.

Pain in SCI patients can arise in relation to spasticity. Therefore, to minimize the possibility of confounding the effects of BTX-A treatment, we excluded patients with musculoskeletal (nociceptive) and visceral pain in the area of injection. In addition, BTX injection was performed in a wide area that covered many muscles with injection routes being subcutaneous, making it hard to assume that the injections influenced spasticity. In regard to spasticity, the spasticity grades using the Ashworth scale for the muscle groups related to the injected area were 1.00 ± 1.17 at baseline, 0.85 ± 1.09 at week 4, and 0.90 ± 1.17 at week 8 in the placebo group and 0.70 ± 0.98 at baseline, 0.65 ± 0.93 at week 4, and 0.70 ± 0.98 at week 8 in the BTX group, showing no statistically significant changes before and after BTX injection.

TABLE 2. Primary and Secondary Outcome Measures at Baseline, 4 Weeks after Injection (Week 4) and 8 Weeks after Injection (Week 8; Intention-to-Treat Analysis)

Measure	BTX-A			Placebo		
	Baseline	Week 4	Week 8	Baseline	Week 4	Week 8
Mean Pain (VAS) ± SD	85.1 ± 13.6	66.5 ± 20.7[a]	63.8 ± 27.5[a]	77.1 ± 14.0	74.5 ± 16.0	76.8 ± 20.4
Pain relief (0–100%) ± SD		22.1 ± 21.1[a]	24.6 ± 32.1[a]		2.1 ± 21.2	−1.2 ± 28.0
At-level pain group, mean pain (VAS) ± SD	85.6 ± 13.5	80.6 ± 12.8	81.2 ± 16.2	77.0 ± 15.9	80.0 ± 11.5	79.7 ± 10.6
Below-level pain group, mean pain (VAS) ± SD	85.0 ± 14.2	61.8 ± 21.0[a]	58.0 ± 28.4[a]	77.2 ± 12.8	72.1 ± 17.4	75.6 ± 23.7
SF-MPQ scores ± SD						
Sensory (0–33)	20.5 ± 9.2	15.4 ± 10.3[a]	17.4 ± 10.7	17.1 ± 9.2	18.3 ± 10.1	18.2 ± 10.8
Affective (0–12)	5.2 ± 3.4	4.3 ± 3.9[a]	4.7 ± 3.6[a]	4.1 ± 3.1	5.1 ± 3.3	5.4 ± 3.6
Total (0–45)	25.7 ± 11.5	19.6 ± 13.6[a]	22.1 ± 13.5[a]	20.1 ± 11.6	23.3 ± 13.0	23.6 ± 14.2
Present pain intensity (0–4)	3.1 ± 0.6	2.7 ± 0.9[a]	2.6 ± 1.0[a]	2.8 ± 0.5	3.1 ± 0.4	3.2 ± 0.6
WHOQOL-BREF scores ± SD						
Physical health domain (0–100)	21.4 ± 12.9	26.4 ± 12.8	24.9 ± 16.4	21.3 ± 11.0	21.1 ± 11.0	18.9 ± 10.9
Psychological domain (0–100)	31.7 ± 15.6	32.0 ± 16.9	30.2 ± 15.1	29.8 ± 15.6	28.9 ± 19.2	28.9 ± 16.0
Social relationships domain (0–100)	29.6 ± 18.6	34.0 ± 16.5	33.2 ± 16.7	31.0 ± 16.2	32.5 ± 17.7	31.1 ± 16.6
Environmental domain (0–100)	33.5 ± 12.9	35.8 ± 12.4	36.5 ± 15.9	41.7 ± 13.4	39.2 ± 18.0	38.5 ± 15.9

Data are presented for intention-to-treat patients (last observation carried forward analysis).
[a]$p < 0.05$ versus placebo.
BTX-A = botulinum toxin type A; SD = standard deviation; SF-MPQ = short-form McGill Pain Questionnaire; VAS = visual analogue scale.

TABLE 3. Degrees of Pain Relief and Neurological Completeness or Types of Pain

	Number of Patients		p	Number of Patients		p
	AIS A	AIS B~D		At-Level Pain	Below-Level Pain	
Pain relief of ≥ 20% at 4 weeks after the BTX-A injection	2	9	0.0216[a]	1	10	0.1273[a]
Pain relief of < 20% at 4 weeks after the BTX-A injection	7	2		4	5	
Pain relief of ≥ 20% at 8 weeks after the BTX-A injection	1	8	0.0098[a]	1	8	0.3189[a]
Pain relief of < 20% at 8 weeks after the BTX-A injection	8	3		4	7	

Data are presented for intention-to-treat patients (last observation carried forward analysis).
[a]p values are based on Fisher exact test.
AIS = American Spinal Injury Association Impairment Scale; BTX-A = botulinum toxin type A.

In this study, the baseline pain intensity according to the VAS score was approximately 80 (85.1 in the BTX-A group and 77.1 in the placebo group), indicating that the pain was very severe and intractable, although the patients had received maximal analgesics to control neuropathic pain. Generally, a 20% change in pain intensity was associated with individuals reporting that they were at least slightly better, and this change can be interpreted as the minimal clinically important difference.[23] However, we propose that a 20% change in pain intensity has a more important clinical significance, considering the limited effectiveness of treatments for chronic intractable pain. The reduction in pain intensity was only approximately 20 according to the VAS score, but this reduction might produce improvements in physical health measures of quality of life and thus implies an additive meaning to mere pain reduction.

Roux et al reported that the preservation of thermal sensibility at baseline was correlated with the analgesic effects of BTX-A in patients with chronic neuropathic pain.[9] Similarly, in our study, the preservation of some motor or sensory function below the neurological level of injury was associated with better outcomes in patients with SCI-associated neuropathic pain at 4 and 8 weeks after the injection ($p = 0.0216$ and $p = 0.0098$, respectively; see Table 3). Subcutaneous BTX-A injections may be more effective in patients with incomplete SCI than in those with complete SCI.

Neuropathic pain can be categorized as at-level neuropathic pain and below-level neuropathic pain according to pain pathology. At-level neuropathic pain manifests in a segmental pattern and includes pain occurring in the dermatome at the associated level of neurologic injury or within 3 dermatomes below this level. Furthermore, at-level neuropathic pain is often perceived as burning, electric, or shooting with accompanying sensory changes of allodynia or hyperalgesia.[20] At-level neuropathic pain occurs most frequently in association with nerve root compression but can also occur after syringomyelia, spinal cord trauma/ischemia, and dual level cord and root trauma.[24] Below-level neuropathic pain occurs >3 dermatomes below the neurological level of injury, and typical characteristics are burning, electric, or shooting qualities with diffuse regional distribution. Allodynia and hyperalgesia are also common sensory features for below-level neuropathic pain. Below-level neuropathic pain is commonly associated with spinal cord trauma and ischemia.[24] In this study, BTX-A injection was effective in SCI patients with below-level neuropathic pain and ineffective in those with at-level neuropathic pain. This is thought to be due to the pathological differences underlying the two pain types.

BTX-A is generally administered intramuscularly to treat spasticity or dystonia. However, recent studies have reported that BTX-A for the treatment of neuropathic pain can be administered subcutaneously or intradermally.[9–11,25,26] The effects on pain may be mediated through direct effects on the sensory system.[16] Therefore, the optimal route of administration of BTX-A for treating neuropathic pain could be different from that for the treatment of spasticity or dystonia. In a previous case report of BTX for neuropathic pain in patients with spinal cord lesions, BTX-A was administered subcutaneously; a subcutaneous route was chosen for this study.[17] In most studies, the fractioned dosage of BTX-A for the treatment of neuropathic pain was between 2.5 and 7.5U/cm^2 per painful surface area, and the maximum total dosage was between 100 and 200U.[9–11,25–27] In the present study, the painful surface area in patients with

SCI was generally larger than that in patients with focal neuropathy such as postherpetic neuralgia and post-traumatic neuropathy. Therefore, the dose of BTX-A was fixed at 200U and the injection area was adjusted so that the maximal injection area was <20% of the total body surface. Despite the larger area of injection, significant improvement of pain was observed in this study, which implies that subcutaneous BTX-A injection may be used for patients with larger areas of neuropathic pain.

Recently, the role of BTX-A for the treatment of painful neuropathic conditions has been emphasized. Initially, the analgesic effects of BTX-A were attributed to a reduction in muscle spasms. However, many preclinical and clinical studies have suggested that BTX-A reduces pain due to mechanisms distinct from muscle spasm inhibition. The proposed mechanisms of the analgesic effect include inhibition of triggered release of neuropeptides, which modulate inflammation and pain.[16] BTX-A may reduce the peripheral release of glutamate, substance P, and calcitonin gene-related peptides, which are involved in neurogenic inflammation and central sensitization.[12,13,28] In addition, BTX-A may prevent SNARE protein-mediated translocation of receptors, such as the N-methyl-D-aspartate (NMDA) receptor and transient receptor potential vanilloid 1, into the plasma membrane in peripheral nociceptor terminals.[29,30] Some evidence of the central mechanisms of the antinociceptive activity of BTX-A has been shown. Although the evidence is limited, axonal transport of BTX-A from the periphery to the central nervous system has been demonstrated.[15,31–35] In a recent study by Marinelli et al, BTX-A was subcutaneously injected into the plantar surface of the hind paw of a sciatic nerve injury mouse model. Immunofluorescence analysis resulted in detection of cleaved SNAP-25, which implies BTX activity, in nerve endings of the hind paw, sciatic nerve, dorsal root ganglion, and spinal cord.[34] Such results demonstrated not merely evidence of retrograde transport of BTX-A, but also a reduction in sciatic nerve injury-related neuropathic pain after subcutaneous injection of BTX-A into the hind paw. Further animal studies of trigeminal neuropathy, diabetic neuropathy, carrageenan-induced hyperalgesia, paclitaxel-induced peripheral neuropathy, and so forth have shown bilateral pain amelioration after either unilateral peripheral or intrathecal injection of BTX-A. These results of neuropathic pain improvement provide evidence of central nervous system involvement in the mechanism of pain reduction by BTX-A in neuropathic pain.[36–38] The underlying mechanisms of neuropathic pain following SCI have remained elusive, but neuronal hyperexcitability is a leading explanation. Reorganization of the nervous system and functional changes in receptors (such as

NMDA and glutamate receptors) and ion channels (such as sodium and calcium channels) may underlie central sensitization after SCI.[39] Furthermore, neuropeptides such as glutamate and substance P play central roles in the peripheral sensitization of nociception.[39–41] Therefore, mechanisms of pain relief by subcutaneous BTX-A injection in patients with SCI may occur through the inhibition of neuropeptides such as glutamate, substance P, and calcitonin gene-related peptide in the periphery as well as through aforementioned central effects on the spinal cord. Because SNAP-25 is a negative regulator of calcium channels, BTX-A may reduce calcium-mediated neurotransmitter release and decrease neuropathic pain.[42]

In this study, the mean VAS scores in the placebo group were 77.1 ± 14.0 at baseline, 74.5 ± 16.0 at week 4, and 76.8 ± 20.4 at week 8, and thus no placebo-related analgesic effect was evident. Previous studies on placebo analgesia research mostly included healthy volunteers who were exposed to experimental pain stimuli or patients with acute postoperative pain.[43–46] Some studies have focused on chronic pain, but the studies mostly involved irritable bowel syndrome patients, and placebo effects in studies regarding chronic neuropathic pain due to nerve lesions have been rare.[47–50] Furthermore, in a previous study by Ranoux et al, where BTX-A showed analgesic effects on chronic neuropathic pain, VAS scores for the placebo group were 60.0 ± 18.9 at baseline, 54.0 ± 22.0 at week 4, and 56.4 ± 26.4 at week 12.[9] In a similar study by Yuan et al, showing effects of BTX-A on diabetic neuropathic pain, decreases in VAS scores for the placebo group were -0.11 ± 2.04, 0.42 ± 1.62, and 0.53 ± 1.57 at week 4, week 6, and week 8, respectively.[10] Both studies showed minimal placebo effects, nor were the differences statistically significant. The placebo effects in healthy volunteers and acute pain patients are thought to be associated with endogenous pain-modulating systems, where the effects originate in the cortical structure and project to the periaqueductal gray, rostral ventromedial medulla, and finally spinal cord. However, the mechanisms differ in patients with neuropathic pain, where pain is thought to be due to defects in pain processing and pain modulation.[45,50–52] Therefore, mechanisms related to placebo effects in those with neuropathic pain may be different from other pain models. In a recent study looking into placebo effects in peripheral nerve injury-induced chronic neuropathic pain, there were placebo effects regarding hyperalgesia but none for spontaneous or evoked pain.[50] Considering that the mechanisms of neuropathic pain in SCI differ from those caused by peripheral nerve injury, the placebo effects may also be different between SCI-induced neuropathic pain and pain caused by injury to the peripheral

nerve.[53,54] The subjects enrolled in this study had moderate to severe neuropathic pain, with VAS scores > 40 and durations > 3 months. Furthermore, these patients were refractory to conventional medications that included opioid analgesics, tricyclic antidepressants, antiepileptic agents, and benzodiazepines. Thus, it is possible that placebo effects may not have occurred due to these clinical characteristics as well as due to differing etiological mechanisms of neuropathic pain from previous studies investigating placebo effects in pain.

This is a proof of concept clinical study and is the first of its kind to investigate the effect of BTX-A in SCI neuropathic pain. However, this study has limitations in that investigations of the effect of BTX-A were performed only twice, at week 4 and week 8 after injection, and the duration of the study was relatively short in comparison to other neuropathic pain-related BTX-A studies. This study was performed on chronic SCI patients with severely limited mobility due to their paralysis, and considering these social and clinical aspects, we tried to minimize revisitations to the clinic and shorten study duration as a whole. Therefore, it was difficult to determine the exact onset and duration of the effects of BTX-A in patients with SCI-associated neuropathic pain. Previous reports on BTX-A injections for neuropathic pain have indicated that the onset of relief and duration of effectiveness were approximately 1 to 2 weeks and 3 to 6 months, respectively.[9–11,25] Our study showed that the effective duration was at least 8 weeks. Further comprehensive trials are required to determine the onset of relief and duration of effectiveness. Furthermore, because the magnitude of responses was small, supplementary scales such as the Patient Global Impression of Scale could have been helpful in this study.

We conclude that subcutaneous BTX-A injections are an effective and safe method of reducing severe neuropathic pain in patients with SCI. Our data show that subcutaneous BTX-A injections might be considered as a treatment regimen for intractable neuropathic pain in patients with SCI. Further studies should be performed to evaluate the underlying mechanisms, onset time, duration, optimal dosage, and optimal route of administration for BTX-A therapy for the treatment of neuropathic pain in patients with SCI.

Acknowledgment

Author Contributions

All authors conceived and designed the study. Acquisition and analysis of data were accomplished by Z.-A.H., D.H.S., and M.E.C. All authors were responsible for writing and drafting the manuscript.

Potential Conflicts of Interest

References

1. Siddall PJ, McClelland JM, Rutkowski SB, et al. A longitudinal study of the prevalence and characteristics of pain in the first 5 years following spinal cord injury. Pain 2003;103:249–257.

2. Turner J, Cardenas D, Warms C, McClellan C. Chronic pain associated with spinal cord injury: a community survey. Arch Phys Med Rehabil 2001;82:501–508.

3. Rintala DH, Holmes SA, Feiss RN, et al. Prevalence and characteristics of chronic pain in veterans with spinal cord injury. J Rehabil Res Dev 2005;42:573–584.

4. Sawatzky B, Bishop CM, Miller WC, at al. Classification and measurement of pain in the spinal cord-injured population. Spinal Cord 2008;46:2–10.

5. Jensen TA, Baron R, Haanpää M, et al. A new definition of neuropathic pain. Pain 2011;152:2204–2205.

6. Westgren N, Levi R. Quality of life and traumatic spinal cord injury. Arch Phys Med Rehabil 1998;79:1433–1439.

7. Murray RF, Asghari A, Egorov DD, et al. Impact of spinal cord injury on self-perceived pre- and postmorbid cognitive, emotional and physical functioning. Spinal Cord 2007;45:429–436.

8. Dodick DW, Turkel CC, DeGryse RE, et al. OnobotulinumtoxinA for treatment of chronic migraine: pooled results from the double-blind, randomized, placebo-controlled phases of the PREEMPT clinical program. Headache 2010;50:921–936.

9. Ranoux D, Attal N, Morain F, Bouhassira D. Botulinum toxin type A induces direct analgesic effects in chronic neuropathic pain. Ann Neurol 2008;64:274–283.

10. Yuan RY, Sheu JJ, Yu JM, et al. Botulinum toxin for diabetic neuropathic pain: a randomized double-blind crossover trial. Neurology 2009;72:1473–1478.

11. Xiao L, Mackey S, Hui H, et al. Subcutaneous injection of botulinum toxin A is beneficial in postherpetic neuralgia. Pain Med 2010;11:1827–1833.

12. Cui M, Khanijou S, Rubino J, et al. Subcutaneous administration of botulinum toxin A reduces formalin-induced pain. Pain 2004;107:125–133.

13. Aoki KR. Review of a proposed mechanism for the antinociceptive action of botulinum toxin type A. Neurotoxicology 2005;26:785–793.

14. Habermann E. 125I-labeled neurotoxin from Clostridium botulinum A: preparation, binding to synaptosomes and ascent to the

spinal cord. Naunyn Schmiedebergs Arch Pharmacol 1974;281: 47–56.

15. Bach-Rojecky L, Lackovic Z. Central origin of the antinociceptive action of botulinum toxin type A. Parmacol Biochem Behav 2009; 94:234–238.

16. Matak I, Lackovic Z. Botulinum toxin A, brain and pain. Prog Neurobiol 2014;119–120:39–59.

17. Jabbari B, Maher N, Difazio MP. Botulinum toxin A improved burning pain and allodynia in two patients with spinal cord pathology. Pain Med 2003;4:206–210.

18. Han ZA, Song DH, Chung ME. Effect of subcutaneous injection of botulinum toxin A on spinal cord injury-associated neuropathic pain. Spinal Cord 2014;52:S5–S6.

19. Haanpää M, Attal N, Backonja M, et al. NeuPSIG guidelines on neuropathic pain assessment. Pain 2011;152:14–27.

20. Widerström-Noga E, Biering-Sørensen F, Bryce T, et al. The international spinal cord injury pain basic data set. Spinal Cord 2008; 46:818–823.

21. O'Sullivan SB, Schmitz TJ. Physical rehabilitation. 5th ed. Philadelphia, PA: F.A. Davis Company, 2007.

22. Min SK, Kim KI, Lee CI, et al. Development of the Korean version of WHO Quality of Life scale and WHOQOL-BREF. Qual Life Res 2002;11:593–600.

23. Farrar JT. Advances in clinical research methodology for pain clinical trials. Nat Med 2010;16:1284–1293.

24. Siddall PJ, Yezierski RP, Loeser JD. Pain following spinal cord injury: clinical features, prevalence, and taxonomy. Int Assoc Study Pain Newlett 2000;3:3–7.

25. Apalla Z, Sotiriou E, Lallas A, et al. Botulinum toxin A in postherpetic neuralgia: a parallel, randomized, double-blind, single-dose, placebo-controlled trial. Clin J Pain 2013;29:857–864.

26. Fabregat G, De Andres J, Villanueva-Perez VL, et al. Subcutaneous and perineural botulinum toxin type A for neuropathic pain: a descriptive review. Clin J Pain 2013;29:1006–1012.

27. Liu HT, Tsai SK, Kao MC, Hu JS. Botulinum toxin A relieved neuropathic pain in a case of post-herpetic neuralgia. Pain Med 2006; 7:89–91.

28. Lucioni A, Bales GT, Lotan TL, et al. Botulinum toxin type A inhibits sensory neuropeptide release in rat bladder models of acute injury and chronic inflammation. BJU Int 2008;101:366–370.

29. Cheng J, Liu W, Duffney LJ, Yan Z. SNARE proteins are essential in the potentiation of NMDA receptors by group II metabotropic glutamate receptors. J Physiol 2013;591:3935–3947.

30. Morenilla-Palao C, Planells-Cases R, Garcia-Sanz N, Ferrer-Montiel A. Regulated exocytosis contributes to protein kinase C potentiation of vanilloid receptor activity. J Biol Chem 2004;279:25665–25672.

31. Wieggand H, Erdman G, Welhoner HH. ^{125}I-labeled botulinum toxin type A neurotoxin: pharmacokinetics in cats after intramuscular injection. Naunyn Schmiedebergs Arch Pharmacol 1976;292:161–165.

32. Black J, Dolly O. Interaction of ^{125}I-labeled botulinum neurotoxins with nerve terminals. I. Ultrastructural autoradiographic localization and quantification of distinct membrane acceptors for types A and B on motor nerves. J Cell Biol 1986;103:521–534.

33. Wiegand H, Erdmann G, Wellhöner HH. ^{125}I-labelled botulinum A neurotoxin: pharmacokinetics in cats after intramuscular injection. Naunyn Schmiedebergs Arch Pharmacol 1976;292:161–165.

34. Marinelli S, Vacca V, Ricordy R, et al. The analgesic effect on neuropathic pain of retrogradely transported botulinum neurotoxin A involves Schwann cells and astrocytes. PLoS One 2012;7:e47977.

35. Antonucci F, Rossi C, Gianfranceschi L, et al. Long-distance retrograde effects of botulinum neurotoxin A. J Neurosci 2008;28:3689–3696.

36. Favre-Guilmard C, Auguet M, Chabrier PE. Different antinociceptive effects of botulinum toxin type A in inflammatory and peripheral polyneuropathic rat models. Eur J Pharmacol 2009;617:48–53.

37. Bach-Rojecky L, Salkovic-Petrisic M, Lackovic Z. Botulinum toxin type A reduces pain supersensitivity in experimental diabetic neuropathy: bilateral effect after unilateral injection. Eur J Pharmacol 2010;633:10–14.

38. Filipović B, Matak I, Bach-Rojecky L, Lacković Z. Central action of peripherally applied botulinum toxin type A on pain and dural protein extravasation in rat model of trigeminal neuropathy. PLoS One 2012;7:e29803.

39. Finnerup NB, Baastrup C. Spinal cord injury pain: mechanisms and management. Curr Pain Headache Rep 2012;16:207–216.

40. Leem JW, Kim HK, Hulsebosch CE, Gwak YS. Ionotropic glutamate receptors contribute to maintained neuronal hyperexcitability following spinal cord injury in rats. Exp Neurol 2010;224:321–324.

41. Marchand F, Perretti M, McMahon SB. Role of the immune system in chronic pain. Nat Rev Neurosci 2005;6:521–532.

42. Pozzi D, Condliffe S, Bozzi Y, et al. Activity-dependent phosphorylation of Ser187 is required for SNAP-25-negative modulation of neuronal voltage-gated calcium channels. Proc Natl Acad Sci U S A 2008;105:323–328.

43. Amanzio M, Pollo A, Maggi G, Benedetti F. Response variability to analgesics: a role for non-specific activation of endogenous opioids. Pain 2001;90:205–215.

44. Benedetti F, Pollo A, Lopiano L, et al. Conscious expectation and unconscious conditioning in analgesic, motor, and hormonal placebo/nocebo responses. J Neurosci 2003;23:4315–4323.

45. Levine JD, Gordon NC, Fields HL. The mechanism of placebo analgesia. Lancet 1978;2:654–657.

46. Benedetti F. Mechanisms of placebo and placebo-related effects across diseases and treatments. Annu Rev Pharmacol Toxicol 2008;48:33–60.

47. Kaptchuk TJ, Friedlander E, Kelley JM, et al. Placebos without deception: a randomized controlled trial in irritable bowel syndrome. PLoS One 2010;5:e15591.

48. Price DD, Craggs J, Verne GN, et al. Placebo analgesia is accompanied by large reductions in pain-related brain activity in irritable bowel syndrome patients. Pain 2007;127:63–72.

49. Vase L, Robinson ME, Verne GN, Price DD. The contributions of suggestion, desire, and expectation to placebo effects in irritable bowel syndrome patients. An empirical investigation. Pain 2003; 105:17–25.

50. Petersen GL, Finnerup NB, Nørskov KN, et al. Placebo manipulations reduce hyperalgesia in neuropathic pain. Pain 2012;153: 1292–1300.

51. Benedetti F, Arduino C, Amanzio M. Somatotopic activation of opioid systems by target-directed expectations of analgesia. J Neurosci 1999;19:3639–3648.

52. Eippert F, Bingel U, Schoell ED, et al. Activation of the opioidergic descending pain control system underlies placebo analgesia. Neuron 2009;63:533–543.

53. Eide PK. Pathophysiological mechanisms of central neuropathic pain after spinal cord injury. Spinal Cord 1998;36:601–612.

54. Siddall PJ. Management of neuropathic pain following spinal cord injury: now and in the future. Spinal Cord 2009;47:352–359.