ORIGINAL ARTICLE

Effect of detrusor botulinum toxin a injection on urothelial dysfunction in patients with chronic spinal cord injury: a clinical and immunohistochemistry study before and after treatment

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Objective: The objective of this study is to investigate the changes of urothelial junction proteins, apoptosis and suburothelial inflammation after detrusor injection of botulinum toxin A (BoNT-A) in patients with spinal cord injury (SCI) and neurogenic detrusor overactivity (NDO).

Methods: A total of 26 patients with chronic suprasacral SCI and NDO were enroled. The urothelium was assessed by cystoscopic biopsy at baseline, 3 and 6 months after a single treatment of 300 U BoNT-A into the detrusor. Immunofluorescence staining of E-cadherin, zonula occludens-1 (ZO-1) and tryptase for mast cell activity were performed. Urothelial apoptosis was also evaluated. The differences in urothelial dysfunction were compared between baseline and 3 and 6 months after treatment. Bladder biopsies from patients undergoing anti-incontinence surgery served as controls.

Results: A single 300-U BoNT-A injection into the detrusor significantly decreased detrusor pressure and increased bladder compliance at 3 and 6 months after treatment. Significantly lower E-cadherin and ZO-1 expressions and increased mast cell and apoptotic cell counts were noted in SCI bladders compared with controls (all $P < 0.001$). Significantly greater distributions of E-cadherin ($P < 0.001$) and ZO-1 ($P = 0.05$) expressions were noted 3 months after BoNT-A injection. However, these changes had declined by 6 months after treatment. Activated mast cells and urothelial apoptosis showed no significant differences between baseline and 3 or 6 months.

Conclusion: Urothelial dysfunction and adhesive and junction protein concentrations in SCI patients' bladders recovered after BoNT-A treatment. However, this effect decreased with time. Thus, neurogenic inflammation after SCI was not adequately improved after a single BoNT-A injection.

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INTRODUCTION

Spinal cord injury (SCI) is a significant cause of morbidity and mortality in developing countries, with a global annual incidence of 1:25 000.[1] Neurogenic detrusor overactivity (NDO) is a consequence of the change in bladder reflex pathways in chronic SCI patients. Neurogenic voiding dysfunction and urinary symptoms in patients with SCI lead to several complications. If not well managed, high intravesical pressure damages the upper urinary tract, causing renal scarring and chronic renal insufficiency, which impair the quality of life.[2] SCI also leads to rapid disruption of the uroepithelial barrier. This manifests in a loss of cell–cell interactions, decreases in transepithelial resistance and increases in water and urea permeability.[3]

We have found decreased expression of urothelial adhesion and junction proteins and increased suburothelial inflammation and apoptosis in patients with chronic SCI, regardless of injury level.[4] Disruption of the urothelial barrier initiates a cascade of bladder dysfunction events, leading to suburothelial inflammation and vulnerability to chronic or recurrent cystitis or infection, or both.

Suburothelial inflammation might also affect urothelial function, leading to a vicious cycle. Botulinum toxin A (BoNT-A) has been useful in the treatment of NDO in SCI patients.[5] It is possible that the decrease of bladder inflammation might also improve the urothelial barrier function after detrusor BoNT-A injection.

BoNT-A is one of the most powerful neurotoxins; it inhibits the release of neurotransmitters from the nerve fibres and urothelium.[6] It may be possible to stem the disruption of the uroepithelium by modulating the release of neurotransmitters and inflammatory mediators. BoNT-A injection decreases bladder nerve growth factor production and urinary nerve growth factor levels, suggesting an anti-inflammatory response can be achieved after intravesical BoNT-A treatment.[7] Besides, our previous study reported BoNT-A treatment improved urothelial dysfunction in interstitial cystitis and overactive bladder.[8,9]

Until now, there is insufficient data to determine definitively whether or not BoNT-A injection into the detrusor improves the urothelial dysfunction in patients with SCI and NDO. The present preliminary study explored the mechanisms of action of BoNT-A

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treatment on urothelial dysfunction in patients with chronic SCI and NDO. We investigated the immunohistochemistry characteristics of bladders from SCI patients at baseline and after BoNT-A treatment by analysing adhesion and junction proteins, inflammation and apoptotic signalling molecules after one single BoNT-A injection treatment.

PATIENTS AND METHODS

In this preliminary, prospective study, 26 patients with chronic SCI and NDO, who were refractory to antimuscarinic treatment were consecutively enroled into the study. Video-urodynamic studies were routinely performed before enrolment, to confirm the presence of detrusor overactivity (DO) with or without detrusor sphincter dyssynergia (DSD). Patients were excluded if they had an active urinary tract infection (UTI) or urinary tract cancer at enrolment, history of lower urinary tract surgery, or chronic systemic diseases such as congestive heart failure and chronic renal failure. All patients had been educated to perform clean intermittent catheterization (CIC) due to possible urinary retention after BoNT-A injection. The study was approved by the Institutional Review Board and Ethics Committee of Buddhist Tzu Chi General Hospital. Each patient was informed about the study rationale and procedures, and written informed consent to participate in the study was obtained before any bladder procedure.

Each patient received 300 U of BoNT-A injection into the detrusor muscle under intravenous general anaesthesia in the operating room. Each vial of BoNT-A (100 U) was diluted with 10 ml of normal saline and 30 injections evenly distributed into detrusor were given using a 23-gauge needle and a rigid cystoscopic injection instrument (22 Fr, Richard Wolf, Knittlingen, Germany). The bladder cold cup biopsies were performed randomly at the posterior wall above the interureteric ridge and only the bladder mucosa and submucosa were taken to prevent bladder perforation.

We also invited 10 patients with stress urinary incontinence, but without urgency frequency symptoms, to serve as controls for comparison. All patients had been proven free of bladder outlet obstruction (BOO) by video-urodynamic study. Male patient was not selected to serve as the controls, because the prostate might cause BOO, which may affect the urothelial function.[10] The BOO in women was diagnosed by radiologic evidence of bladder outlet narrowing plus a voiding detrusor pressure > 35 cm H_2O and a maximum flow rate < 15 ml s^{-1}, or a voiding detrusor pressure > 40 cm H_2O.[11] In male patients, BOO was defined by the Abrams Griffiths number (Pdet.Qmax $- 2 \times$ Qmax). An Abrams Griffiths number > 40 was defined as having BOO.[12] For SCI patients, a BOO was defined if there was radiological evidence of bladder outlet narrowing during voiding cystourethrography, regardless of the voiding pressure. The bladder biopsy specimens were sent to the pathology department, to exclude the possibility of carcinoma *in situ*. At 3 and 6 months after the BoNT-A treatment, patients underwent another bladder biopsy. Specimens were stored frozen in liquid nitrogen for further investigation.

Immunofluorescence staining and quantification of protein expression in the bladder tissues of SCI patients and controls were investigated for urothelial adhesion and junction proteins by measuring E-cadherin expression and zonula occludens-1 (ZO-1) expression, respectively. Mast cell activation was assessed by measuring tryptase levels. Urothelial cell apoptosis was evaluated using the TUNEL (TdT dUTP nick end labelling) assay as described previously.[13,14]

The urinary bladder specimens were immersed and fixed for 1 h in an ice-cold solution of 4% formaldehyde in phosphate-buffered saline (pH 7.4). They were then rinsed with ice-cold phosphate-buffered saline containing 15% sucrose for 12 h. Biopsy specimens were embedded in optimum cutting temperature medium and stored at $-80\,^{\circ}C$. Four sections per specimen were cut using a cryostat at a thickness of 8 μm and collected on new silane III-coated glass slides (Muto Pure Chemicals Co. Ltd, Tokyo, Japan). The expression of E-cadherin and ZO-1 in the urothelium was quantified using Image J software, developed by the National Institutes of Health (Bethesda, MD).[15] Immunofluorescence (tryptase and TUNEL assays) was quantified by counting the number of positively stained cells/total cells per unit area (4 μm^2). The results are shown as the percentage of positive cells per 100 total cells. Differences in expressions of the proteins in the urothelium at baseline and 3

and 6 months after the BoNT-A injection were analysed using the paired *t*-test. All calculations were performed using SPSS for Windows, version 16.0 (SPSS, Chicago, IL, USA). $P < 0.05$ was considered to indicate statistical significance.

RESULTS

A total of 26 patients with chronic SCI and 10 controls were enroled in the study. The patients included 17 men and 9 women with a mean age of 42.7 ± 13.1 years. Among them, 20 had complete injuries, while 6 had incomplete injuries. The injury level was at cervical spinal cord in 12 patients and the thoracic cord in 14. Among them, 10 cervical SCI and 8 thoracic SCI patients had DSD. About the bladder management of SCI patients, 16 voided spontaneously or by reflex, while 5 used percussion to void, 3 voided by abdominal pressure and 2 used CIC. No patient having indwelling catheters or cystostomy was enroled in this study. The control group included 10 women with stress urinary incontinence with a mean age of 51.4 ± 12.1 years.

No severe side effects such as general weakness or severe autonomic dysreflexia occurred after BoNT-A injection. Five patients had slight hematuria, which was spontaneously resolved in 3 days without intervention. UTI was carefully monitored during the follow-up visits and antibiotic treatment was given in six (23%) patient who had symptomatic or asymptomatic UTI. Seven (27%) patients developed urinary retention and needed CIC. In patients with quadriplegia, who could not perform CIC by himself, we taught the family or caregiver to perform CIC, to prevent autonomic dysreflexia.

Table 1 shows the clinical characteristics of the SCI patients at baseline and 3 and 6 months after treatment with 300 U BoNT-A detrusor injection. Compared with the baseline data, the detrusor pressure significantly decreased at 3 and 6 months after BoNT-A injection (17.1 ± 20.4 and 23.6 ± 23.1 cm H_2O, respectively, versus 35.7 ± 23.3 cm H_2O, all $P < 0.05$). Bladder compliance showed a significant increase (48.2 ± 46.5 and 55.9 ± 78.3 ml cm H_2O^{-1} versus 41.7 ± 54.8 ml cm H_2O^{-1}, all $P < 0.05$). The post-void residual volume also significantly increased after treatment (375.0 ± 157.4 and 312.2 ± 177.6 ml, respectively, versus 229.6 ± 138.7 ml, all $P < 0.05$). However, the cystometric bladder capacity, glomerular filtration rate and serum creatinine levels did not change significantly after BoNT-A

Table 1 Clinical features of the spinal cord-injured patients at baseline, 3 months and 6 months after BoNT-A injection

	Baseline	3 Months	6 Months
Cystometric bladder capacity (ml)	289.8 (255.6)	422.0 (131.5) $P=0.156$	361.1 (152.81) $P=0.269$
Detrusor pressure (cmH$_2$O)	35.7 (23.3)	17.1 (20.4) $P=0.001$	23.6 (23.1) $P=0.06$
Qmax (ml s^{-1})	3.92 (4.75)	2.92 (5.14) $P=0.499$	3.71 (5.71) $P=0.906$
PVR (ml)	229.6 (138.7)	375.0 (157.4) $P<0.0001$	312.2 (177.6) $P=0.033$
Compliance	41.7 (54.8)	48.2 (46.5) $P=0.017$	55.9 (78.3) $P=0.003$
Detrusor overactivity	24/26	14/26	16/26
Glomerular filtration rate (ml min^{-1})	84.17 (19.97)	84.68 (24.17) $P=0.836$	81.96 (23.81) $P=0.553$
Serum creatinine (mg dl^{-1})	0.72 (0.16)	0.77 (0.24) $P=0.183$	0.77 (0.22) $P=0.007$

Abbreviations: BoNT-A, botulinum toxin A; PVR, post-void residual volume; Qmax, maximum flow rate.
Data are expressed as mean (s.d.). *P*-values indicate difference between baseline and time points.

Table 2 Changes in bladder expression of E-cadherin, activated mast cells, apoptosis (TUNEL) and ZO-1 in the controls and SCI patients after a single BoNT-A injection at 3 and 6 months compared with baseline

	Control (N = 10)	*SCI_Baseline* (N = 26)	*SCI_3M* (N = 26)	*SCI_6M* (N = 26)
E-cadherin	42.4 ± 16.73	31.27 ± 19.84[a]	52.88 ± 19.55[a]	43.64 ± 19.88[a]
			$P < 0.001$	$P = 0.07$
Mast cell	1.25 ± 1.15	16.46 ± 5.9[a]	16.1 ± 4.64[a]	14.48 ± 4.21[a]
			$P = 0.75$	$P = 0.30$
TUNEL	0.08 ± 0.26	3.6 ± 2.14[a]	4.05 ± 2.16[a]	4.58 ± 3.03[a]
			$P = 0.16$	$P = 0.06$
ZO-1	11.01 ± 5.66	6.50 ± 6.35[a]	8.65 ± 3.86[a]	7.3 ± 4.44[a]
			$P = 0.05$	$P = 0.55$

Abbreviations: BoNT-A, botulinum toxin A; SCI, spinal cord injured; TUNEL, TdT dUTP nick end labelling; ZO-1, zonula occludens-1.
P-values indicate difference between baseline and time points.
[a]Significantly different from the control; data are expressed as mean ± s.d.

treatment. The therapeutic effectiveness showed no significant difference between patients with different SCI level or with and without DSD, or patients with and without performing CIC.

At baseline, expression of E-cadherin, ZO-1, apoptosis and mast cell activity in the patients differed significantly compared with the control group (Table 2). A significant increase in mast cell infiltration was observed in the bladder specimens from SCI patients ($P < 0.001$). The expressions of E-cadherin and ZO-1 in the bladders of SCI patients were significantly lower compared with the corresponding expressions in the control bladders ($P < 0.001$). TUNEL staining showed significantly higher numbers of apoptotic cells in SCI bladder tissue compared with the control bladder tissue (3.6 ± 2.14 versus 0.08 ± 0.26 cells per 100 total cells, $P < 0.001$).

At 3 months after BoNT-A injection, a significantly higher distribution of E-cadherin expression compared with the baseline was observed (52.88 ± 19.55 versus 31.27 ± 19.84 fluorescence intensity units per 4 μm², $P < 0.001$). At 6 months after treatment, the distribution was 43.64 ± 19.88 versus 31.27 ± 19.84, $P = 0.07$. A significantly higher distribution of ZO-1 (8.65 ± 3.86 versus 6.50 ± 6.35 cells per 100 total cells, $P = 0.05$) at 3 months after BoNT-A injection compared with the baseline level was also noted. However, ZO-1 expression was not significantly increased at 6 months compared with baseline (7.3 ± 4.44 cells per 100 total cells, $P = 0.55$). The mast cell activity showed no significant difference between baseline and 3 months (16.46 ± 5.9 versus 16.1 ± 4.64 cells per 100 total cells, $P = 0.75$), nor did urothelial cell apoptosis differ significantly at 3 months after BoNT-A treatment compared with baseline (4.05 ± 2.16 versus 3.6 ± 2.14 cells per 100 total cells, $P = 0.16$). At 6 months after BoNT-A injection, the changes in expression of E-cadherin, mast cell activation, apoptosis and ZO-1 did not differ compared with baseline. Nevertheless, all bladder parameters of SCI patients at 3 and 6 months significantly differed from the controls. The changes of urothelial dysfunction parameters showed no significant difference between different level SCI patients or between patients with and without DSD.

Figure 1 shows the expression of ZO-1, E-cadherin, mast cell counts and apoptotic cell counts (TUNEL staining) in bladder tissues from SCI patients at baseline and 3 and 6 months after a single injection of 300 U of BoNT-A.

DISCUSSION
The results of this study revealed impaired expression of the adhesion protein E-cadherin and tight junction protein ZO-1, and significantly higher mast cell activation and cell apoptosis in the urothelium of patients with chronic SCI and NDO. Although detrusor pressure and

compliance were improved after a single BoNT-A injection, only E-cadherin and ZO-1 expressions were increased at 3 months. However, these changes were not significant at 6 months after treatment, indicating that the effect of single BoNT-A injection on urothelial dysfunction is short term.

Low urinary tract dysfunction and complications are major concerns in the management of SCI patients. Although C-fibre afferents have a role in the development of hyperactivity in NDO due to SCI, there is evidence for the involvement of the urothelium in the development and symptoms of NDO.[16]

Apodaca *et al*.[3] found that SCI also led to rapid disruption of the uroepithelial barrier. This was manifested in loss of cell–cell interactions, decreases in transepithelial resistance and increases in water and urea permeability.[3] One of the significant changes observed is the occurrence of disruption of the urothelium accompanied by reduction in transepithelial resistance. This happened as early as 2 h after SCI, but some recovery of transepithelial resistance occurred at later time points.[3] In addition, changes in the morphology of the urothelium have been detected, including the disappearance of apical cells, disorganization of cell layers and reduction of cellular volume.[3] Studies have shown that the expression of the purinergic receptor P2X2 in the human urothelium of SCI patients is increased.[17] In addition, rats with SCI also had increased release of excitatory mediators including prostaglandins, adenosine triphosphate and nitric oxide.[18,19] These mediators could contribute to increased excitability of bladder sensory afferents present in the suburothelial region or among urothelial cells. Another study also revealed that nerve growth factor mRNA and protein levels are increased in the urinary bladders of rats with SCI.[20] A number of ion channels including transient receptor potential vanilloid receptor 1 also increase in patients with neurogenic bladder disease.[21] Another major consequence of SCI is increased expression of connexin 26 in the urothelium, which could have a role in DO.[22] These evidence show the importance of urothelial dysfunction, which induces inflammation and deteriorates in a vicious cycle.

Studies of animals with SCI revealed that factors released from the mucosal bladder layer influence spontaneous detrusor contractions.[23] This activity may involve a number of factors including release of adenosine triphosphate from the urothelium acting on the underlying cells within the suburothelial space. In addition, chronic SCI in animals (and in patients with urge symptoms) shows an increase in connexin-hemichannel expression and increased intercellular communication within the low urinary tract compared with healthy control groups.[24,25] These findings suggest that altered low urinary tract junction proteins, perhaps by contributing to the amplification of
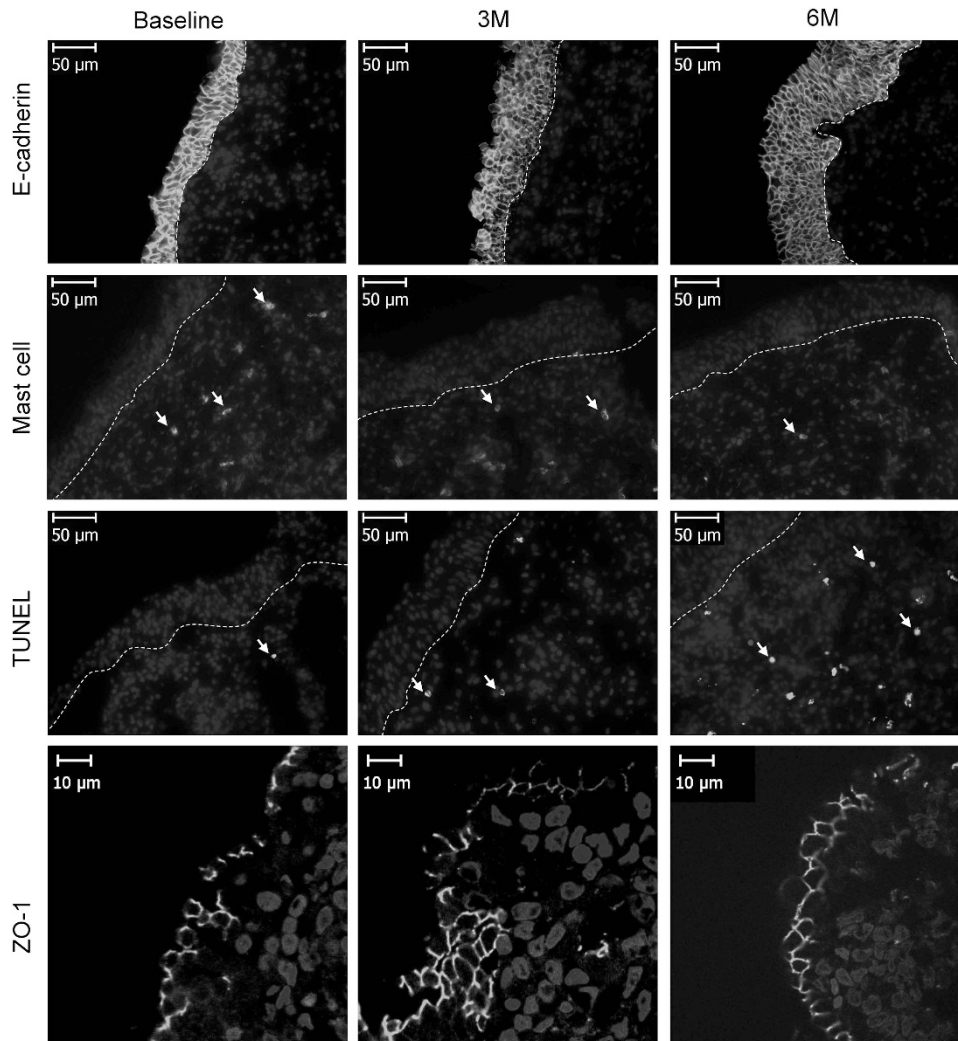
Figure 1 E-cadherin expression, mast cell counts, apoptotic cell counts (TUNEL staining) and ZO-1 expression in bladders of patients with SCIs at baseline and 3 and 6 months after a single 300-U BoNT-A injection. The target cells are labelled in bright (as the arrows indicate) and the white dotted lines indicate the boundary between the urothelium and suburothelium.

chemical signalling between and within low urinary tract cells, may have a role in the development of DO following SCI.

SCI is associated with a number of changes to the urothelium, including both structural changes in addition to defects in urothelial signalling changes.[26] Pretreatment with hexamethonium could block the acute effects of SCI on uroepithelial function, indicating that the release of neurotransmitters by bladder efferent nerves is at least partially responsible for the disruption of the uroepithelium.[3] BoNT-A inhibits adenosine triphosphate release from the urothelial, but not the serosal side of the bladder. This suggests that BoNT-A treatment inhibits transmitter release not only from efferent nerve endings but sensory nerve terminals and the urothelium as well.[6]

E-cadherin is a calcium-dependent glycoprotein, which has a critical role in cell-to-cell adhesion. Loss of E-cadherin function can trigger cancer progression and metastasis.[14] ZO-1 is a tight junction protein that maintains the highly resistant urothelial barrier.[27] Previous study has also established a molecular connection between E-cadherin and TRPV4, suggesting that E-cadherin is associated with bladder sensation and barrier function.[28] In the current study, the results showed the urothelial barrier recovered after BoNT-A injection by improving the adhesion and tight junction protein levels. This could explain the

mechanism of decreasing the hyperexcitability of C-fibre bladder afferents, thereby reducing NDO. However, the effect was not sustained over time.

The mast cells degranulate and release pre-formed mediators of inflammation including histamine, heparin and tryptase.[29] Apoptosis is a stepwise process characterized by a series of stereotypical morphological changes that eventually lead to cell death. Urothelial cell apoptosis in patients with interstitial cystitis/bladder pain syndrome resulted from upregulation of the inflammatory signals, including p38 mitogen-activated protein kinase and tumor necrosis factor-α.[30] In the present study, we showed that the apoptotic process was highly activated in the bladders of patients with SCI compared with the control group. However, the active mast cell activity and urothelial apoptosis showed no significant difference after single BoNT-A injection. These results indicate that BoNT-A injection cannot have an adequate anti-inflammatory effect in a single injection.

The bladder urothelium has a significant role in the sensory transduction mechanisms modulating micturition, in particular in conditions of increased sensory nerve transmission following chronic inflammation and SCI. Animal studies have shown that BoNT-A decreases transmitter release from the urothelium and neuropeptide

release from the bladder afferents.[22,23] In patients with NDO, the increasing nerve growth factor in bladder tissue decreased or normalized with detrusor injection of BoNT-A.[31] BoNT-A has been shown to inhibit contraction of both smooth and skeletal muscle by blocking neurotransmitter release. In humans with NDO, the bladder muscarinic receptors M2 and M3, and purinergic receptors P2X2 and P2X3 are reduced after detrusor BoNT-A injections, suggesting that BoNT-A inhibits DO by acting through inhibition of both the sensory and motor arms of the micturition reflex.[30]

Since 2000, Schurch et al.[32] reported the good effect of BoNT-A toxin detrusor injection for treating NDO in spinal cord-injured patients. Ongoing improvement in urodynamic parameters and incontinence was already present in all patients re-evaluated at 16 and 36 weeks. In addition to the benefit of decreased detrusor pressure to prevent renal damage, it significantly improved quality of life.[33] In the past 10 years, most of the study used 300 U of BoNT-A, usually 30 injections of 10 U ml^{-1} in the bladder (excluding the trigone) under cystoscopic guidance and with different types of anaesthesia.[34] In this study, we used 300 U BoNT-A for treatment of NDO with SCI bladders since 2006. Several phase 3, large-scale, randomized, double-blind, placebo-controlled, multicentre trials were conducted to investigate the efficacy and safety of detrusor BoNT-A injections in patients with NDO, due to SCI or multiple sclerosis (MS). BoNT-A 200 or 300 U significantly reduced urinary incontinence episodes compared with placebo. The effects were equally observed in both SCI and multiple sclerosis patients, and less side effects occurred in the 200-U treatment group.[5] Based on these phase 3 trials, the regulatory approved dose of BoNT-A is 200 U for patients with NDO. Haferkamp et al.[35] reported regeneration of the nerve endings after an interval of about 9 months after BoNT-A detrusor injection, with no significant ultrastructural detrusor changes found in the biopsy tissue. In addition to inhibiting detrusor contraction, there is evidence that the BoNT-A inhibits bladder sensory pathways contributing to storage symptoms.[36–38]

Our previous study revealed that BoNT-A injection decreased detrusor pressure and increase bladder compliance after repeat treatments every 6 months.[39] The therapeutic effectiveness showed no significant difference between patients with different SCI level or with and without DSD.[40] Patients with different clinical demographics and urodynamic parameters had similar treatment outcomes and UDI-6 scores.[36,39] The performance of CIC or not did not affect the satisfactory effect of Botox as well.[40] According to the results of this study, urothelial barrier dysfunction in SCI patients' bladders recovered after BoNT-A treatment; however, this effect decreased with time. Thus, neurogenic inflammation after SCI was not adequately improved after a single 300-U BoNT-A injection; it seems rational to repeat BoNT-A injection at a 6-month interval if the treatment target is at the urothelial dysfunction. In this study, the result showed the urothelial dysfunction improvement after BoNT-A injection in the expression of the adhesion protein E-cadherin and the tight junction protein ZO-1. Therefore, we supposed that BoNT-A injection improved NDO in SCI bladder not only by anticholinergic effects but urothelium function may be recovered after treatment.

UTI is a possible cause for urothelial dysfunction in patients with or without SCI. Most SCI patients may have asymptomatic bacteriuria and might effect on urothelial dysfunction. There has been no study on this topic, However, the asymptomatic bacteriuria might result in increased excitability of suburothelial sensory fibres and induce DO at a small bladder volume. Our previous study also showed the changes of urothelial function after recurrent UTI was similar to that occuring in this study.[41] In this study, patients were excluded if they had an active UTI at enrolment. Although six patients developed symptomatic or asymptomatic UTI after BoNT-A injection, the improvement of E-cadherin and ZO-1 are still present at 3 months after BoNT-A injection. However, the early disappearance of BoNT-A effect on the urothelial dysfunction might, in part, be affected by the UTI episodes.

A limitation of the present study is the small case number and lack of a control arm. However, this preliminary study demonstrated the mechanisms of action of BoNT-A treatment to improve urothelial dysfunction in the bladders of patients with chronic SCI. Another limitation is that there is no histologic assessment, which may provide compatible information to support our results. Further investigation of repeated injections and long-term follow-up is ongoing. In addition, the therapeutic effects of sensory protein improvements of transient receptor potential vanilloid receptor 1, P2X receptors, connexin and endothelial nitric oxide synthase have not yet been thoroughly investigated and require further study.

CONCLUSION

Urothelial dysfunction and adhesive and junction protein concentrations in SCI bladders recovered at 3 months after a single 300-U BoNT-A injection. However, this effect had decreased at 6 months after treatment. Thus, a single BoNT-A injection treatment to the detrusor produced an inadequate therapeutic response without a long lasting impact on chronic inflammation and urothelial apoptosis.

DATA ARCHIVING

There were no data to deposit.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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