



Clinical application of intravesical botulinum toxin type A for overactive bladder and interstitial cystitis

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After decades of clinical and basic science research, the clinical application of botulinum toxin A (Botox) in urology has been extended to neurogenic detrusor overactivity (NDO), idiopathic detrusor overactivity, refractory overactive bladder (OAB), interstitial cystitis/bladder pain syndrome (IC/BPS), lower urinary tract symptoms, benign prostatic hyperplasia, and neurogenic or non-neurogenic lower urinary tract dysfunction in children. Botox selectively disrupts and modulates neurotransmission, suppresses detrusor overactivity, and modulates sensory function, inflammation, and glandular function. In addition to motor effects, Botox has been found to have sensory inhibitory effects and anti-inflammatory effects; therefore, it has been used to treat IC/BPS and OAB. Currently, Botox has been approved for the treatment of NDO and OAB. Recent clinical trials on Botox for the treatment of IC/BPS have reported promising therapeutic effects, including reduced bladder pain. Additionally, the therapeutic duration was found to be longer with repeated Botox injections than with a single injection. However, the use of Botox for IC/BPS has not been approved. This paper reviews the recent advances in intravesical Botox treatment for OAB and IC/BPS.

Keywords: Botulinum toxins, type A; Cystitis, interstitial; Therapeutics; Urinary bladder, overactive

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INTRODUCTION

Botulinum toxin A (Botox) has been applied in lower urinary tract dysfunction (LUTD) since 30 years previously. OnabotulinumtoxinA (BOTOX[®], Allergan, Irvine, CA, USA) was first used to treat patients with spinal cord injury having neurogenic detrusor overactivity (NDO) or detrusor-sphincter dyssynergia [1-4]. Later, Botox was used for the treatment of idiopathic detrusor overactivity (IDO) and overactive bladder syndrome (OAB) refractory to conventional treatment [5-7]. Botox has been approved for these two

clinical conditions, and it has been widely used to restore urinary continence and improve quality of life [8,9]. Clinical trials for LUTD, including interstitial cystitis/bladder pain syndrome (IC/BPS), lower urinary tract symptoms, and benign prostatic hyperplasia, have been performed, but Botox use remains off label.

MECHANISMS OF BOTOX FOR OAB AND IC/BPS

Botox is a potent neurotoxin produced by the bacterium

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Clostridium botulinum [10]. When the toxin is cleaved into a 100-kDa heavy chain and a 50-kDa light chain by proteolytic cleavage, it becomes biologically active [11]. Botox enters neuronal cell membrane by binding to the synaptic vesicle protein SV2 [12]. After endocytosis of the toxin, it is cleaved into heavy and light chains. The light chain binds to the SNAP25 protein and can inhibit the release of neurotransmitters from vesicles [13]. The neurotransmitters and neuropeptides that can be inhibited by Botox include acetylcholine (ACh), adenosine triphosphate (ATP), nitric oxide (NO), substance P, and calcitonin gene-related peptide [14,15]. Through pharmacological actions on neuropeptides, Botox causes relaxation of the striated or smooth muscles and controls local inflammation. It can also block transient receptor potential vanilloid subfamily-1 (TRPV1) and purinergic receptor P2X₃-IR expressions in cases of bladder inflammation and decrease the sensitization of TRPV1 and P2X₃ [16]. Thus, intravesical Botox injection can reduce bladder sensation as well as bladder pain. It has been postulated that Botox might control chronic pain by acting on peripheral nociceptive neurons and causing central desensitization through retrograde toxin transport to the central nervous system (CNS) [17,18].

The clinical symptoms and pathogenesises of OAB and IC/BPS show overlaps. Recent investigations have found that the expression of nerve growth factor (NGF) is increased in the bladder of patients with OAB and those with IC/BPS. NGF is believed to be involved in the regulation of neural function, inflammation, and bladder pain [19,20]. Afferent nerve hyperactivity can be elicited in acute bladder inflammation, and it results in neural plasticity after repeated stimulation [21,22]. Urinary NGF levels have been found to decrease after intravesical Botox injection in both patients with OAB and those with IC/BPS [23,24]. Additionally, the Botox dose has been shown to be associated with decreases in bladder NGF levels and increases in NGF, TrkA, p75, and TRPV1 gene expressions [25]. Chronic bladder inflammation can lead to central sensitization, resulting in sensory nerve activation and bladder hypersensitivity [26]. Intravesical Botox injection can effectively control the inflammatory process by modulating neurotransmitter release from the sensory nerves [27,28]. Moreover, intravesical Botox injections might reduce the sensory urgency in patients with OAB and reduce pain in patients with IC/BPS, suggesting that the sensory and anti-inflammatory effects of Botox, rather than the motor effect alone, are involved in the treatment of patients with OAB and those with IC/BPS [29,30].

BOTOX INJECTION FOR OAB

OAB is highly prevalent and has an impact on patient quality of life. The current oral medications for OAB include antimuscarinics and beta-3 adrenoceptor agonists [6]. In patients refractory to these OAB medications, intravesical Botox injection has been documented to act as a third-line treatment according to the American Urological Association (AUA) and European Association of Urology guidelines [31,32].

The mechanism of Botox treatment for OAB involves the inhibition of the abnormal release of neurotransmitters, such as ACh, ATP, and substance P, and abnormal expression of TRPV1 and P2X₃ [33-35]. These neurotransmitters are associated with bladder sensation and inflammation, and they modulate detrusor contraction in OAB and DO [36]. Therefore, Botox treatment might reduce pain and urgency sensations in inflammatory bladder conditions, including OAB and IC/BPS [37].

Initially, the Botox dose for patients with IDO was 200 U of onabotulinumtoxinA [38]. At this dose, the daily frequency, urgency, and urgency urinary incontinence significantly decreased and the bladder capacity, voiding pressure, and quality of life significantly improved. However, the post-void residual (PVR) volume increased and clean intermittent catheterization (CIC) was required. Another type of Botox called abobotulinumtoxinA (Dysport, Ipsen Biopharm, Wrexham, UK) at a dose of 500 U showed similar results in the treatment of OAB [39,40]. Because the doses of onabotulinumtoxinA and abobotulinumtoxinA are not equivalent, it is difficult to compare the treatment outcomes between groups. However, most clinical trials on OAB used onabotulinumtoxinA as the treatment agent.

A previous clinical trial showed equal improvement in urodynamic parameters between 200 U of onabotulinumtoxinA for IDO and 300 U for NDO [41]. On comparing treatment outcomes among different doses of Botox, it was found that 100 U of onabotulinumtoxinA had a 73.3% success rate for IDO on suburothelial injection and that adverse events were less common at this dose than at 150 U or 200 U [42]. According to these findings and the findings of several other clinical studies, 100 U of onabotulinumtoxinA has become a standard dose for IDO and has shown satisfactory results [43]. Considering the high incidence of adverse events, such as a large PVR volume and subsequent urinary tract infection (UTI), low doses of onabotulinumtoxinA, such as 100 U, are more appropriate in patients with OAB [44].

A double-blind, placebo-controlled, randomized, dose-ranging trial using 50, 100, 150, and 200 U of onabotulinum-

toxinA also found that the dose of 100 U was effective for the treatment of OAB [45]. Moreover, a clinical trial in Europe confirmed that onabotulinumtoxinA injections at doses of 100 U and 150 U had similar tolerability and patient satisfaction [46]. With regard to regulatory approval for use, a phase 3 clinical trial revealed that injection of 100 U of onabotulinumtoxinA at 20 sites effectively treated OAB symptoms and improved quality of life [47,48].

A systemic review of the therapeutic efficacy and adverse events of onabotulinumtoxinA for OAB revealed that Botox has superior effects with regard to improvements in daily frequency, urgency, and urinary incontinence. However, the incidence of UTI and the risk of CIC requirement after Botox treatment significantly increased when compared to the findings with placebo [49]. With Botox, patients experience less urgency and urinary incontinence, which are associated with better quality of life. However, because of the high risk of adverse events, patients should be well informed and consent should be obtained before Botox treatment, especially for frail patients and patients with low detrusor contractility. OAB guidelines warn that Botox injection should be used carefully to prevent urinary retention and subsequent UTI [31,32].

Patients with CNS diseases, such as cerebrovascular accident, Parkinson's disease, and multiple sclerosis, usually have OAB. In these patients, a large PVR volume and UTI are specifically concerning conditions. Intravesical Botox injection at a dose of 100 U has been demonstrated to be safe and effective [50-52]. However, a large PVR volume remains a serious concern after Botox treatment [53]. Additionally, Botox treatment is a concern in patients with OAB and diabetes mellitus (DM). Although Botox injection is safe in DM patients with refractory DO, the possibility of an increase in the PVR volume and the risk of acute urinary retention in DM patients with low detrusor contractility should be considered. Patients should be informed about this risk prior to Botox injection [54]. In patients with OAB receiving Botox treatment, female sex, low OAB symptom score, and OAB-wet were shown to be associated with better therapeutic efficacy, and low baseline voiding efficiency was shown to be associated with a large PVR volume [55].

INTRAVESICAL BOTOX INJECTION FOR IC/BPS

The true pathogenesis of IC/BPS has not been well elucidated. The common histopathological findings of IC/BPS include denuded epithelium, mucosal ulceration, submucosa inflammation, granulation, and perineural infiltrate, sug-

gesting an inflammatory process in the bladder [56]. Recent studies have found that increased urothelial cell apoptosis is mediated by suburothelial inflammation [57,58], and Botox injection could inhibit inflammation and therefore improve the barrier function of the urothelium in patients with IC/BPS [59]. Increased submucosal nerve fiber proliferation, mast cell count, and nerve fiber count have been shown to be associated with high amounts of histamine [60]. A recent study suggested that neuron-mediated inflammation plays an important role in the pathophysiology of IC/BPS and that it might trigger secretion from mast cells [61].

Increased urothelial cell apoptosis has been considered to be involved in the pathogenesis of IC/BPS. Additionally, high numbers of apoptotic endothelial cells with morphological changes associated with nuclear fragmentation have been identified in the bladders of patients with IC/BPS [62]. Our previous study found high cell apoptosis levels and abnormal E-cadherin expression in the urothelium, which are associated with inflammation in the bladders of patients with IC/BPS [58]. Bladder inflammation might modulate the signaling pathway of increased urothelial cell apoptosis in patients with IC/BPS [57]. Immunofluorescence staining of phospho-p38 was positive in the urothelium of patients with IC/BPS, suggesting that abnormal urothelial apoptosis induced by chronic suburothelial inflammation might participate in the pathophysiology of IC/BPS [57].

Neural upregulation has been considered to play a role in the pathophysiology of IC/BPS. Urothelial release of ATP on bladder distention can activate ligand-gated ion channel P2X₃ receptors to evoke a neural discharge [63]. Upregulation of P2X₃ receptors during stretching of bladder urothelial cells has been found in patients with IC/BPS [32]. In addition, increased mRNA expression of TRPV1 was found in the bladders of patients with IC/BPS [64]. These findings suggest that bladder inflammation might alter the neuropeptide expression and increase bladder excitability and sensitization, resulting in increased pain sensation during bladder distension [65].

Since 2004, Botox has been used for treating IC/BPS. Smith et al. [66] found that satisfactory symptomatic improvement could be achieved by injecting 100 U or 200 U of onabotulinumtoxinA at 20 to 30 sites on the bladder base. Our pilot study also revealed that Botox could relieve bladder pain in patients with IC/BPS in whom conventional treatments failed [67]. Later, Giannantoni et al. [68] used 200 U of Botox injected submucosally in the trigone and bladder floor and reported a subjective improvement in 80% of patients with IC/BPS. Moreover, Ramsay et al. [69] reported satisfactory outcomes in patients with IC/BPS who received

200 U to 300 U of Botox. Furthermore, Giannantoni et al. [70] found significant improvements in anxiety, depression, and quality of life among patients with IC/BPS after Botox injection, although the therapeutic duration was only 3 months.

In our previous prospective, randomized, controlled study involving patients with refractory IC/BPS, we found that patients who received suburothelial Botox injection (200 U or 100 U) plus cystoscopic hydrodistention showed significant improvements in the visual analog score of bladder pain and the functional and cystometric bladder capacity at 3 months after treatment when compared with the findings in patients who received cystoscopic hydrodistention alone [59]. The adverse event of difficult urination was less common in the 100 U Botox group than in the 200 U Botox group. Additionally, the successful treatment outcome was better in the Botox groups than in the control group. A further study revealed that suburothelial Botox injection at 100 U with cystoscopic hydrodistention effectively improved IC symptom scores and the global response assessment index at 3 and 6 months of follow-up [71]. Based on the results of our clinical trials, we suggest that intravesical injection of 100 U of Botox is effective for relieving IC symptoms and bladder pain in the short term. The therapeutic duration was longer with repeated Botox injections than with a single injection. Repeated Botox injections every 6 months increased the therapeutic duration, and a long effective period was noted in patients who received four treatments [72].

There is currently no consensus on whether Botox should be injected into the trigone or bladder body in patients with IC/BPS. Giannantoni et al. [68] and Pinto et al. [73] performed trigonal injection and found that the treatment outcome was satisfactory. Additionally, repeated trigonal Botox injections were shown to have persistent therapeutic effects [74]. In our recent clinical trial, we found that Botox injection into the suburothelium of the bladder body was associated with favorable treatment outcomes in all IC/BPS cases, except ulcer-type IC/BPS cases [75]. In another report, the therapeutic response of Botox injection into the trigone was comparable between ulcer-type and non-ulcer-type IC/BPS [76].

Although recent clinical trials on Botox injection for the treatment of IC/BPS have shown promising therapeutic effects, including reductions in bladder pain and IC symptoms, there have been few clinical trials to demonstrate the superiority of Botox over placebo. A recent randomized, double-blind, placebo-controlled, multicenter trial demonstrated that intravesical suburothelial injection of 100 U of Botox significantly reduced bladder pain symptoms. Additionally,

the cystometric bladder capacity was higher in the Botox group than in the normal saline group. Moreover, the overall success rates were 63% in the Botox group and 15% in the normal saline group [77]. Although Botox treatment for IC/BPS has not been approved by regulatory authorities, it has been documented in the treatment guidelines of the AUA and Asian Urological Association [78,79].

BOTOX TREATMENT FOR ULCER/ NON-ULCER-TYPE IC/BPS

IC/BPS can be classified as non-ulcer-type and ulcer-type (classic) IC/BPS according to the characteristic cystoscopic findings under hydrodistention [80]. About 10% to 20% of IC/BPS cases are classified as ulcer-type IC/BPS [81]. Patients with ulcer-type IC/BPS are usually older and have smaller bladder capacity and greater bladder pain when compared with the findings in patients with non-ulcer-type IC/BPS [82]. Ulcer-type and non-ulcer-type IC/BPS are considered as two distinct diseases because of their different underlying pathophysiologies and treatment strategies [83]. The AUA guidelines recommend that ulcer-type IC/BPS should be treated with fulguration by laser or electrocautery of Hunner's lesion [84]. With regard to Botox injection, we found that ulcer-type IC/BPS did not respond well to four repeated intravesical injections of 100 U of onabotulinumtoxinA every 6 months [75]. This finding suggests that Botox treatment might not be suitable for ulcer-type IC/BPS. However, a Portuguese group found that Botox trigonal injection provided satisfactory outcomes in patients with ulcer-type IC/BPS and that treatment outcomes with regard to symptom intensity did not differ between ulcer-type and non-ulcer-type IC/BPS [76]. As ulcer-type IC/BPS has not been well defined, the treatment outcomes of Botox injections in ulcer-type and non-ulcer-type IC/BPS might not be appropriately compared. Nevertheless, considering the poor response of ulcer-type IC/BPS to conventional treatment, it should be treated as a disease different from non-ulcer-type IC/BPS.

ADVERSE EVENTS OF BOTOX INJECTION IN OAB AND IC/BPS

Repeated Botox injections for the treatment of lower urinary tract disorders and pelvic floor dysfunction have been recommended by a European consensus report [85]. The period of retreatment should not be shorter than 3 months, and a 6 to 9-month interval is recommended to prevent residual circulating antibodies that decrease the therapeutic effect of subsequent Botox injections [86]. Considering the potential

adverse events of a large PVR volume and acute urinary retention after intravesical Botox injection, patients who are planning to receive Botox injection should be informed about the potential adverse events, and the possibility of CIC should be conveyed [31,32]. Patients with OAB might develop a large PVR volume in the first month after injection, and they might not appreciate the increase in bladder capacity and reduction in urgency severity if they develop bothersome adverse events and need CIC. The most common reasons for the discontinuation of Botox treatment in patients with OAB are poor efficacy (13%) and issues related to the requirement of CIC (11%) [87].

There has been no report of the occurrence of serious systemic adverse events, such as respiratory depression and generalized muscle weakness, after Botox injection in patients with OAB or IC/BPS. Few patients have reported mild fatigue (2.8%) after Botox treatment. The most common local adverse events after Botox injection are gross hematuria (7.8%), a large PVR volume >150 mL (47.5%), difficult urination (46.5%), and UTI (14.3%) [88]. Interestingly, adverse events were more common in OAB patients than in IC/BPS patients who received 100 U of onabotulinumtoxinA (58.3% vs. 42.7%), including a large PVR volume >200 mL (31.9% vs. 6.7%) and acute urinary retention (1.4% vs. 0%) [89]. Differences in the effects of Botox on disorder pathophysiology between OAB and IC/BPS and differences in the susceptibility of detrusor muscle contractility to Botox might account for these findings. Most clinical studies reported an increase in the PVR volume and a decrease in voiding efficiency among patients with OAB after Botox injection [66,90] and reported decreases in the maximum flow rate and detrusor pressure among patients with IC/BPS [66,67,70]. Injecting Botox into the trigone and not the bladder body might effectively prevent the increase in the PVR volume and the occurrence of acute urinary retention after treatment [73,90]. Our large cohort study on IC/BPS treated with Botox injection found that about 30% of patients complained of dysuria after each injection and that only one episode of acute urine retention occurred [72].

EXPERIMENTAL TRIAL OF LIPOSOME-ENCAPSULATED ONABOTULINUMTOXINA

Botox is a large molecule protein that cannot pass the urothelial cell membrane and act on the nerve plexus unless it is injected into the suburothelium [11]. Tyagi et al. [91] found that a liposome could encapsulate the Botox protein and penetrate the urothelial cell membrane after intravesi-

cal instillation. The lipidic bilayer structure of liposomes facilitates their adherence to the apical membrane surface of luminal cells in the bladder. Intravesical instillation of liposomes alone was shown to treat hypersensitive bladder in a rat model [92]. Liposome-encapsulated Botox could improve acetic acid-induced bladder hyperactivity, and the inflammatory reaction and SNAP25 expression were found to significantly decrease [93]. It is likely that Botox can be delivered into urothelial cells through liposome encapsulation and fusion with the phospholipids of the cell membrane without injection trauma. If this hypothesis is true, Botox could be used to treat OAB and IC/BPS through intravesical instillation.

In a pilot study, we found that liquid liposomal delivery of Botox (Liposome-Botox) could penetrate the bladder urothelium in patients with refractory OAB [94]. After intravesical instillation of Lipotoxin (containing 80 mg of liposome and 200 U of onabotulinumtoxinA) or normal saline in patients with OAB, we found that Lipotoxin effectively reduced frequency episodes and the urgency severity score when compared to the findings with placebo at 1 month after treatment. There was no increase in the PVR volume or the risk of UTI after Lipotoxin instillation. We also found that SV2A receptors were present in the human urothelial cell lysate. However, SNAP25 did not show a significant decrease in OAB tissue [94,95]. In another multicenter clinical trial using Lipotoxin for the treatment of OAB, Lipotoxin was associated with significant decreases in frequency (three-day diary) and urinary urgency episodes without an increase in the PVR volume [96]. These results suggest that Lipotoxin might block the release of sensory neurotransmitters from the urothelium and inhibit sensory hyperactivity, but might not influence detrusor contractility.

Intravesical liposomes were found to have beneficial effects in a bladder hypersensitivity rat model [97]. Recent clinical studies have tested multilamellar liposomes composed entirely of sphingomyelin as a novel intravesical therapy for IC. Although no significant difference in clinical efficacy was noted with regard to pentosan polysulphate, liposome instillation into the bladder significantly decreased urinary frequency and nocturia in patients with IC/BPS [98]. In a recent clinical trial on bladder instillation of liposome-formulated onabotulinumtoxinA for IC/BPS, we did not note the therapeutic superiority of Lipotoxin when compared with placebo. However, a single Lipotoxin instillation was associated with a decrease in symptoms from baseline among patients with moderate-to-severe IC/BPS [99].

Although liposome-encapsulated Botox for the treatment of OAB or IC/BPS seems rational and promising, only 50%

of patients with OAB responded to this treatment, and the therapeutic effect on IC/BPS was not superior to that of placebo. The short therapeutic duration with regard to OAB might limit the wide clinical application of this treatment approach in real-world practice. Technical improvements in formulation and instillation as well as dosing of Botox might increase the response rate in the future.

CONCLUSIONS

Recent evidence has demonstrated that intravesical Botox injection is effective for the treatment of patients with OAB refractory to antimuscarinic therapy and those with IC/BPS. OAB symptoms improve and the bladder capacity increases after Botox injection; however, the incidences of a large PVR volume, UTI, and need for CIC increase after treatment, especially in frail elderly patients. Recent results show that Botox is effective for relieving bladder pain and bothersome bladder symptoms in patients with refractory IC/BPS. Short-term improvements in bladder pain, frequency, and bladder capacity could be achieved with Botox injection, but long-term therapeutic effects are not achieved. Repeat Botox injections could provide successful long-term outcomes. Although Botox injection is promising for the treatment of OAB and IC/BPS patients refractory to conventional therapies, the need for anesthesia, occurrence of local complications, and risks of a large PVR volume and UTI limit its clinical application. Intravesical instillation of liposome-encapsulated Botox might become an alternative treatment approach in the future. However, the short therapeutic duration is a major drawback. Further well-designed randomized trials with a placebo control are needed.

CONFLICTS OF INTEREST

The authors have nothing to disclose.

AUTHORS' CONTRIBUTIONS

Research conception and design: Hann-Chorng Kuo. Drafting of the manuscript: Jing-Ling Chen. Revision of the manuscript: Hann-Chorng Kuo. Approval of the final manuscript: Jing-Ling Chen and Hann-Chorng Kuo.

REFERENCES

1. Dykstra DD, Sidi AA, Scott AB, Pagel JM, Goldish GD. Effects of botulinum A toxin on detrusor-sphincter dyssynergia in spinal cord injury patients. *J Urol* 1988;139:919-22.
2. Schurch B, Hauri D, Rodic B, Curt A, Meyer M, Rossier AB. Botulinum-A toxin as a treatment of detrusor-sphincter dyssynergia: a prospective study in 24 spinal cord injury patients. *J Urol* 1996;155:1023-9.
3. Dykstra DD, Sidi AA. Treatment of detrusor-sphincter dyssynergia with botulinum A toxin: a double-blind study. *Arch Phys Med Rehabil* 1990;71:24-6.
4. de Sèze M, Petit H, Gallien P, de Sèze MP, Joseph PA, Mazaux JM, et al. Botulinum a toxin and detrusor sphincter dyssynergia: a double-blind lidocaine-controlled study in 13 patients with spinal cord disease. *Eur Urol* 2002;42:56-62.
5. Kuo HC. Botulinum A toxin urethral sphincter injection for neurogenic or nonneurogenic voiding dysfunction. *Ci Ji Yi Xue Za Zhi* 2016;28:89-93.
6. Kuo HC. Individualizing medical treatment of overactive bladder. *Ci Ji Yi Xue Za Zhi* 2018;30:195-9.
7. Kuo HC. Clinical effects of suburothelial injection of botulinum A toxin on patients with nonneurogenic detrusor overactivity refractory to anticholinergics. *Urology* 2005;66:94-8.
8. Schurch B, Stöhrer M, Kramer G, Schmid DM, Gaul G, Hauri D. Botulinum-A toxin for treating detrusor hyperreflexia in spinal cord injured patients: a new alternative to anticholinergic drugs? Preliminary results. *J Urol* 2000;164(3 Pt 1):692-7.
9. Reitz A, Stöhrer M, Kramer G, Del Popolo G, Chartier-Kastler E, Pannek J, et al. European experience of 200 cases treated with botulinum-A toxin injections into the detrusor muscle for urinary incontinence due to neurogenic detrusor overactivity. *Eur Urol* 2004;45:510-5.
10. Erbguth FJ. Historical notes on botulism, *Clostridium botulinum*, botulinum toxin, and the idea of the therapeutic use of the toxin. *Mov Disord* 2004;19 Suppl 8:S2-6.
11. Schiavo G, Rossetto O, Santucci A, DasGupta BR, Montecucco C. Botulinum neurotoxins are zinc proteins. *J Biol Chem* 1992;267:23479-83.
12. Dong M, Yeh F, Tepp WH, Dean C, Johnson EA, Janz R, et al. SV2 is the protein receptor for botulinum neurotoxin A. *Science* 2006;312:592-6.
13. Simpson LL. Molecular pharmacology of botulinum toxin and tetanus toxin. *Annu Rev Pharmacol Toxicol* 1986;26:427-53.
14. Smith CP, Gangitano DA, Munoz A, Salas NA, Boone TB, Aoki KR, et al. Botulinum toxin type A normalizes alterations in urothelial ATP and NO release induced by chronic spinal cord injury. *Neurochem Int* 2008;52:1068-75.
15. Lucioni A, Bales GT, Lotan TL, McGehee DS, Cook SP, Rapp DE. Botulinum toxin type A inhibits sensory neuropeptide release in rat bladder models of acute injury and chronic inflammation. *BJU Int* 2008;101:366-70.
16. Yiangou Y, Facer P, Ford A, Brady C, Wiseman O, Fowler CJ, et al. Capsaicin receptor VR1 and ATP-gated ion channel P2X3

- in human urinary bladder. *BJU Int* 2001;87:774-9.
17. Dolly JO, O'Connell MA. Neurotherapeutics to inhibit exocytosis from sensory neurons for the control of chronic pain. *Curr Opin Pharmacol* 2012;12:100-8.
 18. Guo BL, Zheng CX, Sui BD, Li YQ, Wang YY, Yang YL. A closer look to botulinum neurotoxin type A-induced analgesia. *Toxicon* 2013;71:134-9.
 19. Steers WD, Kolbeck S, Creedon D, Tuttle JB. Nerve growth factor in the urinary bladder of the adult regulates neuronal form and function. *J Clin Invest* 1991;88:1709-15.
 20. Dupont MC, Spitsbergen JM, Kim KB, Tuttle JB, Steers WD. Histological and neurotrophic changes triggered by varying models of bladder inflammation. *J Urol* 2001;166:1111-8.
 21. Chuang YC, Fraser MO, Yu Y, Chancellor MB, de Groat WC, Yoshimura N. The role of bladder afferent pathways in bladder hyperactivity induced by the intravesical administration of nerve growth factor. *J Urol* 2001;165:975-9.
 22. Lamb K, Gebhart GF, Bielefeldt K. Increased nerve growth factor expression triggers bladder overactivity. *J Pain* 2004;5:150-6.
 23. Giannantoni A, Di Stasi SM, Stephen RL, Bini V, Costantini E, Porena M. Intravesical resiniferatoxin versus botulinum-A toxin injections for neurogenic detrusor overactivity: a prospective randomized study. *J Urol* 2004;172:240-3.
 24. Liu HT, Tyagi P, Chancellor MB, Kuo HC. Urinary nerve growth factor but not prostaglandin E2 increases in patients with interstitial cystitis/bladder pain syndrome and detrusor overactivity. *BJU Int* 2010;106:1681-5.
 25. Giannantoni A, Conte A, Farfariello V, Proietti S, Vianello A, Nardicchi V, et al. Onabotulinumtoxin-A intradetrusorial injections modulate bladder expression of NGF, TrkA, p75 and TRPV1 in patients with detrusor overactivity. *Pharmacol Res* 2013;68:118-24.
 26. Aoki KR. Evidence for antinociceptive activity of botulinum toxin type A in pain management. *Headache* 2003;43 Suppl 1:S9-15.
 27. Cui M, Aoki KR. Botulinum toxic type a (BTX-A) reduces inflammatory pain in the rat formalin model. *Cephalgia* 2000;20:414.
 28. Durham PL, Cady R, Cady R. Regulation of calcitonin gene-related peptide secretion from trigeminal nerve cells by botulinum toxin type A: implications for migraine therapy. *Headache* 2004;44:35-42; discussion 42-3.
 29. Kuo HC. Reduction of urgency severity is associated with long-term therapeutic effect after intravesical onabotulinumtoxin A injection for idiopathic detrusor overactivity. *Neurourol Urodyn* 2011;30:1497-502.
 30. Jiang YH, Liu HT, Kuo HC. Decrease of urinary nerve growth factor but not brain-derived neurotrophic factor in patients with interstitial cystitis/bladder pain syndrome treated with hyaluronic acid. *PLoS One* 2014;9:e91609.
 31. Gormley EA, Lightner DJ, Faraday M, Vasavada SP; American Urological Association; Society of Urodynamics, Female Pelvic Medicine. Diagnosis and treatment of overactive bladder (non-neurogenic) in adults: AUA/SUFU guideline amendment. *J Urol* 2015;193:1572-80.
 32. Nambiar AK, Bosch R, Cruz F, Lemack GE, Thiruchelvam N, Tubaro A, et al. EAU Guidelines on assessment and nonsurgical management of urinary incontinence. *Eur Urol* 2018;73:596-609.
 33. Yoshida M, Miyamae K, Iwashita H, Otani M, Inadome A. Management of detrusor dysfunction in the elderly: changes in acetylcholine and adenosine triphosphate release during aging. *Urology* 2004;63(3 Suppl 1):17-23.
 34. Sun Y, Chai TC. Up-regulation of P2X3 receptor during stretch of bladder urothelial cells from patients with interstitial cystitis. *J Urol* 2004;171:448-52.
 35. Apostolidis A, Brady CM, Yiangou Y, Davis J, Fowler CJ, Anand P. Capsaicin receptor TRPV1 in urothelium of neurogenic human bladders and effect of intravesical resiniferatoxin. *Urology* 2005;65:400-5.
 36. Avelino A, Cruz C, Nagy I, Cruz F. Vanilloid receptor 1 expression in the rat urinary tract. *Neuroscience* 2002;109:787-98.
 37. Lawrence GW, Aoki KR, Dolly JO. Excitatory cholinergic and purinergic signaling in bladder are equally susceptible to botulinum neurotoxin a consistent with co-release of transmitters from efferent fibers. *J Pharmacol Exp Ther* 2010;334:1080-6.
 38. Sahai A, Khan MS, Dasgupta P. Efficacy of botulinum toxin-A for treating idiopathic detrusor overactivity: results from a single center, randomized, double-blind, placebo controlled trial. *J Urol* 2007;177:2231-6.
 39. Jeffery S, Fynes M, Lee F, Wang K, Williams L, Morley R. Efficacy and complications of intradetrusor injection with botulinum toxin A in patients with refractory idiopathic detrusor overactivity. *BJU Int* 2007;100:1302-6.
 40. Rajkumar GN, Small DR, Mustafa AW, Conn G. A prospective study to evaluate the safety, tolerability, efficacy and durability of response of intravesical injection of botulinum toxin type A into detrusor muscle in patients with refractory idiopathic detrusor overactivity. *BJU Int* 2005;96:848-52.
 41. Popat R, Apostolidis A, Kalsi V, Gonzales G, Fowler CJ, Dasgupta P. A comparison between the response of patients with idiopathic detrusor overactivity and neurogenic detrusor overactivity to the first intradetrusor injection of botulinum-A toxin. *J Urol* 2005;174:984-9.
 42. Kuo HC. Will suburothelial injection of small dose of botulinum A toxin have similar therapeutic effects and less adverse events for refractory detrusor overactivity? *Urology* 2006;

- 68:993-7; discussion 997-8.
43. Werner M, Schmid DM, Schüssler B. Efficacy of botulinum-A toxin in the treatment of detrusor overactivity incontinence: a prospective nonrandomized study. *Am J Obstet Gynecol* 2005; 192:1735-40.
 44. Dmochowski R, Chapple C, Nitti VW, Chancellor M, Everaert K, Thompson C, et al. Efficacy and safety of onabotulinumtoxinA for idiopathic overactive bladder: a double-blind, placebo controlled, randomized, dose ranging trial. *J Urol* 2010;184:2416-22.
 45. Denys P, Le Normand L, Ghout I, Costa P, Chartier-Kastler E, Grise P, et al.; VESITOX study group in France. Efficacy and safety of low doses of onabotulinumtoxinA for the treatment of refractory idiopathic overactive bladder: a multicentre, double-blind, randomised, placebo-controlled dose-ranging study. *Eur Urol* 2012;61:520-9.
 46. Rovner E, Kennelly M, Schulte-Baukloh H, Zhou J, Haag-Molkenteller C, Dasgupta P. Urodynamic results and clinical outcomes with intradetrusor injections of onabotulinumtoxinA in a randomized, placebo-controlled dose-finding study in idiopathic overactive bladder. *Neurourol Urodyn* 2011;30:556-62.
 47. Nitti VW, Dmochowski R, Herschorn S, Sand P, Thompson C, Nardo C, et al.; EMBARK Study Group. OnabotulinumtoxinA for the treatment of patients with overactive bladder and urinary incontinence: results of a phase 3, randomized, placebo controlled trial. *J Urol* 2013;189:2186-93.
 48. Chapple C, Sievert KD, MacDiarmid S, Khullar V, Radziszewski P, Nardo C, et al. OnabotulinumtoxinA 100 U significantly improves all idiopathic overactive bladder symptoms and quality of life in patients with overactive bladder and urinary incontinence: a randomised, double-blind, placebo-controlled trial. *Eur Urol* 2013;64:249-56.
 49. Mangera A, Apostolidis A, Andersson KE, Dasgupta P, Giannantoni A, Roehrborn C, et al. An updated systematic review and statistical comparison of standardised mean outcomes for the use of botulinum toxin in the management of lower urinary tract disorders. *Eur Urol* 2014;65:981-90.
 50. Giannantoni A, Conte A, Proietti S, Giovannozzi S, Rossi A, Fabbrini G, et al. Botulinum toxin type A in patients with Parkinson's disease and refractory overactive bladder. *J Urol* 2011;186:960-4.
 51. Anderson RU, Orenberg EK, Glowe P. OnabotulinumtoxinA office treatment for neurogenic bladder incontinence in Parkinson's disease. *Urology* 2014;83:22-7.
 52. Çetinel B, Tarcan T, Demirkesen O, Özyurt C, Şen İ, Erdoğan S, et al. Management of lower urinary tract dysfunction in multiple sclerosis: a systematic review and Turkish consensus report. *Neurourol Urodyn* 2013;32:1047-57.
 53. Kuo HC. Therapeutic effects of suburothelial injection of botulinum a toxin for neurogenic detrusor overactivity due to chronic cerebrovascular accident and spinal cord lesions. *Urology* 2006;67:232-6.
 54. Wang CC, Liao CH, Kuo HC. Diabetes mellitus does not affect the efficacy and safety of intravesical onabotulinumtoxinA injection in patients with refractory detrusor overactivity. *Neurourol Urodyn* 2014;33:1235-9.
 55. Hsiao SM, Lin HH, Kuo HC. Factors associated with therapeutic efficacy of intravesical onabotulinumtoxinA injection for overactive bladder syndrome. *PLoS One* 2016;11:e0147137.
 56. Ke QS, Kuo HC. Pathophysiology of interstitial cystitis/bladder pain syndrome. *Tzu Chi Med J* 2015;27:139-44.
 57. Shie JH, Liu HT, Kuo HC. Increased cell apoptosis of urothelium mediated by inflammation in interstitial cystitis/painful bladder syndrome. *Urology* 2012;79:484.e7-13.
 58. Shie JH, Kuo HC. Higher levels of cell apoptosis and abnormal E-cadherin expression in the urothelium are associated with inflammation in patients with interstitial cystitis/painful bladder syndrome. *BJU Int* 2011;108(2 Pt 2):E136-41.
 59. Kuo HC, Chancellor MB. Comparison of intravesical botulinum toxin type A injections plus hydrodistention with hydrodistention alone for the treatment of refractory interstitial cystitis/painful bladder syndrome. *BJU Int* 2009;104:657-61.
 60. Lundeberg T, Liedberg H, Nordling L, Theodorsson E, Owzarski A, Ekman P. Interstitial cystitis: correlation with nerve fibres, mast cells and histamine. *Br J Urol* 1993;71:427-9.
 61. Li WW, Guo TZ, Liang DY, Sun Y, Kingery WS, Clark JD. Substance P signaling controls mast cell activation, degranulation, and nociceptive sensitization in a rat fracture model of complex regional pain syndrome. *Anesthesiology* 2012;116:882-95.
 62. Yamada T, Nishimura M, Mita H. Increased number of apoptotic endothelial cells in bladder of interstitial cystitis patients. *World J Urol* 2007;25:407-13.
 63. Rong W, Spyer KM, Burnstock G. Activation and sensitisation of low and high threshold afferent fibres mediated by P2X receptors in the mouse urinary bladder. *J Physiol* 2002;541(Pt 2):591-600.
 64. Homma Y, Nomiya A, Tagaya M, Oyama T, Takagaki K, Nishimatsu H, et al. Increased mRNA expression of genes involved in pronociceptive inflammatory reactions in bladder tissue of interstitial cystitis. *J Urol* 2013;190:1925-31.
 65. Shea VK, Cai R, Crepps B, Mason JL, Perl ER. Sensory fibers of the pelvic nerve innervating the Rat's urinary bladder. *J Neurophysiol* 2000;84:1924-33.
 66. Smith CP, Radziszewski P, Borkowski A, Somogyi GT, Boone TB, Chancellor MB. Botulinum toxin a has antinociceptive effects in treating interstitial cystitis. *Urology* 2004;64:871-5; discussion 875.

67. Kuo HC. Preliminary results of suburothelial injection of botulinum A toxin in the treatment of chronic interstitial cystitis. *Urol Int* 2005;75:170-4.
68. Giannantoni A, Costantini E, Di Stasi SM, Tascini MC, Bini V, Porena M. Botulinum A toxin intravesical injections in the treatment of painful bladder syndrome: a pilot study. *Eur Urol* 2006;49:704-9.
69. Ramsay AK, Small DR, Conn IG. Intravesical botulinum toxin type A in chronic interstitial cystitis: results of a pilot study. *Surgeon* 2007;5:331-3.
70. Giannantoni A, Cagini R, Del Zingaro M, Proietti S, Quartesan R, Porena M, et al. Botulinum A toxin intravesical injections for painful bladder syndrome: impact upon pain, psychological functioning and Quality of Life. *Curr Drug Deliv* 2010;7:442-6.
71. Chung SD, Kuo YC, Kuo HC. Intravesical onabotulinumtoxinA injections for refractory painful bladder syndrome. *Pain Physician* 2012;15:197-202.
72. Kuo HC. Repeated onabotulinumtoxin-a injections provide better results than single injection in treatment of painful bladder syndrome. *Pain Physician* 2013;16:E15-23.
73. Pinto R, Lopes T, Frias B, Silva A, Silva JA, Silva CM, et al. Trigonal injection of botulinum toxin A in patients with refractory bladder pain syndrome/interstitial cystitis. *Eur Urol* 2010;58:360-5.
74. Pinto R, Lopes T, Silva J, Silva C, Dinis P, Cruz F. Persistent therapeutic effect of repeated injections of onabotulinum toxin a in refractory bladder pain syndrome/interstitial cystitis. *J Urol* 2013;189:548-53.
75. Lee CL, Kuo HC. Intravesical botulinum toxin a injections do not benefit patients with ulcer type interstitial cystitis. *Pain Physician* 2013;16:109-16.
76. Pinto R, Lopes T, Costa D, Barros S, Silva J, Silva C, et al. Ulcerative and nonulcerative forms of bladder pain syndrome/interstitial cystitis do not differ in symptom intensity or response to onabotulinum toxin A. *Urology* 2014;83:1030-4.
77. Kuo HC, Jiang YH, Tsai YC, Kuo YC. Intravesical botulinum toxin-A injections reduce bladder pain of interstitial cystitis/bladder pain syndrome refractory to conventional treatment - a prospective, multicenter, randomized, double-blind, placebo-controlled clinical trial. *Neurourol Urodyn* 2016;35:609-14.
78. Hanno PM, Erickson D, Moldwin R, Faraday MM; American Urological Association. Diagnosis and treatment of interstitial cystitis/bladder pain syndrome: AUA guideline amendment. *J Urol* 2015;193:1545-53.
79. Homma Y, Ueda T, Tomoe H, Lin AT, Kuo HC, Lee MH, et al. Clinical guidelines for interstitial cystitis and hypersensitive bladder updated in 2015. *Int J Urol* 2016;23:542-9.
80. Nigro DA, Wein AJ, Foy M, Parsons CL, Williams M, Nyberg LM Jr, et al. Associations among cystoscopic and urodynamic findings for women enrolled in the Interstitial Cystitis Data Base (ICDB) Study. *Urology* 1997;49(5A Suppl):86-92.
81. Denson MA, Griebing TL, Cohen MB, Kreder KJ. Comparison of cystoscopic and histological findings in patients with suspected interstitial cystitis. *J Urol* 2000;164:1908-11.
82. Tomaszewski JE, Landis JR, Russack V, Williams TM, Wang LP, Hardy C, et al.; Interstitial Cystitis Database Study Group. Biopsy features are associated with primary symptoms in interstitial cystitis: results from the interstitial cystitis database study. *Urology* 2001;57(6 Suppl 1):67-81.
83. Peters KM, Killinger KA, Mounayer MH, Boura JA. Are ulcerative and nonulcerative interstitial cystitis/painful bladder syndrome 2 distinct diseases? A study of coexisting conditions. *Urology* 2011;78:301-8.
84. Hanno PM, Burks DA, Clemens JQ, Dmochowski RR, Erickson D, Fitzgerald MP, et al.; Interstitial Cystitis Guidelines Panel of the American Urological Association Education and Research, Inc. AUA guideline for the diagnosis and treatment of interstitial cystitis/bladder pain syndrome. *J Urol* 2011;185:2162-70.
85. Apostolidis A, Dasgupta P, Denys P, Elneil S, Fowler CJ, Giannantoni A, et al.; European Consensus Panel. Recommendations on the use of botulinum toxin in the treatment of lower urinary tract disorders and pelvic floor dysfunctions: a European consensus report. *Eur Urol* 2009;55:100-19.
86. Schurch B, de Sèze M, Denys P, Chartier-Kastler E, Haab F, Everaert K, et al.; Botox Detrusor Hyperreflexia Study Team. Botulinum toxin type a is a safe and effective treatment for neurogenic urinary incontinence: results of a single treatment, randomized, placebo controlled 6-month study. *J Urol* 2005;174:196-200.
87. Dowson C, Watkins J, Khan MS, Dasgupta P, Sahai A. Repeated botulinum toxin type A injections for refractory overactive bladder: medium-term outcomes, safety profile, and discontinuation rates. *Eur Urol* 2012;61:834-9.
88. Kuo HC, Liao CH, Chung SD. Adverse events of intravesical botulinum toxin a injections for idiopathic detrusor overactivity: risk factors and influence on treatment outcome. *Eur Urol* 2010;58:919-26.
89. Kuo YC, Kuo HC. Adverse events of intravesical onabotulinumtoxinA injection between patients with overactive bladder and interstitial cystitis--different mechanisms of action of botox on bladder dysfunction? *Toxins (Basel)* 2016;8:E75.
90. Kuo HC. Bladder base/trigone injection is safe and as effective as bladder body injection of onabotulinumtoxinA for idiopathic detrusor overactivity refractory to antimuscarinics. *Neurourol Urodyn* 2011;30:1242-8.
91. Tyagi P, Chancellor MB, Li Z, De Groat WC, Yoshimura N, Fraser MO, et al. Urodynamic and immunohistochemical eval-

- uation of intravesical capsaicin delivery using thermosensitive hydrogel and liposomes. *J Urol* 2004;171:483-9.
92. Fraser MO, Chuang YC, Tyagi P, Yokoyama T, Yoshimura N, Huang L, et al. Intravesical liposome administration--a novel treatment for hyperactive bladder in the rat. *Urology* 2003; 61:656-63.
 93. Chuang YC, Tyagi P, Huang CC, Yoshimura N, Wu M, Kaufman J, et al. Urodynamic and immunohistochemical evaluation of intravesical botulinum toxin A delivery using liposomes. *J Urol* 2009;182:786-92.
 94. Kuo HC, Liu HT, Chuang YC, Birder LA, Chancellor MB. Pilot study of liposome-encapsulated onabotulinumtoxinA for patients with overactive bladder: a single-center study. *Eur Urol* 2014;65:1117-24.
 95. Liu HT, Chen SH, Chancellor MB, Kuo HC. Presence of cleaved synaptosomal-associated protein-25 and decrease of purinergic receptors P2X3 in the bladder urothelium influence efficacy of botulinum toxin treatment for overactive bladder syndrome. *PLoS One* 2015;10:e0134803.
 96. Chuang YC, Kaufmann JH, Chancellor DD, Chancellor MB, Kuo HC. Bladder instillation of liposome encapsulated onabotulinumtoxinA improves overactive bladder symptoms: a prospective, multicenter, double-blind, randomized trial. *J Urol* 2014;192:1743-9.
 97. Tyagi P, Hsieh VC, Yoshimura N, Kaufman J, Chancellor MB. Instillation of liposomes vs dimethyl sulphoxide or pentosan polysulphate for reducing bladder hyperactivity. *BJU Int* 2009;104:1689-92.
 98. Chuang YC, Lee WC, Lee WC, Chiang PH. Intravesical liposome versus oral pentosan polysulfate for interstitial cystitis/painful bladder syndrome. *J Urol* 2009;182:1393-400.
 99. Chuang YC, Kuo HC. A prospective, multicenter, double-blind, randomized trial of bladder instillation of liposome formulation onabotulinumtoxinA for interstitial cystitis/bladder pain syndrome. *J Urol* 2017;198:376-82.