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Review Article

Pathologic mechanism of the therapeutic effect of botulinum toxin A on interstitial cystitis and painful bladder syndrome

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ABSTRACT

Interstitial cystitis/painful bladder syndrome (IC/PBS) is a chronic bladder condition characterized by bladder pain, frequency and nocturia. There is no definite treatment providing a long-term cure for IC/PBS. Recent studies have demonstrated that intravesical botulinum toxin A (BoNT-A) has promising effects on IC/PBS. Repeated BoNT-A injections might provide long-term symptom relief and decrease glomerulations after cystoscopic hydrodistention. Our previous studies demonstrated bladder tissue nerve growth factor is elevated in IC/PBS bladders and decreased in responders to BoNT-A injection associated with decreased visual analog pain scores. Another study revealed that increased urothelial cell apoptosis, decreased cell proliferation, increased mast cell activation, and impaired expression of junction protein E-cadherin were significant in IC/PBS bladders. Further study of apoptotic markers and inflammatory protein expression also revealed that apoptotic signaling molecules, including Bad, Bax, and caspase 3, were increased in the bladder tissues of patients with IC/PBS. The apoptosis and growth arrest of bladder tissues of IC/PBS patients could be due to upregulation of inflammatory signals, including p38 mitogen-activated protein kinase and tumor necrosis factor alpha. We reviewed the possible pathologic mechanisms of the therapeutic effects of intravesical BoNT-A injection and presented the results of our pilot studies of BoNT-A injections for IC/PBS.

1. Introduction

Interstitial cystitis/painful bladder syndrome (IC/PBS) is a chronic bladder condition characterized by bladder pain, frequency, and nocturia. There is no definite treatment providing a long-term cure of IC/PBS. Recent studies have demonstrated that intravesical botulinum toxin A (BoNT-A) has promising effects on IC/PBS. Repeated BoNT-A injections might provide long-term symptom relief and decrease glomerulations after cystoscopic hydrodistention. We reviewed the possible pathologic mechanisms of the therapeutic effects of intravesical BoNT-A injection and presented the results of our pilot studies on improvement of bladder inflammation and urothelial cell growth in bladder tissue specimens after BoNT-A injections.

2. Pathogenesis of IC/PBS

The cause of IC/PBS may be the result of long-standing inflammation of the bladder; however, the actual pathophysiology remains unclear. The most common clinical presentations of IC/PBS are bladder and pelvic pain, glomerulations under cystoscopic hydrodistention, denudation, or thinning of the bladder epithelium, suggesting bladder inflammation and urothelial dysfunction [1,2]. However, the molecular mechanisms are still not well elucidated. Yamada and colleauges [3] demonstrated an apoptotic process in the microvascular endothelial cells of bladders with IC/PBS. A recent study further revealed that urothelial homeostasis in IC/PBS bladders was significantly impaired, and the abnormal urothelial function was associated with chronic inflammation of the bladder [4].

The chronic pain in IC/PBS may occur because of central nervous system sensitization and persisting abnormalities in the urinary bladder that activate the afferent sensory system [5]. Recent findings have proposed several pathophysiologic mechanisms including epithelial dysfunction, activation of mast cells, neurogenic inflammation, autoimmunity and occult infection. Evidence

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also indicates that IC/PBS is a heterogeneous syndrome and that the two subtypes, the ulcer type (classic) and nonulcer type, may represent different disease entities [6]. The diagnosis of IC/PBS can only be made by clinical symptoms and cystoscopic hydrodistention under anesthesia and exclusion of other bladder disorders [7].

Recent studies have shown that the lamina propria of the bladder plays an important role in transmitting bladder sensation and the response of the bladder to chemical stimuli and inflammation [8–10]. In the urinary tract, nerve growth factor (NGF) is produced by bladder smooth muscle and urothelium [11]. Previous studies have found that NGF is involved in the ongoing regulation of neural function in conditions such as spinal cord injury and denervation, as well as inflammation, and produces pain [5,11,12]. Increased levels of NGF have also been reported in the bladder tissue and urine of patients with painful inflammatory conditions of the lower urinary tract, such as sensory urgency and IC/PBS [13,14].

Bladder inflammation in chemically induced cystitis or intravesical NGF instillation leads to acute afferent nerve activity and long-term plasticity that lowers the threshold for nociceptive and mechanoreceptive afferent fibers [15–17]. Chronic sensitization of afferent fibers might involve both peripheral and central mechanisms. A rise in bladder NGF in the muscle or urothelium initiates signals had been found to transport along the afferent nerves of the bladder to the dorsal root ganglion (DRG) or the spinal cord, which may further trigger bladder overactivity during subsequent stimulation [18,19]. Intravesical injection of BoNT-A has been found to reduce bladder pain in IC/PBS patients refractory to conventional treatment [20]. The NGF levels in the bladder tissue have been found significantly increased in patients with IC/BPS and decreased to the normal range after BoNT-A treatment [21].

Histologic investigations of the bladder urothelium and suburothelium have found that signs of chronic inflammation were present in 60% of baseline biopsies of patients with overactive bladder (OAB) [22]. The expression of inflammatory markers such as mast cells in IC/PBS is similar to that in OAB [23]. OAB could be a subtype of neurogenic inflammation characterized by a series of vascular and nonvascular inflammatory responses triggered by the activation of primary sensory neurons and the subsequent release of inflammatory neuropeptides, including substance P and calcitonin gene-related peptide [24]. The inflammatory response-induced overexpression of transient receptor potential vanilloid receptor subfamily type 1 in the suburothelium as well as c-fos protein in the DRG have been demonstrated in rat models of OAB and in human bladder biopsies [8,10,18]. Urinary inflammatory biomarkers such as NGF were also noted to elevate in patients with OAB as well as IC/BPS [25]. A recent investigation of urinary chemokines in OAB patients also showed increased monocyte chemotactic protein-1 (MCP-1) and some proinflammatory cytokines. A greater than tenfold elevation in the levels of MCP-1 and the soluble fraction of the CD40 ligand was obtained from the urine of OAB patients relative to controls. At least fivefold elevations were detected in the levels of macrophage inflammatory protein, interleukin (IL)-12p70/p40, IL-5, epidermal growth factor (EGF), and growth-related oncogene-α compared with controls. A significant threefold elevation was also noted in the urine levels of sIL-2Rα, and IL-10 in patients with OAB [26].

IC/BPS involves an aberrant differentiation program in the bladder urothelium that leads to altered synthesis of several proteoglycans, cell adhesion and tight junction proteins, and bacterial defense molecules such as GP51. These findings have led to the rationale for selecting some urinary markers to detect the presence of IC/BPS [27]. The potential biomarkers for detection of IC/PBS in recent studies are antiproliferative factor (APF), EGF, heparin-binding epidermal growth factor, glycosaminoglycans, and bladder nitric oxide [28].

3. Chronic inflammation and apoptosis in IC/PBS

Chronic inflammation is a medical condition characterized by persistent inflammatory processes in the tissue. A number of diseases can be included in this category, such as atherosclerosis, cancer, heart valve dysfunction, obesity, diabetes, congestive heart failure, digestive system diseases, Alzheimer disease, and bladder disorders such as IC/PBS. Excessive levels of one or more of the inflammatory cytokines, e.g., tumor necrosis factor alpha (TNFα), IL-6, or IL-8, are usually found in chronic inflammatory diseases [29]. TNFα also promotes apoptosis through binding to TNF-receptor 1, resulting in many effects ranging from inflammation to apoptosis [30]. The primary role of TNFα is in the regulation of immune cells, and it can also induce apoptotic cell death through activation of the transcription factor nuclear factor kappa B and c-Jun N-terminal kinase [31].

Several previous studies have indicated that p38 mitogen-activated protein kinase (MAPK) activation is implicated in inflammation, tissue fibrosis and mediating apoptosis in different cell types in various species [32,33]. Apoptosis is a major form of cell death, characterized initially by a series of stereotypic morphological changes. It is well known that the p38 MAPK pathway participates in the apoptotic process through the regulation of P53 activation [34,35]. Although this signaling pathway is important in the coordination of cellular responses to many stimuli, the effect of the p38 MAPK pathway in IC/PBS has not been well defined [36]. P38 MAPK was recently found to be an important mediator of the anti-proliferative effects of APF in bladder urothelial cells [37]. Our recent study showed that apoptotic signaling molecules, including Bad, Bax, and cleaved caspase-3, were increased in the bladder tissues of IC/PBS patients [38]. The results suggest that apoptosis in IC/PBS bladders might be due to upregulation of inflammatory signals. Moreover, the onset of inflammatory signals of TNFα and p38 might transfer to downstream molecules, even though p38 MAP kinase activities are inhibited. Thus, the molecular mechanism of apoptosis in IC/PBS specimens is likely TNFα and p38 MAPK -mediated [38].

One previous study indicated that phosphorylation of p53 by p38α could play a role in p53-dependent transcription [39]. In addition, p53 translocates into the mitochondria to interact with B-cell lymphoma-2 (Bcl-2) or Bcl-extra large (Bcl-xL), thereby activating Bax directly or indirectly, leading to caspase-dependent apoptosis. Therefore, the extranuclear role of p53 in inducing apoptosis is through mechanisms involving Bad and Bax, which are the main factors that mediate mitochondrial dysfunction and cell apoptosis [40]. Our evidence suggested that the apoptotic process stimulated by p38α was through p53 or regulated by TNFα concurrently [38].

In various inflammatory conditions, the p38 kinases play important roles in regulating cellular signaling initiated via a variety of proinflammatory cytokines including TNFα, IL-1β, and IL-6, as well as in modulating the release of such cytokines. However, both the phosphorylation and activation of p38 MAPK were enhanced after stimulation with several doses of TNFα (10–10000 U/mL), indicating that TNFα was the upstream signal of the p38 MAPK pathway [41]. A previous study indicated that the activation of p38 MAPK attenuated apoptosis induced by TNFα in differentiated neuronal PC12 cells [42]. This evidence suggests multiple roles for TNFα in p38 MAPK activation.

4. Glomerulations and angiogenesis

The vascular system has the critical function of supplying tissues with nutrients and clearing waste products. Vascular permeability by any measure is dramatically increased in acute and chronic inflammation, cancer, and wound healing. Angiogenesis also includes vessel penetration into avascular regions of the tissue and is crucially dependent on the correct interactions between endothelial cells (which form the vessel lining), pericytes (which interact externally with the endothelium) and stromal cells (such as fibroblasts), and their association with the extracellular matrix and the vascular basement membrane. Endothelial cells are guided into a vascular area by macromolecules such as vascular epidermal growth factor (VEGF), a potent proangiogenic factor and endothelial cell mitogen. This hyperpermeability is mediated by acute or chronic exposure to vascular permeabilizing agents, particularly vascular permeability factor/VEGF [43]. These vasoactive and proinflammatory mediators hereby released, such as histamine, prostaglandins, leukotrienes and tryptase, may possibly play a role in the pathogenesis of IC/PBS [44].

Glomerulations during hydrodistention are highly associated with the overexpression of angiogenic growth factors such as platelet derived endothelial cell growth factor/thymidine phosphorylase in the bladder. Thus, it seems likely that neovascularization promoted by angiogenic growth factors has an important role in the pathogenesis of IC/PBS, inducing glomerulations during hydrodistention [43]. There is increased VEGF and immature vascularization in patients with IC/PBS, and VEGF expression is associated with the degree of pain described by patients. Taken together, VEGF might contribute to pain and promote the formation of immature vessels in IC/PBS, and the increased immature vascularization might have a role in glomerulations in patients with IC/PBS [44].

The degree of glomerulations in IC/PBS patients has been noted to decrease after BoNT-A injection. Although the actual mechanism for this BoNT-A effect on angiogenesis is not known, it is important to elucidate which signal molecules are responsible for the regulation of angiogenesis after BoNT-A treatment. In our preliminary studies we showed that the angiogenesis markers IL-8 and VEGF were suppressed under BoNT-A therapy (Fig. 1). The glomerulations and increased vascular permeability of IC/PBS may be due to inflammatory stimulation. BoNT-A may block the expression of angiogenic markers and human umbilical vein endothelial cell (HUVEC) permeability. According to preliminary results (protein array and western blotting), our study indicated that the degree of glomerulations and angiogenic markers could be reduced because of inflammatory suppression after intravesical BoNT-A injection in some IC/PBS patients. However, not all patients have a decrease in the degree of glomerulations after BoNT-A injection. Therefore, it is important to find the underlying pathophysiology between different subgroups of patients to realize the possible mechanism of successful BoNT-A treatment.

5. Mast cell activity

Mast cell tryptase cleaves protease-activated receptors whose neotermini then engage G-protein-coupled receptors on mast cells, sensory nerve endings, the endothelium and neutrophils [45]. This further activates mast cells and neurons, makes the endothelium sticky for leukocytes and leaky to fluid, and prompts leukocytes to release platelet-activating factor (PAF). PAF reinforces the proadhesive conversion of the endothelium, which results in leukocyte emigration from the vasculature [46]. Mast cells are triggered by numerous stimuli, including infections agents, cytokines, certain drugs, hormones, and growth factors; when stimulated, they release mediators of inflammation that may contribute to the pathology of IC/PBS [47].

Protease activated receptor (PAR)-2 is activated by a limited proteolysis at its amino-terminal exodomain by trypsin (but not thrombin) and tryptase. Trypsin can activate PAR-2 and PAR-4, while tryptase can only activate PAR-2 [47]. We recently found that PAR-2 is expressed on ECV304 cells and can be activated by tryptase. The increased expression of IL-8 induced by tryptase is inhibited by the specific monoclonal antibody 11, indicating that PAR-2 is probably involved in the tryptase-induced inflammatory reaction. Tryptase can activate the MAPK/activator protein-1 (AP-1) pathway in human peripheral blood eosinophils, causing cytokine production and release. These results indicate tryptase may activate AP-1 through inducing phosphorylation of p38 MAPK in ECV304 cells. MAPKs are upstream activators of AP-1/MAPK signaling pathways that influence AP-1 activity by both increasing the abundance of AP-1 components and by stimulating their activity directly [45]. It is well known that BoNT-A not only inhibits the release of acetylcholine and norepinephrine, but also NGF, adenosine triphosphate (ATP), and substance P [21,48].

An increased number of activated mast cells are often seen in the urothelium, lamina propria and detrusor of IC/PBS patients, which has led to the theory that mast cells play a major pathogenic role in some cases of IC/PBS [23]. Mast cells are often increased in classic ulcerative IC bladders (6 to 10-fold) and approximately twofold in nonulcer IC bladders compared with controls. Mast cells are triggered by numerous stimuli, including infectious agents, cytokines, certain drugs, hormones, and growth factors; when stimulated, they release mediators of inflammation that may contribute to the pathophysiology of IC/PBS [45]. One study showed that a high mast cell count, fibrosis, and severe inflammation are associated with poor treatment outcomes for IC/PBS patients. Biopsies to assess mast cells, fibrosis, and inflammation in the bladder must be performed in a standardized way [49]. The epithelium and lamina propria should be examined for ulceration, inflammation, granulations, and fibrosis, as well as nonspecific inflammation, hyperemia, and hemorrhage. Detrusor muscle abnormalities, the presence of intrafascicular fibrosis, and the mast cell count should also be reported. Detrusor muscle myopathy is observed in other bladder diseases but appears to be associated with more severe IC/PBS. Intrafascicular fibrosis in IC is reproducible and may be correlated with clinical outcome and stage. The European Society for the Study of Interstitial Cystitis has developed a number of recommendations concerning how to perform biopsies and evaluate the morphologic findings of biopsies [50].

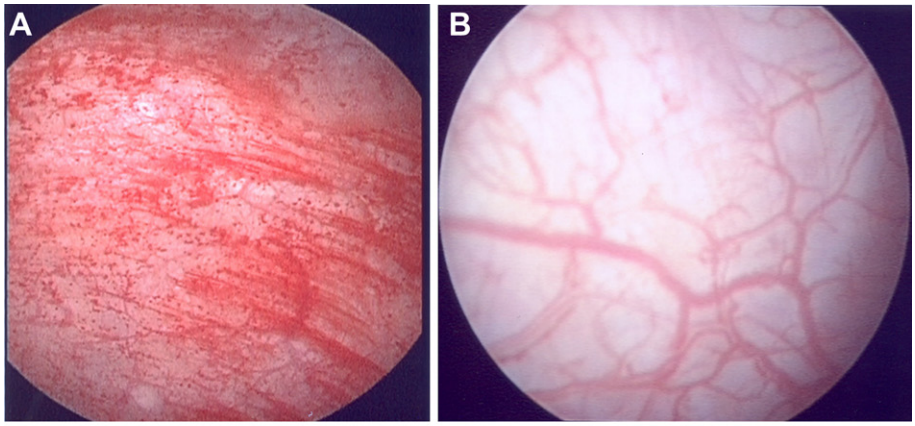
6. Bladder pain

The nature of neurogenic pain was studied in animal models, and these pain models indicated that cytokines (TNFα and NGF) were upregulated in the injured nerves, and neutralizing TNFα reduced pain behavior [51–53]. Moreover, important research showed that TNFα and TNF receptor1 mediated the urothelial apoptotic process in inducible neurogenic cystitis, and TNFα recombinant protein treatment could induce apoptosis in urothelial cells [54]. In the past few years, some p38 inhibitors have been used in the treatment of inflammatory diseases in human and animal models [55,56]. The study of animal models also showed p38 inhibitors strongly interfered with production of TNFα and other cytokines [56]. However, IC/PBS is considered a heterogeneous disease, which may have different causes, diagnoses, and symptoms. Therapeutic treatments for different types of pathophysiology should not be identical. Our previous study showed that p38α- and TNFα–induced apoptosis was present in patients with nonulcerative/G2–3 IC/PBS [38]. We hypothesize that there are

different molecular mechanisms in different subgroups of IC/PBS patients.

7. Clinical experience with BoNT-A injection for IC/PBS

Previous results suggest that BoNT-A has an antinociceptive effect on bladder afferent pathways in patients with IC/PBS, producing both symptomatic and functional (i.e., urodynamic) improvements [48]. In a rat chemical cystitis model, detrusor injection of BoNT-A increased bladder capacity [57]. Previous studies indicated that BoNT-A not only inhibited the release of acetylcholine and norepinephrine, but also NGF, ATP and substance P [48,58]. We suggested that inhibition of neuroplasticity of the sensory fibers in the suburothelial space by intravesical BoNT-A



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Protein	Fold
TIMP-4	0.713
VEGF	0.644
VEGF-C	0.712
Pentraxin 3	0.689
platelet factor 4	0.625
PlGF	0.685
GDNF	0.685
GM-SCF	0.724
IL-1β	0.678
IL-8	0.600
TGF-β1	0.673
coagulation factor III	0.746
CXCL16	0.639
amphiregulin	0.741

Fig. 1. The angiogenesis protein array in an IC/PBS patient at baseline and after intravesical BoNT-A treatment. The results were compared with baseline measurements in the same patient. The fold changes in protein expression between baseline and post-treatment in the urine samples of the IC/PBS patient were calculated. Glomerulations at baseline were (A) Grade 2 before; and (B) Grade 0 after BoNT-A treatment. Angiogenesis molecules and vascular inflammatory proteins in the array membrane were suppressed by 92%; (C) protein expression, such as that of VEGF, platelet factor 4, IL-1β, IL-8, CXCL16, and TIMP-4, was dramatically decreased after BoNT-A injections. BoNT-A = botulinum toxin A; CXCL = (C-X-C motif) ligand; GDNF = germ cell nuclear factor; GM-SCF = granulocyte macrophage colony-stimulating factor; IC/PBS = interstitial cystitis/painful bladder syndrome; IL = interleukin; PlGF = placental growth factor; TGF = transforming growth factor; TIMP = tissue inhibitor of metalloproteinases; VEGF = vascular endothelial growth factor.

injections might have good therapeutic targeting for relief of bladder pain and sensory urgency in patients with IC/PBS.

BoNT-A is one of the most powerful neurotoxins inhibiting the release of neurotransmitters from nerve fibers and the urothelium [58–61]. BoNT-A is widely reported as effective in the treatment of neurogenic and idiopathic detrusor overactivity [62,63]; however, the application of BoNT-A in IC/PBS has been reported in only a few studies [20,48,64]. BoNT-A reduced bladder pain, impaired bladder sensation, and chronic inflammation in the central nervous system in animal and human experiments [59,60,64,65]. Although BoNT-A injections seem promising in treating symptoms of IC/PBS, long-term follow-up does not show successful outcomes after single injections [66]. The limited long-term success is possibly due to the poorly sustained therapeutic effect of a single BoNT-A injection on chronic bladder inflammation.

In our preliminary data, we treated 32 patients who received four repeated injections and the clinical data showed significant improvement not only in subjective symptoms but also in objective urodynamic parameters (Table 1). The long-term success rate was also significantly higher in patients who received repeated BoNT-A injections than in those who received a single injection (Fig. 2). The results indicate repeated intravesical injections of BoNT-A increase functional bladder capacity and provide long-term pain relief in patients with IC/PBS who are refractory to conventional treatment. The long-term effect of repeated intravesical BoNT-A injections is better than that of a single injection. These therapeutic effects could involve not only inhibiting release of acetylcholine in the neuromuscular junctions of the detrusor, but also an antiinflammatory response.

8. Preliminary laboratory evidence of the antiinflammatory effects of BoNT-A on IC/PBS

Our laboratory performed several pilot studies of the molecular pathology of BoNT-A effects on IC/PBS urothelial dysfunction, characterized by decreases in inflammation, apoptosis, and angiogenesis after BoNT-A injections. These molecular findings are compatible with the clinical characteristics of IC/PBS patients after repeated BoNT-A injections.

There was a reduction of activated mast cells in IC/PBS bladders after BoNT-A injection. However, apoptotic urothelial cells did not vanish completely after a single BoNT-A injection, suggesting repeated BoNT-A injections are necessary to achieve complete

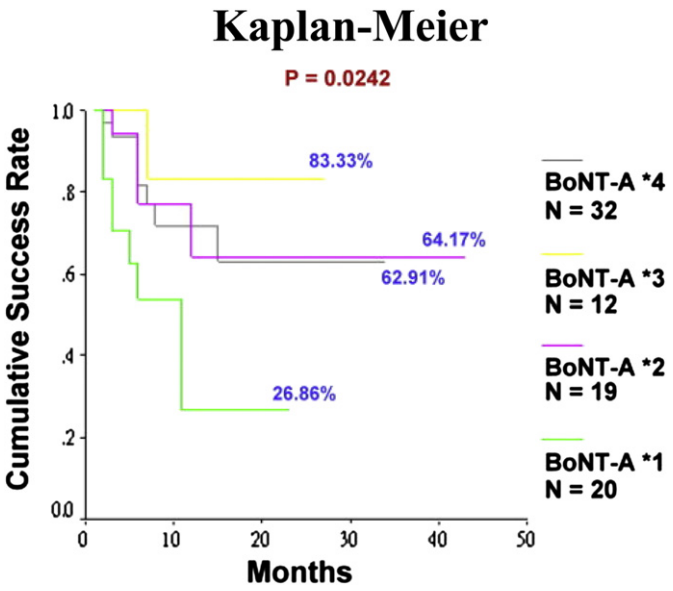

Fig. 2. Cumulative success rates of 83 patients receiving single or repeated BoNT-A injections. BoNT-A = botulinum toxin A.

resolution. To investigate the effect of BoNT-A and prove the pathologic mechanism of IC/PBS we need to prove that (a) urothelial cell apoptosis, (b) mast cell activity, and (c) angiogenesis and the degree of glomerulation all decrease after repeated BoNT-A injections. Patients with good responses to repeated BoNT-A injections should have a higher degree of improvement and those who receive repeated BoNT-A injections should show a higher degree of improvement than those receiving a single injection.

Our study indicated that inflammatory effects could be reduced with intravesical BoNT-A injection and urothelial apoptosis is also inhibited in IC/PBS. These results could provide evidence for the existing pathophysiology of IC/PBS, as well as the actual mechanism of action of BoNT-A in treating IC/PBS. Previous research has indicated that tryptase induced phosphorylation of p38 MAPK, causing cytokine production and release [67]. According to this result, we suggested that phosphorylation of p38 might be induced by tryptase in IC/PBS. We will investigate whether the decrease of mast cells leads to p-p38 downregulation after BoNT-A injection.

To investigate the molecular changes of angiogenesis and inflammation at baseline and after BoNT-A treatment and provide evidence for the actual mechanism of action of BoNT-A treatment for IC/PBS, it must be proved that VEGF expression and mast cell activity in IC/PBS are decreased after repeated BoNT-A injections. Mast cell tryptase may exert its angiogenic effects in part through selective stimulation of angiogenic chemokines [68]. In our

Table 1
Changes of parameters in 32 patients receiving four repeated BoNT-A injections.

BoNT-A × 4	BoNT-A-1	BoNT-A-2	BoNT-A-3	BoNT-A-4	*p* value
(*N* = 32)	Baseline	Baseline	Baseline	Baseline	
ICSI	12.9 ± 3.47	9.27 ± 4.55	8.9 ± 3.66	8.57 ± 3.94	0.000
ICPI	11.8 ± 3.0	8.8 ± 4.47	8.2 ± 4.13	6.63 ± 4.6	0.000
OSS (ICSI + ICPI)	24.8 ± 6.18	18.1 ± 8.68	17.1 ± 7.57	15.2 ± 8.38	0.000
VAS	5.8 ± 2.27	4.03 ± 2.34	3.70 ± 2.35	3.03 ± 2.3	0.000
FBC	139 ± 81.2	170 ± 87.6	210 ± 100	228 ± 118	0.000
Frequency	13.8 ± 5.1	11.3 ± 6.31	11.5 ± 5.93	10.3 ± 4.94	0.029
Nocturia	3.73 ± 2.12	3.13 ± 1.85	3.13 ± 2.33	3.4 ± 2.79	0.384
Qmax	14.4 ± 4.95	13.5 ± 7.13	13.6 ± 4.99	12.4 ± 6.22	0.535
Volume	2527 ± 111	279 ± 144	296 ± 137	283 ± 150	0.289
PVR	9.84 ± 21.92	41.2 ± 67.4	38.8 ± 82.3	47.3 ± 66.7	0.127
CBC	262 ± 116	323 ± 126	338 ± 113	335 ± 145	0.009
MBC	711 ± 217	752 ± 218	756 ± 202	739 ± 186	0.472
GRA	0.30 ± 0.92	1.40 ± 1.19	1.53 ± 0.78	1.77 ± 1.25	0.000

BoNT-A = botulinum toxin A; CBC = cystometric bladder capacity; FBC = functional bladder capacity; GRA = global response assessment; ICPI = interstitial cystitis problem index; ICSI = interstitial cystitis symptom index; MBC = maximal bladder capacity; OSS = O'Leary-Sant symptom score; PVR = postvoid residual; Qmax = maximum flow rate; VAS = visual analog score.

Table 2
Changes of parameters in patients receiving repeated BoNT-A injection.

	Baseline	Repeated BoNT-A injections	P value
VAS	5.80 ± 2.27	3.03 ± 2.30	0.000
MBC	711.7 ± 217.2	738.7 ± 186.3	0.46
Glomerulation	1.80 ± 1.06	1.20 ± 1.06	0.026
GRA	0.30 ± 0.92	1.20 ± 1.06	0.000
Tryptase	100	64.2 ± 17.4	0.001
Bax	100	84.3 ± 21.9	0.050
P-p38	100	66.3 ± 15.9	0.000
SNAP25	100	53.1 ± 11.7	0.001

GRA = global response assessment; MBC = maximal bladder capacity; VAS = visual analog score.

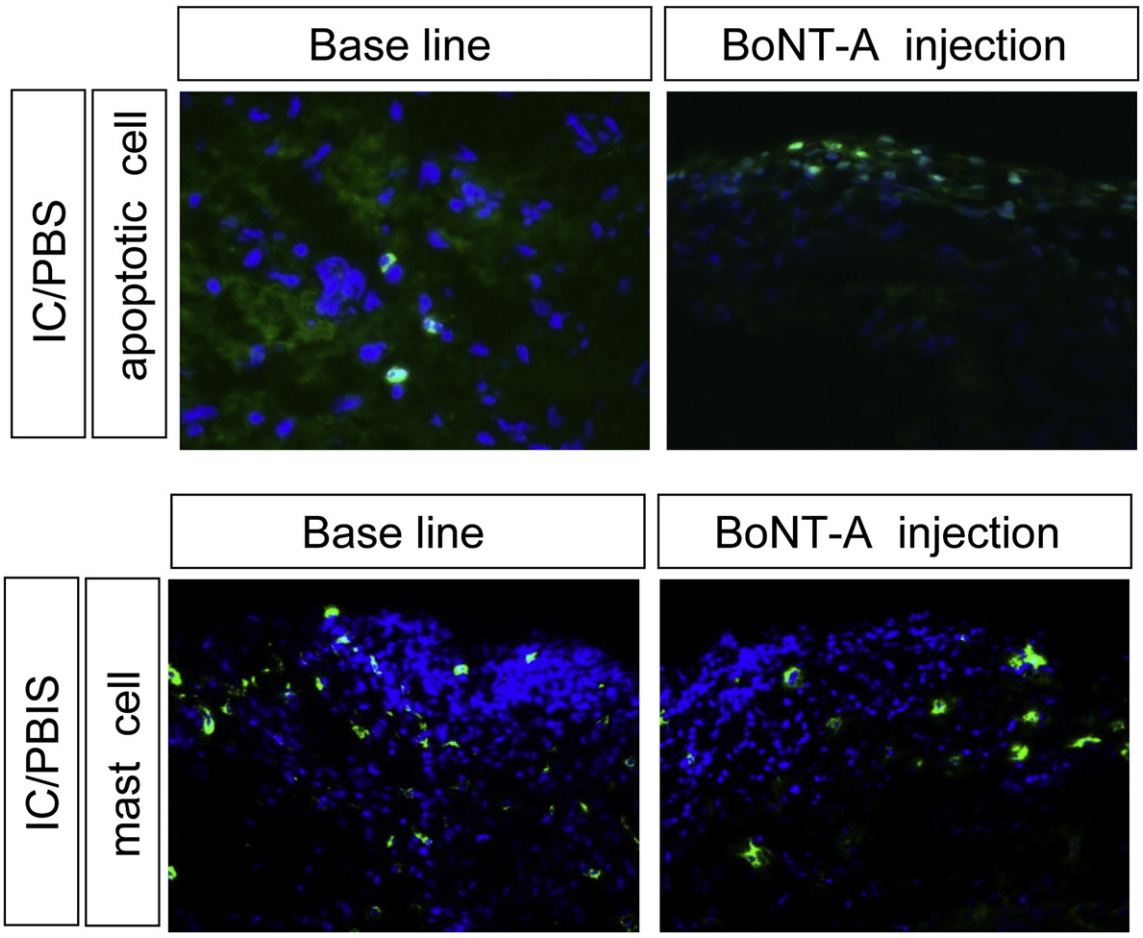

Fig. 3. Apoptotic cells and mast cells are decreased in the bladder after repeated BoNT-A injections. BoNT-A = botulinum toxin A.

preliminary results, several factors, including tryptase, induced the expression of Intracellular adhesions molecules, which accompany increases in vascular permeability. In addition, the phosphorylation of p38 can be upregulated by TNFα, but not tryptase in HUVEC.

We performed a pilot study investigating 28 patients with IC/PBS who received repeated BoNT-A injections. Our pilot study demonstrated that the degree of glomerulations, inflammatory markers (tryptase) and apoptotic markers (Bax and p38) could be reduced because of inflammatory suppression after repeated intravesical BoNT-A injections in some IC/PBS patients. The bladder tissues also showed the intensity of synaptosome- associated protein of 25 kd was significantly decreased after three repeated BoNT-A injections, suggesting a BoNT-A effect on the bladder specimens. These results could provide evidence for the existing pathophysiology of IC/PBS as well as the actual mechanism of action of BoNT-A in treating IC/PBS (Table 2).

In a histologic analysis, the results of apoptotic cell and mast cell activity stains indicated that inflammation was decreased in bladder tissue after repeated BoNT-A injections (Fig. 3). Although statistical analysis showed no significant decrease in urothelial cell apoptosis overall in patients after BoNT-A injections, western blotting showed significant reductions in tryptase, Bax and phospho-p38α in these bladders specimens after repeated BoNT-A injections. (Fig. 4; Table 2). Based on these results, our pilot study indicated that the inflammatory effect in IC/PBS bladders could be reduced after repeated intravesical BoNT-A injections but urothelial apoptosis was not inhibited completely after a single BoNT-A injection in some IC/PBS patients. These results could provide evidence for the existing pathophysiology of IC/PBS, as well as the actual mechanism of action of BoNT-A in treating IC/PBS.

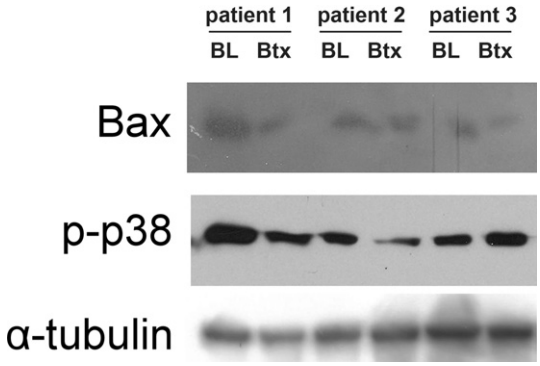

Fig. 4. Changes in the proapoptotic proteins Bax and phosphorylated p38 in patients from baseline to 6 months after BoNT-A injection. BL = baseline; BoNT-A = botulinum toxin A; Btx = 6 months after BoNT-A injection.

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