Understanding the Role of Botulinum Toxin A in the Treatment of the Overactive Bladder—More than Just Muscle Relaxation

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Abstract

The established use of botulinum neurotoxin type A (BoNT/A) therapy has been based on its reversible inhibition of acetylcholine (ACh) and associated muscle relaxation. Greater understanding of the pathophysiology of detrusor overactivity (DO), as well as macromolecular analysis and analysis of the duration of the effects of BoNT/A treatment and of data generated in pain models, has suggested that, in DO, a mechanism of action based solely on the inhibition of ACh-mediated muscular contraction is too simplistic. Evidence suggests that the sensory pathways involving the urothelium and suburothelium have a significant role in the coordination of bladder activity. BoNT/A treatment has been shown to reduce raised levels of sensory receptors in suburothelial nerve fibres, but not in the urothelium, without causing a degeneration of the fibres themselves. No signs of significant histologic changes in muscle-fibre density, muscle atrophy, or other degenerative changes have been noted following BoNT/A treatment. In contrast to the effects seen with skeletal muscle, limited evidence of axonal sprouting following BoNT/A treatment has been seen in the detrusor muscle, further supporting differences in the BoNT/A mechanism of action between skeletal and smooth muscles.

1. Introduction

Botulinum neurotoxins (BoNTs) inhibit the release of acetylcholine (ACh) from the motor nerve terminals into the neuromuscular junction, causing temporary chemodenervation and assisting with muscle relaxation. BoNTs each consist of a two-chain polypeptide that is joined by a disulphide bond. The seven BoNT subtypes (A, B, C1, D, E, F, and G) differ only by their light chains, which determine
their different modes of action. Only types A (BOTOX®, Dysport®) and B (Myobloc®/Neurobloc®) have been approved for use in the treatment of conditions that are characterised by excessive or inappropriate muscle contractions. Botulinum toxin type A (BoNT/A) was first investigated for its effects on the parasympathetic nervous system in the 1920s [1]. Since its approval in the late 1980s for the treatment of strabismus and blepharospasm, BoNT/A has increasingly been used for several other disorders, including cervical dystonia, spasmodic torticollis, and hyperhidrosis [2–4].

Over the past seven years, focal injections of BoNT/A into the detrusor have shown promise as an effective means of reducing uninhibited detrusor contractions and the consequential symptoms of urinary incontinence (UI), as well as avoiding the side-effects that are associated with systemic drug uptake. Differences in duration of activity, as well as ultrastructural changes seen following treatment, have suggested that the way in which BoNT/A exerts its effects in smooth muscles differs from that seen in skeletal muscles. A summary of the evidence that supports the rationale for use of BoNT/A in detrusor overactivity (DO), and the possible mechanism of action, is presented below.

2. Lower urinary tract

The functions of the lower urinary tract—to store and periodically release urine—depend on the coordinated activity of smooth and striated muscles in the urinary bladder, urethra, and external urethral sphincter. This activity is, in turn, controlled by neural circuits in the brain, spinal cord, and peripheral ganglia. Within the detrusor muscle, a single nerve innervates several muscle fibres, which produces coordinated bladder contraction. Cholinergic fibres are predominant in the innervation of the smooth muscle of the bladder body, whereas adrenergic fibres are present in both the bladder body and neck.

In the bladder suburothelium, a wide plexus of afferent nerves have been identified immediately beneath the epithelial lining [5,6]. This innervation has become the focus of increasing interest in studies of bladder function in health and disease [5–8]. Animal experiments have demonstrated that the sensation of bladder fullness is transmitted to the brain via the spinal cord by two types of afferent axons: the myelinated Aδ-fibres, which are sensitive to mechanical stimuli (e.g., distention, stretch) and the primarily nociceptive, unmyelinated C-fibres. This signal is relayed via the periaqueductal grey matter to the pontine micturition centre, which stimulates micturition via excitatory parasympathetic outflow to the bladder [9,10] (Fig. 1).

Immunohistochemical studies have confirmed the presence of sensory receptors, such as the vanilloid receptor TRPV1 and the adenosine triphosphate (ATP)-gated purinergic receptor P2X3, in suburothelial nerves [11,12]. Further animal experiments have indicated that both receptors have a mechanosensory role in normal bladder activity. In urodynamic studies, TRPV1-deficient mice show increased frequency of nonvoiding contractions, increased cystometric capacity, and inefficient voiding [13]. P2X3-deficient mice also exhibit increased cystometric capacity and decreased voiding frequency [14]. In the rat bladder wall, 90% of the TRPV1-immunoreactive nerve fibre population were found to coexpress the sensory neuropeptides substance P (SP) and calcitonin gene-related peptide (CGRP) [15]. Treatment with intravesical vanilloids, which are considered to be C-fibre toxins, resulted in dramatic decreases in the immunoreactivity of TRPV1, SP, and CGRP [11].

The urothelium has also been found to express TRPV1 and P2X3 [16–19] and release tachykinins [20],
nitric oxide [21], and ATP [22] in response to distention. Further evidence for a possible cross-talk between the urothelium and suburothelium exists, such that mechanical and irritant stimuli may be perceived by the bladder. TRPV1 seems to be a prerequisite for urothelial ATP release during bladder distention [13]. The urothelium was shown to be the main source of ATP release in response to bladder stretch [23]. ATP released from the urothelium is believed to bind to P2X3 receptors in the suburothelium to initiate afferent firing to the spinal cord [22,24]. The bladder urothelium also represents the main nonneuronal source of ACh release during bladder filling [25]. Urothelial release of ACh increases with detrusor stretch [25]. It was proposed that, in health, a basal release of ACh from the urothelium during bladder filling acts on muscarinic receptors in smooth muscle cells to regulate bladder tone, or may even exert an effect via such receptors in suburothelial nerves.

3. Changes in micturition in DO

Neurogenic detrusor overactivity (NDO) is a common cause of UI in disorders in which neural pathways are disrupted, including spinal cord injury and myelomeningocele. In these conditions, higher neural controls are bypassed, leading to imbalances between sympathetic and parasympathetic activity in the bladder detrusor muscle, which results in uncontrolled contractions and subsequent voiding [26]. A growing body of evidence suggests that the parasympathetic overactivity, which results in an overactive detrusor, may be due to increased activity/excitability of the suburothelial nerve network, via spinally mediated reflexes in NDO [9,26].

Data from animal studies suggest that, under conditions of spinal disruption, C-fibres in the suburothelium undergo hypertrophy and display increased excitability. As a result, these aberrant C-fibres become sensitive to mechanical stimuli, leading to an increased parasympathetic input to the bladder and DO (Fig. 2) [10].

Evidence to support a similar C-fibre-mediated reorganisation of the micturition reflex in human spinal NDO comes from studies showing increased suburothelial innervation in these patients. Bladder biopsies from patients with intractable NDO showed a significant increase in the numbers of nerve fibres expressing TRPV1 and P2X3 compared with control subjects [27,28]. Instillations of the C-fibre toxin, resiniferatoxin (RTX), were found to reduce suburothelial innervation and TRPV1- and P2X3-immunoreactive nerve fibre density in NDO patients who responded to RTX therapy, but not in non-responders.

Evidence also suggests involvement of suburothelial sensory innervation in the pathology of idiopathic detrusor overactivity (IDO) [19]. Involuntary detrusor contractions in patients with IDO are delayed or suppressed by RTX [29]. An increase was seen in the densities of suburothelial nerve fibres that were immunoreactive to either TRPV1 or P2X3 in patients with IDO compared with controls [19]. Additionally, significantly increased density of SP and CGRP immunoreactive nerve fibres, which would not be explained by an increase in overall nerve density, have been reported in patients with IDO compared with controls [30].

Alterations in the interactions between the urothelium and suburothelium may contribute to the pathophysiology of bladder overactivity. Raised urothelial levels of TRPV1 have been demonstrated in patients with spinal NDO [16–19]. They were significantly reduced in patients who responded to intravesical RTX, with a parallel reduction in suburothelial TRPV1-expressing afferent fibres also being reported [18,19].

Stretch-evoked release of ATP by the urothelium becomes even more prominent with age and in
cases of NDO [23,25]. Similar age- and stretch-related increases in nonneuronal release of ACh from the urothelium have been reported [31]. It has been proposed that such increases in urothelial release of ACh may contribute to DO by stimulating suburothelial afferent activity during the bladder storage phase [25,31,32].

4. Possible effect of BoNT/A on human bladder afferent pathways

BoNT/A binds to and enters the presynaptic end plate of cholinergic neurons by receptor-mediated endocytosis and selectively cleaves synaptosomal-associated protein 25 (SNAP-25), thereby preventing normal vesicle docking and fusion to the presynaptic plasma membrane and inhibiting the secretion of ACh into the synaptic space. This leads to temporary chemodenervation and muscle relaxation. Although this may account for some of the activity of BoNT/A in the overactive bladder, recent investigations have also shown differences in ultrastructural changes following treatment in smooth and striated muscle [33]. Furthermore, in rat bladders BoNT/A inhibited the abnormal urothelial release of ATP in NDO-simulated conditions [34], reduced the capsaicin-evoked detrusor contractions [35], and inhibited the mucosal release of CGRP [36], evidence for BoNT/A-induced modulation of bladder pathways served by sensory innervation. In addition, because in vitro data from sensory ganglia showed a BoNT/A-induced inhibition of the release of SP [37] and blockade of mechanisms involved in the axonal expression of TRPV1 [38], it was becoming progressively clearer that inhibition of ACh release may not be the sole mechanism of action of BoNT/A in DO.

The effect of BoNT/A in human bladder afferent pathways was investigated in both NDO and IDO patients who were treated with BOTOX® injections (NDO, 300 U; IDO, 200 U). Flexible bladder cystoscopic bladder biopsies were obtained at baseline and 4 and 16 wk after treatment and subjected to immunohistochemical analysis using antibodies to the pan-neuronal marker PGP 9.5 and the sensory receptors P2X3 and TRPV1 [19].

All patients responded to BoNT/A treatment with improvements in at least two of the following parameters that were evaluated using voiding diaries and urodynamics: 24-h frequency, urgency, urgency incontinence, maximum cystometric capacity, and maximum filling detrusor pressure.

Immunohistochemical results showed an increase at baseline of, presumably, sensory suburothelial fibres in NDO and IDO compared with controls. These initial levels were higher in patients with NDO than in those with IDO. Following treatment, there was no change in overall nerve fibre count (by PGP 9.5 immunostaining), but there was a progressive decrease in suburothelial fibres expressing P2X3 and TRPV1, with increasing significance over the 16-wk period of the study and with similar changes for NDO and IDO patients. By week 16, levels of P2X3- and TRPV1-expressing suburothelial fibres were similar to those of control patients (Fig. 3). However, there was no change in sensory-receptor immunoreactivity in the urothelium following BoNT/A treatment.

There were significant correlations between the decrease in suburothelial P2X3 receptor expression and a decrease in voids associated with urgency after BoNT/A treatment. Such a result further

Fig. 3 – Intradetrusor BoNT/A injections produced progressive decrease and eventual ‘normalisation’ of mean P2X3 (a) and TRPV1 (b) immunoreactivity in suburothelial nerve fibres at 16 weeks after successful treatment in a group of patients suffering from intractable NDO or IDO.
supports the notion that urgency, now identified as the core symptom of the overactive bladder syndrome [39], is due to aberrant afferent activity resulting from increased excitability/activity of the urothelio-suburothelial proposed functional syncytium [40]. In the context of DO it is thought that the abnormal afferent activity that leads to detrusor contraction is perceived by consciousness as the pathologic sensation of urgency that immediately precedes a detrusor contraction and associated incontinence.

These results indicate that BoNT/A injections to the bladder reduce the levels of sensory receptors in suburothelial fibres, without causing degeneration of the fibres themselves, in contrast to the reduction of suburothelial sensory nerve fibre density induced by intravesical RTX. This effect does not extend to the urothelium, which, in previous studies, has shown significant reductions in TRPV1 immunoreactivity after instillation of the C-fibre toxin RTX [18]. These differences suggest that the mechanism of action of BoNT/A differs from that of intravesical vanilloids.

5. Ultrastructural changes in bladder smooth muscle

Because continuous therapy requires BoNT/A injections to be repeated at approximately 10-mo intervals, the possible long-term effects of extended treatment are of great importance. Although the effects of BoNT/A on smooth muscle have not previously been investigated, its effects on striated muscle have been studied in mice and humans. Studies on the striated sternomastoid muscle of mice used video microscopy to sequentially compare the same cholinergic nerve terminals before and for 28 d after BoNT/A injection. During this time, new nerve sprouts formed on disabled neurons but disappeared after the effects of BoNT/A wore off [41]. In a study into the effects of BoNT/A on the human striated orbicularis muscle, sprouting axon profiles were found in 10 patients who were treated for blepharospasm, 6 wk to 3 yr after receiving 2–18 injections of BoNT/A. No changes were found in five subjects who were never exposed to BoNT/A [42]. Although this study was conducted without a within-subjects comparison, it suggests that human striated muscle may be subject to some neural-sprouting effects with BoNT/A treatment.

To investigate the effects of BoNT/A on neural growth in human detrusor muscle, biopsies of the bladder wall were taken from 13 patients immediately prior to BoNT/A treatment (group 1) and from 17 patients immediately after treatment (group 2). Group 2 included six biopsies from patients within 3 mo after their first injection and 11 biopsies at the time of decreasing efficacy of BoNT/A [33]. Results showed no significant differences between the two groups in muscle cell fascicle structure (p = 0.445), width of intercellular space (p = 0.482), or number and kind of muscle cell junctions (p = 0.443). In group 1, a median of 70% of intrinsic axon terminals showed signs of degeneration, compared with a median of 66% in group 2 (p = 0.840). Of 309 evaluated axon terminals in both groups, one sprouting axon was found in group 1 and three were found in group 2 (p = 0.864). Only limited collagen deposits within the detrusor were found in both groups. No changes in the ultrastructure of the detrusor were observed in those biopsies that were obtained before and after BoNT/A injection of the same patient.

These results confirm that the main structural changes associated with NDO are associated with severe axon degeneration in the detrusor. No signs were seen of any significant histologic changes in muscle fibre density, muscle atrophy, or other degenerative changes, even after repeated injections of BoNT/A. Unlike reports of BoNT/A activity in the striated muscle of mice and humans, axonal sprouting within the smooth muscle of the detrusor was limited after BoNT/A injection, indicating pathophysiologically different reactions to the toxin, either between striated and smooth muscle or between different treated diseases.

Adding further to these results, a recent study of excised neurogenic overactive bladders investigating possible effects of intradetrusor BoNT/A on human bladder histology identified significantly less fibrosis in those previously treated with BoNT/A, but no differences in inflammation and oedema, compared to untreated ones [43]. Specimens were examined for both urothelial/suburothelial and detrusor changes. Despite the fact that the time interval between BoNT/A injections and cystectomy was not specified, such findings may imply phenotypic changes—lesser production of collagen by fibroblasts or transcriptional collagen alterations—in support of the changes suggested in suburothelial sensory receptor expression in intact nerve fibres [19] and the reduction in the levels of neurotrophic factors [44].

6. Efferent effect of BoNT/A

Despite the absence of gross ultrastructural changes in the detrusor muscle following at least a single treatment with BoNT/A, clinical evidence also
suggests an effect on detrusor function via direct modulation of the efferent innervation. Significant decreases in detrusor pressure during both filling and voiding have been recorded both in NDO and IDO patients [45–47], and are accompanied by significant improvements in bladder compliance [45,48]. In support of an effect on the motor innervation, post-BoNT/A decrease in the levels of sensory receptors were not found to correlate with respective changes in either detrusor pressures or cystometric capacity [19]. Further evidence for an effect of BoNT/A on detrusor function is provided by the posttreatment increased postvoid residual urine volumes, which may require the need for intermittent self-catheterisation [47,49]. In support, a study comparing posttreatment residuals between patients who had received detrusor injections only and those who also received urethral sphincter injections showed a significant increase in the former group of patients, whereas no change was noted in the latter. No differences were seen in detrusor pressures between the two groups [49]. Animal studies have already shown that BoNT/A can inhibit the release of ACh from parasympathetic (motor) nerve endings in the detrusor in experimental conditions simulating parasympathetic nerve overactivity that occurs in human DO [50,51]. However, in addition to a direct effect on the nerves providing efferent input to the bladder, BoNT/A may also exert its actions on the detrusor via blocking the proposed “efferent” function of sensory nerves. Sensory fibres convey the afferent impulses arising from the bladder and urethra, which determine urine continence and voiding, modulate pain sensation, and activate cardiovascular reflexes. At the same time the release from their endings of neurotransmitters and neuropeptides, which have an excitatory role in the activation of detrusor smooth muscle cells, immuno-cell migration, mast cell degradation, and plasma protein extravasation, is regarded as the “efferent” element in the function of these nerves (see the review by Andersson [52]). Animal bladder studies already suggest that BoNT/A blocks the “efferent” function of capsaicin-sensitive sensory nerves by inhibiting the release of such excitatory neuropeptides/neurotransmitters [36,53].

7. Conclusions

The established mode of action of BoNT/A in treating muscle disorders is the blockage of ACh release from motor nerves, which causes muscle relaxation. Greater understanding of NDO patho-

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Fig. 4 – Proposed ‘cascade’ mechanism of action of intravesically injected BoNT/A via multiple inhibition of the vesicular release of neurotransmitters and neuropeptides by the urothelium and suburothelial nerves and reduction of the axonal expression of SNARE-complex dependent proteins that are thought to be involved in bladder mechanosensation (reproduced with permission from Eur Urol 2006;49:644–650).
physiology, combined with data generated in pain models and analysis of bladder tissue following BoNT/A treatment, has shown that the traditional theories of mechanism of action of BoNT/A in bladder dysfunction are incomplete.

Positive correlations between posttreatment changes in the sensory receptors TRPV1 and P2X3 and symptomatic improvement suggest that BoNT/A partly exerts its therapeutic effects in patients with NDO/IDO by reducing the levels of sensory receptors in the suburothelium. These changes occur without causing significant degeneration or sprouting of suburothelial nerve fibres. The reduction of sensory receptor levels, particularly the reduction of P2X3 to control levels, may reduce the sensitivity of the aberrant C-fibres to mechanical stimulation and hence reduce overactivity of the detrusor and the frequency of incontinence episodes.

Complementary in vivo and in vitro data showing an effect of BoNT/A on sensory receptors [19,38], neuropeptides [36,37,54,55], and other neurotransmitters [34,35], which are thought to be involved in the bladder afferent pathways, support the novel notion that the clinical effects of BoNT/A in the treatment of DO may at least, in part, involve effects on the afferent limb of the bladder signalling pathway [19,56] (Fig. 4).

Treatment with BoNT/A does not seem to alter the structure of the detrusor and does not induce muscle cell degeneration. In addition, BoNT/A did not appear to induce axonal sprouting. This is contrary to previous observations in striated muscle, which may reflect differences in the reaction of striated and smooth muscle to BoNT/A injections, or the effect of the pathology underlying NDO on axon regeneration. However, the absence of ultrastructural changes does not exclude an effect of the toxin on the detrusor, either directly or via inhibition of the proposed “efferent” function of the suburothelial sensory nerves.

The absence of axon sprouting, both in the detrusor and suburothelium, suggests that the therapeutic effects of BoNT/A may be longer lasting in the treatment of NDO than for some other indications. A hypothetical model of BoNT/A-induced peripheral and central desensitisation proposed a cascade mechanism of inhibition of bladder afferent pathways [56] (Fig. 4), in conjunction with earlier in vitro observations of prolonged persistence of the product cleavage by BoNT/A (SNAP-25A), which acts as a further inhibitor of vesicular exocytosis [57]. In addition, the absence of changes in histomorphology and ultrastructure following BoNT/A therapy suggests that this treatment is unlikely to have detrimental effects in the long term. However, further studies are required to confirm the absence of long-term effects, especially following repeated injections.

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References


