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Review Article

Mechanism of action of onabotulinumtoxinA on lower urinary tract dysfunction

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ABSTRACT

Botulinum neurotoxins (BoNTs) are known for their ability to induce chemical denervation, modulation of neurotransmission, and successful long-term treatment of muscle hypercontractility. Recent basic science studies as well as clinical studies suggest that BoNT affects the modulation of sensory processing, inflammation, and glandular function. Causes of lower urinary tract symptoms (LUTS) include abnormalities of the bladder, prostate, urethra, and neurological function that controls the lower urinary tract. Urologists have become interested in the potential application of BoNTs in patients with LUTS including detrusor and sphincter overactivity, bladder hypersensitivity, interstitial cystitis/painful bladder symptoms, and benign prostatic hyperplasia. In this study we review the biological action of BoNTs in the bladder and prostate.

1. Introduction

Botulinum neurotoxin (BoNT) is one of the most potent toxins known to date. BoNT is produced by a Gram-positive, rod-shaped anaerobic bacterium—*Clostridium botulinum*. It was first used effectively for different conditions involving muscular hypercontraction [1,2] by inhibiting acetylcholine (ACh) release at the presynaptic cholinergic neuromuscular junction. Seven immunologically distinct neurotoxins are available, which are designated as types A, B, C, D, E, F, and G [1–3]. All these serotypes block transmission at neuromuscular junctions to various degrees. Effects of type A botulinum neurotoxin (BoNT-A), which is the most used neurotoxin, have been studied most extensively.

Although BoNT has seven serotypes, only BoNT-A and BoNT-B are in clinical use. Available brands of BoNT-A include onabotulinumtoxinA (Botox; Allergan, Inc., Irvine, CA, USA) and abobotulinumtoxinA (Dysport; Ipsen Ltd., Berkshire, UK), and the brand of BoNT-B includes rimabotulinumtoxinB (Myobloc; Elan Pharmaceuticals, Inc., Princeton, NJ, USA). The potency of each toxin is expressed in units of activity. However, these toxins have different doses, efficacy, and safety profiles and should not be considered generic equivalents comparable by single dose ratios [3].

BoNT has been applied for the treatment of lower urinary tract symptoms (LUTS) since the late 1980s. Dykstra et al [4] demonstrated that an injection of BoNT into the external urethral sphincter induces chemical sphincterotomy and lowers detrusor-sphincter dyssynergia in patients with spinal injuries. A resurgence of interest in BoNT was led by Schurch et al [5], who reported successful treatment of spinal-cord-injured patients with neurogenic detrusor overactivity (NDO). Maria et al [6] reported the therapeutic effects of BoNT in patients with benign prostatic hyperplasia (BPH). Interestingly, all three pioneers were not urologists. Chancellor and his group played a major role for urologists to step into this fascinating field [1]. As the uses of BoNT continue to expand in the field of urology, it is important to understand the mechanism and clinical effects by which the toxin works on different tissue types and disease entities.

2. Working mechanisms of BoNT

BoNT-A was originally synthesized as an inactive chain of 1285 amino acids and is activated when the single chain is cleaved by an endogenous clostridial protease [1,7]. A dichain polypeptide containing a 50 kDa light chain and a 100 kDa heavy chain linked covalently by a single disulfide bond [7] is created. BoNT-A inhibits signal transmission at the neuromuscular and neuroglandular junctions in the following four discrete stages: (1) binding, the

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heavy chain of the toxin adheres to a specific nerve terminal receptor; (2) internalization, the toxin gets into the nerve terminal; (3) translocation, the light chain of the toxin translocates into the cytosol; and (4) inhibition of neurotransmitter release, the toxin attacks active neurons in a receptor-mediated endocytotic process.

BoNT-A is taken up in a neuron activity-dependent manner by utilizing synaptic vesicle protein SV2 as its protein receptor and attacking active neurons in a receptor-mediated endocytotic process. In nerve terminals, synaptic vesicles fuse with the presynaptic membrane where they release the neurotransmitter into the neuromuscular or neuroglandular junction and more active receptors are exposed [8]. Vesicle fusion is mediated by a set of SNARE (soluble *N*-ethylmaleimide-sensitive fusion attachment protein receptor) proteins. In nerve terminals affected by BoNT, light-chain proteolytic fragments are released into the cytosol, which cleave specific peptide bonds presenting in the synaptic fusion complex and prevent exocytosis of the neurotransmitters containing vesicle at the nerve terminal [1,2,9]. Each botulinum serotype cleaves a distinct protein site. BoNT-A cleaves soluble *N*-ethylmaleimide-sensitive fusion attachment protein-25 (SNAP-25), and BoNT-B cleaves synaptobrevin [1–3]. Patients who are refractory to BoNT-A may possibly respond to BoNT-B because of the different targeting proteins.

3. Duration of BoNT effects

Each toxin inhibits exocytosis of neurotransmitters effectively for various lengths of time, depending on differences in the SNARE-binding profiles among the botulinum toxin serotypes. When used clinically for the treatment of dystonias, BoNT-A has by far the longest duration of activity, inducing clinical effects on neuromuscular activity for longer than 4 months, compared with the durations of approximately 2 months for BoNT-B and less than 4 weeks for BoNT-E [3]. Recovery of neurotransmission is dependent on the protease that removes BoNT as well as on the restoration of intact SNARE proteins. In addition, structural differences in end organs lead to effects of different durations even with the same toxin.

4. Rationale for using BoNT for lower urinary tract dysfunction

BoNT affects motor, sensory, and glandular functions and induces anti-inflammation through the modulation of release of various neurotransmitters in different kinds of tissues.

4.1. Effects on motor function

Smith et al [10] revealed significant decreases in the release of ACh in normal rat bladders after a BoNT-A injection, suggesting that BoNT-A can inhibit cholinergic nerve-induced bladder activity. In addition, contractile data suggest that onabotulinumtoxinA may impair adenosine triphosphate (ATP) release in addition to ACh release from isolated bladder tissue [11]. The effects of BoNT on detrusor muscle are not permanent. Studies have shown that onabotulinumtoxinA produces no persistent changes in the internal architecture of muscle fiber after recovery from paralysis.

4.2. Effects on detrusor morphology

Morphological changes after onabotulinumtoxinA injection include subsequent compensatory nerve sprouting and creation of extrajunctional synapses [12]. The sprouts are retracted and endplate functioning returns to normal when exocytosis at the parent terminal eventually recovers [12].

Multiple bladder injections may induce mechanical trauma. Haferkamp et al [13] collected 30 bladder biopsies from 24 patients with a diagnosis of neurogenic overactive bladder. They observed no significant changes in muscle cell fascicles, intercellular collagen content, or muscle cell degeneration between biopsies taken prior to and 3 months after BoNT-A administration. Surprisingly, in another study, bladder specimens obtained from cystectomy in 45 patients with neurogenic overactive bladders showed that patients who had received onabotulinumtoxinA injection had significantly less fibrosis of the bladder wall than those who had not received the toxin injection. In addition, a trend showed that responders to the toxin therapy had less fibrosis and edema of the bladder wall than nonresponders [14]. Apostolidis et al [15] reported that BoNT-A injections did not produce significant inflammatory changes, fibrosis, or dysplastic changes in human bladder urothelium/suburothelium after a single injection or in a limited number of repeat treatment biopsies in patients with NDO or idiopathic detrusor overactivity (IDO). In conclusion, BoNT bladder injection may reverse tissue fibrosis and edema in NDO.

4.3. Sensory effects

Treatment using BoNT has expanded beyond the original concept of inhibition of muscle overactivity. Increasing evidence exists that BoNT may also inhibit afferent neurotransmission and have analgesic properties [16]. Changes in afferent activity may influence pain through both direct sensory effects and indirect central sensitization in the central nervous system. OnabotulinumtoxinA has been shown to inhibit the release of calcitonin gene-related peptide, substance P, glutamate, nerve growth factor (NGF), and ATP, which are mediators of pain sensation. In a model of formalin-induced somatic pain and inflammation, rats pretreated with BoNT-A displayed significantly reduced pain behaviors and glutamate release from 5 hours to 12 days post injection [17]. Similar effects were observed in an acetic acid-induced bladder pain model [18]. The concepts have been proved in the clinical use of BoNT for interstitial cystitis, radiation cystitis, and chemical cystitis, as well as for migraine.

Bladder urothelium not only is a barrier, but also plays an important role in the sensory transduction mechanisms modulating micturition, particularly in conditions of increased sensory nerve transmission following chronic inflammation and spinal-cord injury [19]. OnabotulinumtoxinA was shown to inhibit ATP release from the urothelium in the bladders of rats with spinal cord injuries [20]. The effects of BoNT are not limited solely to inhibiting neurotransmitter release. For example, laboratory studies have shown that transient receptor potential vanilloid 1 (i.e., capsaicin-sensitive) receptors are released by SNARE-dependent processes and can be inhibited by onabotulinumtoxinA treatment [21]. Giannantoni et al [22] reported that intravesical onabotulinumtoxinA injection reduces NGF content in the bladder tissue of patients with NDO. The reduction of NGF leads to decreased hyperexcitability of C-fiber bladder afferents, thereby reducing NDO. Liu et al [23] showed that NDO patients responsive to BoNT had reduced urine NGF levels, but nonresponders had no significant changes. Thus, the inhibitory effects of BoNT on sensory function may relieve somatic and visceral irritative symptoms.

4.4. Anti-inflammation

Chuang et al [24,25] reported that painful behavioral changes, polymorphonuclear cell accumulation, and cyclooxygenase (COX)-2 expression in the prostate gland and in the L6 ventral and dorsal horns induced by capsaicin injection into the rat prostate were inhibited in a dose-dependent fashion by BoNT. OnabotulinumtoxinA pretreatment

can inhibit capsaicin-induced COX-2 expression from the peripheral organ to the L6 level of the spinal cord, prostatic pain, and inflammation. This finding suggests a potential clinical benefit of onabotulinumtoxinA for the treatment of nonbacterial prostatitis. Furthermore, a previous study also demonstrated that intravesical onabotulinumtoxinA administration blocked cyclophosphamide-induced bladder inflammation and hyperactivity, and inhibited COX-2 and prostaglandin E(4) expression in the bladder as well as in the spinal cord [26]. Taken together, these findings suggest a potential benefit of BoNT treatment for prostate and bladder inflammatory conditions.

5. Clinical use in the bladder

Patients with symptoms of NDO; IDO; overactive bladder, hypersensitive bladder, or interstitial cystitis/painful bladder syndrome; and noninfectious cystitis have been treated with a BoNT injection into the bladder. An injection of 200–300 U onabotulinumtoxinA is most commonly used for NDO, whereas 100–200 U onabotulinumtoxinA has been applied in treating IDO; overactive bladder, hypersensitive bladder, or interstitial cystitis/painful bladder syndrome; and noninfectious cystitis.

5.1. Rationale for the application of BoNT in the prostate

In a rat model, surgical denervation of the prostate by sectioning the hypogastric nerve has been shown to induce prostatic atrophy [27]. Previous studies using chemical denervation by injection of BoNT into the rat prostate showed generalized atrophy and apoptosis of glandular elements [28,29]. Cholinergic innervation of the prostate gland has an important role in regulating the functions of the prostate epithelium, with effects on growth and secretion, whereas noradrenergic innervation has been implicated in the contraction of smooth muscle and the etiology of outflow obstruction accompanying BPH [29]. BoNT-induced prostatic gland atrophy, and in some cases apoptosis, was identified in rats as well as in dogs [28,30]. In addition, Lin et al [31] reported that an injection of 200 U onabotulinumtoxinA into the canine prostate significantly reduced the prostate urethral pressure response to intravenous norepinephrine and electrostimulation. They concluded that BoNT reduces the contractile function of the prostate and may attenuate the dynamic component of BPH. Intraprostatic BoNT injection appears to be effective in relieving the symptoms of BPH to different degrees. However, a recent double-blind randomized control study has not shown significant clinical effects in comparison with those in the placebo group [32]. The clinical effects of BoNT on LUTS suggestive of BPH are still controversial. Different doses, dilution volumes, and prostate components may lead to different results.

5.2. Rationale for instillation of BoNT for bladder disorders

The bladder is a hollow organ that is easy to access or treat locally through the urethra. Instillation as a mode of administering BoNT may decrease side effects and treatment costs drastically.

Recent studies suggested the potential of liposomes as a promising vehicle for delivery of neurotoxins into the bladder [33]. Liposomes have been proved to be biocompatible delivery agents in the bladder. The transport of BoNT into the urothelium from liposomes (Lipella Pharmaceuticals, Pittsburgh, PA, USA) was confirmed by detection of its unique effects on neurotransmitters and proteolysis of synaptosomal-associated protein SNAP-25 through immunohistochemistry and western blotting. BoNT entrapped inside liposomes was protected from degradation in urine without compromising efficacy, which was demonstrated by attenuation of acetic acid-induced bladder irritation in rats. These results support the use of liposomes as an efficient vehicle for delivering botulinum toxin without injection. Clinical trials should be conducted to assess the efficacy of liposomal formulation of BoNT and investigate whether it can reduce the risk of retention.

6. Conclusion

BoNT has been proved to provide therapeutic benefits across a wide variety of LUTS that involve muscular hypercontractility, hypersensitivity, and glandular hypertrophy. Advances have been made in our understanding of how BoNT works in LUTS. However, currently, it has been proved to be effective only for neurogenic and idiopathic overactive bladder.

References

[1] Smith CP, Chancellor MB. Emerging Role of botulinum toxin in the treatment of voiding dysfunction. J Urol 2004;171:2128–37.
[2] Chuang YC, Kuo HC, Chancellor MB. Botulinum toxin for the lower urinary tract. BJU Int 2010;105:1046–58.
[3] Aoki KR. A comparison of the safety margins of botulinum neurotoxin serotypes A, B, and F in mice. Toxicon 2001;39:1815–20.
[4] 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.
[5] Schurch B, Stohrer 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;164:692–7.
[6] Maria G, Brisinda G, Civello IM, Bentivoglio AR, Sganga G, Albanese A. Relief by botulinum toxin of voiding dysfunction due to benign prostatic hyperplasia: results of a randomized, placebo-controlled study. Urology 2003;62:259–64.
[7] Kozaki SA, Miki A, Kamata Y, Oqasawara J, Sakaquchi G. Immunological characterization of papain-induced fragments of *Clostridium botulinum* type A neurotoxin and interaction of the fragments with brain synaptosomes. Infect Immun 1989;57:2634–9.
[8] 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.
[9] Modugno N, Priori A, Berardelli A, Vacca L, Mercuri B, Manfredi M. Botulinum toxin restores presynaptic inhibition of group Ia afferents in patients with essential tremor. Muscle Nerve 1998;21:1701–5.
[10] Smith CP, Boone TB, de Groat WC, Chancellor MB, Somogyi GT. Effect of stimulation intensity and botulinum toxin isoform on rat bladder strip contractions. Brain Res Bull 2003;61:165–71.
[11] Thesleff S, Molgo J, Tågerud S. Trophic interrelations at the neuromuscular junction as revealed by the use of botulinal neurotoxins. J Physiol Paris 1990;84:167–73.
[12] de Paiva A, Meunier FA, Molgo J, Aoki KR, Dolly JO. Functional repair of motor endplates after botulinum neurotoxin type A poisoning: biphasic switch of synaptic activity between nerve sprouts and their parent terminals. Proc Natl Acad Sci U S A 1999;96:3200–5.
[13] Haferkamp A, Schurch B, Krengel U, Grosse J, Kramer G, Schumacher S, et al. Lack of ultrastructural detrusor changes following endoscopic injection of botulinum toxin type a in overactive neurogenic bladder. Eur Urol 2004;46:784–91.
[14] Compérat E, Reitz A, Delcourt A, Capron F, Denys P, Chartier-Kastler E. Histologic features in the urinary bladder wall affected from neurogenic overactivity—a comparison of inflammation, oedema and fibrosis with and without injection of botulinum toxin type A. Eur Urol 2006;50:1058–64.
[15] Apostolidis A, Jacques TS, Freeman A, Kalsi V, Popat R, Gonzales G, et al. Histological changes in the urothelium and suburothelium of human overactive bladder following intradetrusor injections of botulinum neurotoxin type A for the treatment of neurogenic or idiopathic detrusor overactivity. Eur Urol 2008;53:1245–53.
[16] Aoki KR. Review of a proposed mechanism for the antinociceptive action of botulinum toxin type A. Neurotoxicology 2005;26:785–93.
[17] Cui M, Khanijou S, Rubino J, Aoki KR. Subcutaneous administration of botulinum toxin type A reduces formalin-induced pain. Pain 2004;107:125–33.
[18] Chuang YC, Yoshimura N, Huang CC, Chiang PH, Chancellor MB. Intravesical botulinum toxin A administration produces analgesia against acetic acid induced bladder pain responses in rats. J Urol 2004;172:1529–32.
[19] Khera M, Somogyi GT, Kiss S, Boone TB, Smith CP. Botulinum toxin A inhibits ATP release from bladder urothelium after chronic spinal cord injury. Neurochem Int 2004;45:987–93.
[20] 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.
[21] Apostolicism A, Poppet R, Yangon Y, Cocaine D, Ford AP, Davis JB, et al. Decreased sensory receptors $P2X_3$ and TRPV1 in suburothelial nerve fibers

following intradetrusor injections of botulinum toxin for human detrusor overactivity. J Urol 2005;174:977–82.
[22] Giannantoni A, Di Stasi SM, Nardicchi V, Zucchi A, Macchioni L, Bini V, et al. Botulinum-A toxin injections into the detrusor muscle decrease nerve growth factor bladder tissue levels in patients with neurogenic detrusor overactivity. J Urol 2006;175:2341–4.
[23] Liu HT, Chancellor MB, Kuo HC. Urinary nerve growth factor levels are elevated in patients with detrusor overactivity and decreased in responders to detrusor botulinum toxin-A injection. Eur Urol 2009;56:700–6.
[24] Chuang YC, Yoshimura N, Huang CC, Chiang PH, Wu M, Chancellor MB. Intraprostatic capsaicin injection as a novel model for non-bacteria prostatitis. Eur Urol 2007;51:1119–27.
[25] Chuang YC, Yoshimura N, Huang CC, Chiang PH, Wu M, Chancellor MB. Intraprostatic botulinum toxin A injection inhibits COX-2 expression and suppresses prostatic pain on capsaicin induced prostatitis model in rat. J Urol 2008;180:742–8.
[26] Chuang YC, Yoshimura N, Huang CC, Chiang PH, Wu M, Chancellor MB. Intravesical botulinum toxin A administration inhibits COX-2 and EP4 expression and suppresses bladder hyperactivity in cyclophosphamide induced cystitis in rats. Eur Urol 2009;56:159–66.
[27] Pennefather JN, Lau WA, Mitchelson F, Ventura S. The autonomic and sensory innervation of the smooth muscle of the prostate gland: a review of pharmacological and histological studies. J Auton Pharmacol 2000;20:193–206.
[28] Doggweiler R, Zermann DH, Ishigooka M, Schmidt RA. Botox induced prostatic involution. Prostate 1998;37:44–50.
[29] Chuang YC, Chancellor MB. The application of botulinum toxin in the prostate. J Urol 2006;176:2376–86.
[30] Chuang YC, Huang CC, Kang HY, Chiang PH, Demiguel F, Yoshimura N, et al. Novel action of botulinum toxin on the stromal and epithelial components of prostate gland. J Urol 2006;175:1158–63.
[31] Lin AT, Yang AH, Chen KK. Effect of botulinum toxin A on the contractile function of dog prostate. Eur Urol 2007;52:582–9.
[32] Marberger M, Chartier-Kastler E, Egerdie B, Lee KS, Grosse J, Bugarin D, et al. A randomized double-blind placebo-controlled phase 2 dose-ranging study of onabotulinumtoxinA in men with benign prostatic hyperplasia. Eur Urol 2013;63:496–503.
[33] 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.