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Basic Pharmacology of Botulinum Toxin in the Lower Urinary Tract

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Abstract

Neurotransmitters formed in the cytosol of presynaptic nerve endings are enclosed in transport vesicles, which are then transported toward plasma membrane. Through interaction with SNARE proteins, transport vesicles fuse with plasma membrane to release neurotransmitters into the synaptic space. SNARE proteins are targets of botulinum toxins (BoNTs) and tetanus toxin. All types of BoNTs consist of a heavy chain and a light polypeptide joined by a disulfide bond. The light chain possesses protease activity. Once inside the cytosol, the light chain is released to cleave the specific component of SNARE protein responsible for transmitter exocytosis. Type A and E cleave to SNAP-25. Cleavage of SNARE protein prevents exocytosis of neurotransmitters, resulting in chemo-denervation. BoNT is known to inhibit the release of acetylcholine from cholinergic nerve terminals in the neuromuscular junctions of striated muscle. BoNT/A has been found to reduce the release of norepinephrine from the urethra. The release of CGRP and substance P is also found to be inhibited by BoNT. Stimulated release of ATP, a mediator for nociception of urinary bladder, from urothelial cells of cyclophosphamide-induced inflammatory rat bladders was significantly reduced by BoNT/A. Release of some neurotransmitters, including neuropeptide Y and nitric oxide, is not affected by BoNTs. BoNTs might influence the presentation of membrane receptor. BoNT/A has been found to reduce the expression of adrenergic receptor in rat prostate. Higher dose of BoNT/A might cleave the SNARE protein responsible for receptor trafficking, with a resultant reduction in receptor presentation on plasma membrane. Lethal BoNT dose for humans is unknown, but referencing data derived from animal research, one would suspect a LD50 of BoNT/A for a 70-kg human to be around 3000 U. Botulism-like side effects are prone to occur in patients with neurological disorder. Serious systemic side effects from BoNT/A injection into the lower urinary tract are not common. Only a few cases of generalized weakness have been reported. Active UTI and known hypersensitivity to BoNTs prohibit BoNT application. Drugs affecting neuromuscular transmission, such as aminoglycosides, should not be used concurrently. Decreased detrusor contractility is expected following bladder injection of BoNT. Patients with significant bladder outlet obstruction should be informed of the possibility of urinary retention with a need for intermittent catheterization after BoNT administration. (*Tzu Chi Med J* 2007;19(3):109–114)

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1. Mechanism of neurotransmitter release from presynaptic nerve endings

The neurotransmitters formed in the cytosol of presynaptic nerve endings are enclosed in transport vesicles, which are then transported toward the plasma membrane. Through interaction with SNARE (N-ethylmaleimide-sensitive factor attachment protein receptor) proteins, transport vesicles fuse with the plasma membrane to release neurotransmitters into the synaptic space, a process called regulated exocytosis. SNARE proteins are a large protein superfamily consisting of more than 60 members. Upon fusion, SNARE proteins form a 4-alpha-helix bundle, consisting of synaptobrevin, syntaxin and two 25 kD synaptosomal associated proteins (SNAP-25). Without an intact SNARE protein complex, neurotransmitter-containing vesicles cannot fuse with the plasma membrane to release neurotransmitters. SNARE proteins are targets of botulinum toxins (BoNTs) and tetanus toxin [1,2].

2. Structure and action mechanisms of BoNTs

There are seven serotypes of BoNT: A, B, C1, D, E, F and G. Only A, B, E and F are poisonous to humans. All types consist of a heavy chain and a light polypeptide joined by a disulfide bond. The light chain possesses protease activity. The heavy chain involves the binding with BoNT receptors located on the plasma membrane to facilitate endocytosis of the toxin molecule. The receptor for BoNTs was not fully recognized until recently. Dong et al identified synaptic vesicle protein SV2 as the membrane receptor for botulinum toxin A (BoNT/A) [3]. Receptors for other types of BoNTs remain to be determined.

Once inside the cytosol, the light chain is released to cleave the specific component of the SNARE protein responsible for transmitter exocytosis. Each serotype of BoNT has its own specific cleavage site on the SNARE protein. Types A and E cleave to SNAP-25; types B, D, F, and G cleave to synaptobrevin, and type C1 to syntaxin. Cleavage of the SNARE protein prevents exocytosis of neurotransmitters, resulting in chemo-denervation [4,5].

3. Release of what neurotransmitter is inhibited by BoNT?

Classically, BoNT is known to inhibit release of acetylcholine from cholinergic nerve terminals in the neuromuscular junction of striated muscle. It is now known that acetylcholine release can also be inhibited from parasympathetic nerve endings of exocrine glands. Ellies et al demonstrated that BoNT decreased acetylcholinesterase expression in the submandibular glands of rats [6]. In addition, decreased release of acetylcholine from the rat detrusor was found following BoNT A injection [7].

Whether adrenergic neurotransmitter release is modified by BoNT is controversial. An earlier study on brain synaptosome could not show a decrease in potassium-stimulated release of norepinephrine [8]. However, later studies showed inhibitory effects of BoNT on the release of norepinephrine from brain neurons [9,10].

BoNT/A has been found to reduce the release of norepinephrine from nerve endings in smooth muscles. Morris et al demonstrated that isometric contractions of isolated vena cava to field stimulation at 20 Hz, mediated by norepinephrine, were reduced significantly by BoNT/A [11]. Smith et al also found that norepinephrine release from the urethra induced by high frequency electrostimulation was inhibited by BoNT/A for at least 30 days after injection [7].

The release of calcitonin gene related peptide (CGRP), a sensory neurotransmitter, is also found to be inhibited by BoNT. Durham et al showed that the stimulated release of CGRP from cultured trigeminal neurons was greatly reduced by BoNT/A [12]. Chuang et al also found that intravesical BoNT/A administration blocked acetic acid-induced bladder pain responses and inhibited CGRP release from afferent nerve terminals [13].

BoNT/A is also capable of inhibiting the release of substance P. Welch et al found that substance P secretion was reduced by BoNT/A in embryonic rat dorsal root ganglia neurons that exhibited calcium-dependent substance P secretion when depolarized with elevated extracellular potassium [14].

Not only do BoNTs inhibit the release of neurotransmitters from nerve endings and neurons, some of them also prevent the release of neurotransmitters from other type of cells. Smith et al found that stimulated release of ATP, a mediator for nociception in the urinary bladder, from urothelial cells in rat bladders with cyclophosphamide-induced inflammation was significantly reduced by BoNT/A [15]. The authors proposed that exocytosis of ATP from urothelial cells required SNARE proteins, which were cleaved by BoNT/A. However, this hypothesis needs further verification since there is no clear evidence showing the existence of BoNT receptors on urothelial cells. Besides, the exact mechanisms involving ATP release from urothelium remain uncertain [16].

Release of some neurotransmitters is clearly not affected by BoNTs. Morris et al found that sustained field-stimulated contractions of guinea pig vena cava mediated by neuropeptide Y were not affected by BoNT/A [11]. In another study, Morris et al

demonstrated that although field-stimulated contraction of the guinea pig uterine artery was markedly reduced by BoNT/A, nitric oxide-induced relaxation was not affected. These results indicate that nitric oxide release does not go through exocytosis or that nitric oxide release is not SNARE protein dependent [17]. Olgart et al also found that nitric oxide release from guinea pig intestine could not be inhibited by BoNT/B, indicating the non-vesicular nature of nitric oxide release [18]. We also found that in canine prostate strips contracted by phenylephrine, field stimulation-induced relaxation was not changed by BoNT/A injection, indicating that nitric oxide release in the prostate is also not SNARE protein dependent.

4. Target sites of BoNT

Target sites of BoNTs other than the plasma membrane of nerve endings or neurons have been proposed. As indicated in the previous section, urothelial cells have been considered to be a target of BoNT/A to inhibit the release of ATP [15].

Some studies suggested smooth and striated muscle cells as the targets of BoNTs. James et al demonstrated that low concentration BoNT/A reduced guinea pig pyloric contractions to electric field stimulation without affecting acetylcholine-induced contractions. However, at higher concentrations (10 U/mL), BoNT decreased pyloric contractile responses to both field stimulation and acetylcholine administration, indicating direct inhibitory effects of BoNT/A on pyloric smooth muscle [19].

In rat skeletal muscle 4 weeks after BoNT injection, vacuoles of variable size were seen in the sarcoplasm near myonuclei, both at and away from the endplates. This phenomenon was not observed following surgical denervation, suggesting direct toxic effects of BoNT on skeletal muscle cells [20]. We also observed extensive vacuolization of stromal smooth muscle cells in dog prostate 4 weeks after injection of BoNT/A, further confirming the direct toxic effects of BoNT/A on smooth muscle.

5. Actions of BoNT other than inhibiting neurotransmitter release

BoNTs might influence the presentation of membrane receptors. Ma et al injected BoNT/A into rat gastrocnemius muscle and found that mRNA from nicotinic cholinergic receptors was upregulated and then returned to normal levels within 2 weeks. This could be a compensatory response of treated muscle to the blockade of acetylcholine release [21]. However, in contrast to Ma et al's finding, BoNT/A has been found to reduce the expression of adrenergic receptors. Chuang et al found that 1 week after injection of BoNT/A into rat prostate, the amount of alpha 1A receptors was dose-dependently reduced [22]. One would expect an increase of alpha adrenergic receptors following the BoNT/A-induced decreased catecholamine in the synaptic space as compensation. One possibility could be that the trafficking of alpha adrenergic receptors from internal pools to the plasma membrane is mediated by some SNARE proteins [2]. Such machinery has been identified in the case of nicotinic receptors [23]. Higher doses of BoNT/A might also cleave the SNARE protein responsible for receptor trafficking, resulting in a reduction of receptor presentation on the plasma membrane.

6. Physiological sequences of BoNT administration

6.1. Induces atrophy with a decrease in contractile function of skeletal muscle

It is well known that BoNT injection reduces contractile function of striated muscle and it has been widely used for dystonia, spasticity, strabismus and blepharospasm.

6.2. Reduces contractile function of smooth muscle

A large number of studies have proved the effectiveness of BoNT/A injection in reducing the contractile function of the smooth muscle of the anal sphincter, esophageal gastric junction and urinary bladder in the treatment of anal fissures, achalasia and detrusor overactivity.

6.3. Induces atrophy with reduced secretion from exocrine glands

Clinical studies showed BoNT injection may decrease sweating and improve symptoms of hyperhydrosis [24–26]. Several papers reported good results in treating sialorrhoea with sonography-guided BoNT injection into the salivary glands [27,28].

Studies indicated that the prostatic gland is richly innervated by cholinergic nerves [29,30]. M3 muscarinic receptors were also found in the outer muscle layer surrounding the prostatic acini [29]. Theoretically, prostatic glandular secretion should also be depressed by BoNT. One study did show atrophic effects of BoNT/A on rat prostate. One and 2 weeks after prostate BoNT injection, there was a significant atrophy of the prostate glands with an abundance of apoptosis [31]. Similar findings were also observed

by Chuang et al [22]. We also saw atrophic changes in the glandular component of canine prostate after BoNT/A injection (unpublished data).

6.4. *Decreases inflammatory response and pain provoked by experimental inflammation*

It has been shown that subcutaneous injection of BoNT/A 5 hours to 12 days before injecting formalin into the plantar surface of rat hind paw inhibited the formalin-induced inflammatory pain response [32]. Further study showed that BoNT/A reduced glutamate release from the rat footpad and also downregulated *fos* gene expression in the spinal cord [33].

Chuang et al induced prostatitis by injecting capsaicin. Pretreatment with BoNT/A reduced polymorphonuclear cell infiltration in the prostate with a reduction of COX-2 expression. The pain from prostatitis was also diminished by BoNT/A application [34].

7. Adverse effects of BoNTs

7.1. *Lethal dose of BoNTs*

The potency of BoNTs is expressed in units (U). One unit represents the dose fatal to 50% (LD50) of a batch of Swiss Webster mice. The lethal dose of BoNT for humans remains undetermined. Data on monkeys might be applicable to humans. In one study, BoNT/A was injected intramuscularly into monkeys. An injection of 33 U/kg began to cause systemic toxicity. The LD50 was approximately 39 U/kg body weight [35]. Another study found the LD50 of an intravenous injection of BoNT/A in monkeys was 40 U/kg, which was similar to that of intramuscular injection [36]. Referencing these data, one would suspect an LD50 of BoNT/A for a 70 kg human is around 3000 U. However, interspecies differences in the susceptibility to toxin make it difficult to confirm that this estimate is a lethal dose of BoNTs for humans. Nevertheless, the maximal dosage clinically used rarely exceeds 300–400 U, which is far below the lethal dose and is unlikely to cause serious systemic side effects.

7.2. *Systemic botulism-like side effects*

Although botulism-like systemic side effects following clinical application of BoNTs is uncommon, it has been found by some investigators. In two separate reports, following injection of BoNT/A (Dysport), five adult patients with dystonia or spasticity developed botulism-like symptoms with generalized weakness with and without ptosis. The side effects emerged from 4 days to 3 weeks after injection and lasted for 3–6 months [37,38]. Wyndaele and Van Dromme reported two cases of generalized muscle weakness following BoNT/A (one with 1000 U Dysport, one with 300 U Botox) injection into the detrusor muscle [39].

Patients with neurological disorders are prone to botulism-like side effects. Wilson reported one case of acute myasthenic crisis after a second injection for blepharospasm in a patient with motor neuron disorder [40].

7.3. *Systemic autonomic side effects*

Some systemic autonomic side effects of BoNT/A have been proposed. Claus et al found that after a second injection of BoNT (Dysport) in patients with spasmodic torticollis, there were changes in some parameters of heart rate variability (HRV). However, no clinically significant arrhythmia was found [41]. In contrast, Nebe et al found no effects of BoNT on HRV [42].

Impaired contractile function of the gallbladder has also been found following BoNT injection. Schnider et al discovered that in four patients who received high dose BoNT/A (Dysport) injections, the emptying function of the gallbladder became abnormal 8 and 15 days after the injection. Although there were no complaints of gastrointestinal symptoms, BoNTs should be used cautiously in patients with gallbladder disorders [43].

More systemic autonomic side effects were found with BoNT/B injection. Dresser et al applied BoNT/B to treat cervical dystonia, and 92% of patients experienced at least one autonomic side effect, such as mouth dryness, swallowing difficulty, and constipation [44].

7.4. *Side effects of BoNT treatment for lower urinary tract dysfunction*

Serious systemic side effects from BoNT/A injection into the lower urinary tract are not common. Only a few cases have been reported. As indicated in a previous section, two patients who received BoNT/A bladder injection experienced generalized weakness [39]. Dykstra and Sidi found that following BoNT/A injection into the urethral sphincter, three patients with detrusor sphincter dyssynergia experienced mild generalized weakness, which lasted for 2–3 weeks [45].

Only a few studies have used BoNT/B in the lower urinary tract. In one study, four of 20 patients receiving BoNT/B bladder injections for detrusor overactivity experienced side effects, including dry mouth, constipation and generalized weakness [46].

7.5. Factors related to the occurrence of side effects

The serotypes of the BoNTs, formulation, dilution factor, injection technique, and individual susceptibility may all influence the occurrence of side effects with BoNT injection.

8. Contraindications and cautions in BoNT administration

Active urinary tract infection precludes urethral instrumentation for bladder and urethral injection. Prostate injections, either transurethrally or transperineally, are also not suitable for patients with urinary tract infection.

Of course, known hypersensitivity to BoNTs prohibits BoNT application. Although one patient with myasthenia gravis has been treated safely with BoNT, BoNT injection is generally not recommended for patients with neuromuscular disorders; side effects in patients with neuromuscular disorders have been noted (47). Drugs affecting neuromuscular transmission, such as aminoglycosides, should not be used concurrently.

Decreased contractile function of the detrusor is expected following bladder injection of BoNT. Patients with significant bladder outlet obstruction should be informed of the possibility of urinary retention with a need for intermittent catheterization after BoNT administration.

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