Botulinum toxin type A: basic pharmacological profile and therapeutic applications

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Abstract

Botulinum toxin Type A (BoNT/A), produced by the Gram-positive anaerobic bacteria *Clostridium botulinum*, is one of the most potent toxins in nature, and a very useful therapeutic tool for combating various neurological and autonomic disorders. The main pharmacological features of BoNT/A are neurospecificity, long-lasting effect, and safety. These features are grounded on its peculiar molecular mechanism of action: after specific binding to the neuronal membrane, it is internalized into the neuronal cytosol, where it specifically cleaves one of the proteins necessary for neurotransmitters release. The consequent reversible neuroparalysis lasts for several months and explains the long-lasting clinical effects after a single local toxin application. Although already approved for the prevention of chronic migraine, the basic and clinical investigations have repeatedly shown the potential of BoNT/A in relieving other chronic pain conditions. Accumulated data from experimental pain models demonstrated that BoNT/A reduces pathological pain hypersensitivity after axonal transport to the central nervous system, where it interferes with complex processes of central sensitization. Future experiments are needed to explain in more depth BoNT/A molecular mechanism of action and pharmacokinetic peculiarities.

Key words: botulinum toxin type A, mechanism of action, therapeutic applications, pain investigation

Introduction

Clostridium botulinum is an anaerobic spore-forming Gram-positive bacteria which under appropriate conditions produces different protein neurotoxins (BoNTs). Bacteria produce seven serotypically distinct BoNTs, denoted as BoNT/A–BoNT/G, which can be further divided into subtypes according to amino acid sequences. Due to their high potency and specificity for neurons, BoNTs are one of the most powerful known toxins in nature (1). Their biological and pharmacological properties have been extensively studied during the last several decades, with special focus on their potential therapeutic applications. The therapeutic potential of BoNT/A was unveiled in the 1970s, and nowadays it has the widest clinical application of all BoNTs.

Structure of BoNT/A

BoNT protein is released from bacteria as a large 900 kDa oligomers consisting of a 150 kDa neurotoxin and auxiliary proteins, which includes a non-toxic nonhemagglutinin (NTNH) and three hemagglutinin proteins (2). NTNH contributes to toxin stability in the acidic environment, while haemagglutinins are involved in translocation across the intestinal epithelial lining into the lymphatic system and the bloodstream (3). The neurotoxic part of this progenitor toxin complexes consists of a light chain (L chain; 50 kDa) and a heavy chain (H chain; 100 kDa), which are held together by non-covalent interactions and a single inter-chain disulfide bond. While the H chain mediates the specific binding of neurotoxin to the presynaptic plasma membrane of nerve terminals, the L chain exerts proteolytic activity against specific intracytosolic proteins (3,4).

Molecular mechanism of action

BoNT/A binds with high affinity to peripheral cholinergic nerve terminals and after entering into the neuronal cytosol blocks the release of acetylcholine from motoneurons and parasympathetic neurons. The consequence is generalized peripheral flaccid paralysis of skeletal muscles and dysfunction of cholinergic nervous systems as the most important pathological features of botulism (4). The mechanism of nerve terminal intoxication by the BoNT/A includes four steps: 1) specific binding to nerve terminals, 2) internalization within an endocytic compartment, 3) translocation of the L chain across the vesicle membrane and its release in the cytosol upon reduction of the interchain disulfide bond; and 4) cleavage of SNAP-25 (synaptosomal-associated protein 25 kDa), one of the proteins which is crucial for neurotransmitter release (4). The final result of this specific series of events is neuroparalysis (Figure 1).



Figure 1. Molecular mechanism of BoNT/A action on motor nerve terminals

In analogy with its known action on the neuromuscular junction, it was proposed that BoNT/A prevents the sensory neurotransmitter release from the peripheral nerve endings as well (5). This was demonstrated with series of in vitro experiments where BoNT/A inhibited the release of neurotransmitters such as glutamate, noradrenaline, serotonin, substance P, calcitonin gene-related peptide, adenosine triphosphate, nicotinamide adenine dinucleotide from cultured cells (4,5).

Clinical applications

BoNT/A has several favorable and unique pharmacological characteristics. As already described, it is very potent and neurospecific, its action is reversible with time and finally, it is very safe because of minimal diffusion after local injection in small volumes. Because of these features, BoNT/A (applied intramuscularly or intradermally in picogram quantities) is one of the safest and most efficacious therapeutic proteins for autonomic disorders, spasticity, and hyperkinetic movement disorders, as well as for cosmetic treatments (6). Since the introduction in human therapy during the 1980s, the

number of clinical indications has been continuously expanding (Table I). Currently, the only approved use of BoNT/A concerning pain is chronic migraine, based on randomized controlled clinical trials (RCTs) and meta-analysis (7).

Neurology	focal dystonias (blepharosmasm, cervical dystonia)	
	non-dystonic disorder (hemifascial spasm)	
	focal spasticity (limb spasticity)	
	cerebral palsy	
	hyperhidrosis	
	hypersalivation (sialorrhea, drooling in parkinsonian	
	syndrome)	
	aesthetics	
Ophtalmology	strabismus	
Urology	overactive bladder (detrusor overactivity)	
Pain	chronic migraine prevention	

Table ITherapeutic indications of BoNT/A (Botox[®]) (4)

Three products that contain BoNT/A are commercially available in this part of the world: onabotulinumtoxin A (ONA), abobotulinumtoxin A (ABO), and incobotulinumtoxin A (INCO). ONA and ABO are neurotoxin complexes with auxiliary proteins (molecular weight 900 kDa) while INCO contains only the purified BoNT/A (molecular weight 150 kDa). The potency of BoNT preparations is expressed as Units (U), where 1U corresponds to 1 LD50 in the mouse bioassay (~48 pg). Regarding relative potency between all three products, in the clinical setting, it was observed that ONA and ABO have non-parallel dose-response curves, where 1 U of ONA is equivalent o 2.5-3 U of ABO (conversion ratio is 1:2.5-3), in contrast to ICNO which is equivalently potent as ONA (8).

After local injection into the muscle, BoNT/A induces paralysis 2–3 days after the application. The paralytic effect is maintained for 3-4 months, while in the autonomic synapses the effect lasts for approximately 1 year (4). As already mentioned, excellent safety profile is one of the key features of BoNT/A use. The adverse effects can be related to the paralysis outside the target muscle because of the local diffusion to adjacent muscles. This depends on the volume and speed of injection, dose, and site of injection. For example, dysphagia can be a side-effect in the treatment of cervical dystonia, while ptosis or a "frozen" face could be seen in facial aesthetics (9,10). After local injections of BoNT/A, the fraction which didn't enter the neurons is probably diluted in the lymphatic circulation and washed away from the injection sites, being unable to affect more distant nerve endings because of too low concentration.

BoNT/A action on pain

An unexpected discovery that BoNT/A injection into glabellar lines reduces migraine headaches, followed by positive results coming from randomized clinical trials, led to regulatory approval of Botox[®] for chronic (not episodic) migraine headaches in 2011 (7). BoNT/A is injected intramuscularly in 31-39 anatomical points distributed across the corrugator, procerus, frontalis, temporalis, occipitalis, cervical paraspinal, and trapezius muscle groups. BoNT/A is injected intramuscularly in 31-39 anatomical points distributed across the corrugator, procerus, frontalis, temporalis, occipitalis, cervical paraspinal, and trapezius muscle groups. BoNT/A reduces migraine frequency and provides modest improvement of migraine symptoms. BoNT/A is investigated in other pain conditions and shows promising results in neuropathic and some other chronic pain disorders (11,12). In contrast to conventional and non-conventional analgesic drugs, longlasting analgesic effect after a single application (several months in humans, more than 15 days in animals) is main advantage of BoNT/A use. While the results are promising, the quality level of evidence due to a low number of participants, lack of standardized dosing and delivery protocols, is not persuasive enough to provide explicit guidelines for pain physicians (12).

Basic research of the action of BoNT/A on pain during the last two decades led to an important insight into the molecular mechanism of its antinociceptive action. BoNT/A was investigated in different inflammatory and neuropathic pain models, as well as on models of "mirror" pain of central origin (Table II).

Table IIPreclinical investigation of BoNT/A in some experimental inflammatory and
neuropathic pain models (according to review of Matak et al. (12)

Pain models	Results
Inflammatory pain models:	• BoNT/A applied s.c. (3.5-7 U/kg) into the rat hind-paw pad
- formalin-induced spontaneous pain	or intrathecally (1 U/kg) into the lumbar segment of the
- carrageenan-induced hypersensitivity	spinal cord reduced:
and paw oedema	- pain behaviours in the second inflammatory phase of the
- capsaicin–induced hypersensitivity	formalin test;
and neurogenic inflammation	- thermal and mechanical hypersensitivity but not the size of
	local oedema in the carrageenan model;
	- thermal and mechanical hypersensitivity but not the local
	neurogenic inflammation in the capsaicin model
Neuropathic pain models:	• BoNT/A applied s.c. (3.5-7 U/kg) into the hind-paw pad or
- partial sciatic nerve transection	intrathecally (1 U/kg) into the lumbar segment of the spinal
- streptozotocin-induced diabetic	cord reduced:
polineuropathy	- mechanical and thermal hyperalgesia induced by partial
- paclitaxel-induced neuropathic	sciatic nerve injury;
polineuropathy	- hypersensitivity to mechanical and thermal stimuli induced
- infraorbital nerve constriction injury-	by streptozotocin and paclitaxel (pain reduction was
induced trigeminal neuropathy	observed on both paws although BoNT/A was applied on
	one side only)
	• BoNT/A applied s.c. (3.5 U/kg) into the vibrissal pad
	reduced:
	- mechanical hypersensitivity after partial infraorbital nerve
	constriction injury (pain reduction was observed on both
	sides of the head although BoNT/A was applied into the
	whisker pad on one side only)

In analogy with action in the neuromuscular junction, first, it was suggested that the antinociceptive effect of BoNT/A is a consequence of inhibition of neurotransmitter release from peripheral sensory nerve endings (13). However, a series of behavioral data from experimental models demonstrated that the BoNT/A action on pain occurs primarily in the central nervous system (CNS), where it inhibits neurotransmitter release from central terminals of primary afferent neurons. This was confirmed by the immunohistochemical detection of cleaved SNAP-25, using antibody specific for BoNT/A-cleaved SNAP-25 (the product of BoNT/A proteolytic activity), within the sensory regions of the brainstem or spinal segment associated with the peripherally injected area (12,14). These experiments have also suggested that axonal transport of BoNT/A from the peripheral site of application to the CNS is a prerequisite for its action on pain (Figure 2).



Figure 2. Proposed mechanism of antinociceptive action of BoNT/A

Furthermore, it was demonstrated that BoNT/A modulates spinal opioid, GABA and glutamate neurotransmitter systems, as well as microglial activation and signaling. Attenuation of the microglia activation and neuroinflammation was proposed to play a role in the overall antinociceptive action of BoNT/A (12,15,16).

In the sensory system, BoNT/A action may be limited to certain neuronal populations mediating nociception, as already demonstrated for capsaicin-sensitive

neurons (17). This could explain the observed selective BoNT/A's antinociceptive action on some types of chronic pain and patient subpopulations. Long-lasting action after a single application is most likely a consequence of BoNT/A cellular localization and escape of intracellular degradation (12). It was demonstrated that BoNT/A light chain does not distribute evenly within the cytosol, but is concentrated at the inner side of the plasma membrane thus interacting with small GTP-ase proteins that polymerize into nonpolar filaments to form a part of cytoskeleton. Another possible explanation is that BoNT/A escapes the ubiquitine-proteasome degradation pathway by recruiting specialized enzymes that remove polyubiquitin chains (12).

Further experiments investigating BoNT/A effects on multiple sites on its way from the periphery to the CNS are needed to explain in more depth its action on pain of different etiologies and to improve its clinical use.

References:

- Rossetto O, Pirazzini M, and Montecucco C. Botulinum neurotoxins: genetic, structural and mechanistic insights. Nat Rev Microbiol. 2014;12:535–549.
- Benefield DA, Dessain SK, Shine N, Ohi MD, and Lacy DB. Molecular assembly of botulinum neurotoxin progenitor complexes. Proc Natl Acad Sci USA. 2013;110:5630–5635.
- Gu S and Jin R. Assembly and function of the botulinum neurotoxin progenitor complex. Curr Top Microbiol Immunol. 2013;364:21–44.
- Pirazzini M., Rossetto O, Eleopra R, Montecucco C. Botulinum Neurotoxins: Biology, Pharmacology, and Toxicology. Pharmacol Rev. 2017;69:200–235.
- McMahon HT, Foran, Dolly JO, Verhage M. Wiegant VM, Nicholls DG. Tetanus toxin and botulinum toxins type A and B inhibit glutamate, gamma-aminobutyric acid, aspartate, and metenkephalin release from synaptosomes. Clues to the locus of action. J Biol Chem. 1992; 267:21338– 21343.
- Safarpour Y, Jabbari B. Botulinum Toxin Treatment of Movement Disorders. Curr Treat Options Neurol. 2018;20:4 doi: 10.1007/s11940-018-0488-3.
- Herd CP, Tomlinson CL, Rick C, Scotton WJ, Edwards J, Ives N, Clarke CE, Sinclair A. Botulinum toxins for the prevention of migraine in adults. Cochrane Database Syst Rev. 2018, doi: 10.1002/14651858.CD011616.pub2.
- 8. Frevert J. Pharmaceutical, biological, and clinical properties of botulinum neurotoxin type A products. Drugs R D. 2015;15:1–9.

- 9. Naumann M, Jankovic J. Safety of botulinum toxin type A: a systematic review and meta-analysis. Curr Med Res Opin. 2004;20:981–990.
- de Almeida AT, De Boulle K. Diffusion characteristics of botulinum neurotoxin products and their clinical significance in cosmetic applications. J Cosmet Laser Ther. 2007;9 (Suppl 1):17–22.
- Safarpour Y, Jabbari B. Botulinum toxin treatment of pain syndromes –an evidence based review. Toxicon. 2018;147:120–128
- Matak I, Bölcskei K, Bach-Rojecky L, Helyes Z. Mechanisms of Botulinum Toxin Type A Action on Pain. Toxins (Basel). 2019;11(8):459. doi:10.3390/toxins11080459
- Aoki KR. Review of a proposed mechanism for the antinociceptive action of botulinum toxin type A. Neuro. Toxicology. 2005;26:785-793.
- 14. Matak I, Bach-Rojecky L, Filipović B, Lacković Z. Behavioral and immunohistochemical evidence for central antinociceptive activity of botulinum toxin A. Neuroscience. 2011;186:201-207.
- Drinovac Vlah V, Filipović B, Bach-Rojecky L, Lacković, Z. Role of central versus peripheral opioid system in antinociceptive and anti-inflammatory effect of botulinum toxin type A in trigeminal region. Eur J Pain. 2018;22:583–591.
- 16. Drinovac Vlah V, Bach-Rojecky L, Lacković Z. Association of antinociceptive action of botulinum toxin type A with GABA-A receptor. J Neural Transm (Vienna). 2014;121:665–669.
- 17. Matak I, Rossetto O, Lacković Z. Botulinum toxin type A selectivity for certain types of pain is associated with capsaicin-sensitive neurons: Pain. 2014;155:1516–1526.

Botulinski toksin tip A: osnovni farmakološki profil i terapijska primena

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Sažetak

Botulin toksin tipa A (BoNT/A) proizvodi gram-pozitivna anaerobna bakterija Clostridium botulinum i jedan je od najpotentnijih toksina u prirodi, ali i vrlo korisno terapijsko sredstvo kod različitih neuroloških i autonomnih poremećaja. Glavne farmakološke karakteristike BoNT/A su neurospecifičnost, dugotrajno dejstvo i sigurnost. Te su karakteristike utemeljene na njegovom posebnom molekularnom mehanizmu delovanja: nakon specifičnog vezanja za membranu neurona, internalizuje se u citosol, gde specifično cepa jedan od proteina potrebnih za egzocitozu neurotransmitera. Posledična reverzibilna neuroparaliza traje nekoliko meseci i objašnjava dugotrajne kliničke učinke nakon jednokratne lokalne primene toksina. BoNT/A je odobren za primenu u prevenciji hronične migrene, a brojna pretklinička i klinička ispitivanja pokazala su njegov potencijal u ublažavanju drugih hroničnih bolnih stanja. Mnogobrojni podaci iz eksperimentalnih modela bola pokazali su da BoNT/A smanjuje patološku preosetljivost na bol nakon aksonskog transporta u centralni nervni sistem, gde interferira sa složenim procesima centralne senzitizacije. Potrebna su dodatna istraživanja koja bi detaljnije opisala molekularni mehanizam delovanja BoNT/A na bol, kao i posebne farmakokinetičke karakteristike.

Ključne reči: botulinski toksin tipa A, mehanizam delovanja, terapijska primena, ispitivanje bola