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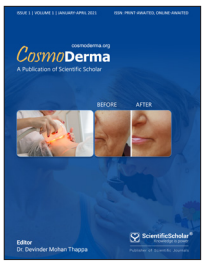

Review Article

Botulinum toxin – Know the product before injecting

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ABSTRACT

Botulinum toxin for injection is a purified and diluted protein which is isolated from the bacterium *Clostridium botulinum*. It is one of the most potent toxins known to humankind. *C. botulinum* is an anaerobic Gram-positive, spore-forming bacterium which is present naturally in soil, plants, static water bodies, and the gastrointestinal tract of mammals and aquatic life. Different formulations of botulinum toxins are available. FDA approval for these formulations varies. This article reviews these factors and the molecule, its mechanism of action, and other pharmacological aspects including dilutions for various indications.

Keywords: Botulinum toxin, Botox, Mechanism of action

INTRODUCTION

Botulinum toxin for injection is a purified and diluted protein which is isolated from the bacterium *Clostridium botulinum*. It is one of the most potent toxins known to humankind.[1] The harnessing of this toxin for therapeutic and cosmetic indications using very small doses has been a breakthrough in medical pharmacology. *C. botulinum* is an anaerobic Gram-positive, spore-forming bacterium which is present naturally in soil, plants, static water bodies, and the gastrointestinal tract of mammals and aquatic life.[2] Alan B Scott first reported the therapeutic use of botulinum toxin for strabismus,[3,4] following which it was used for other medical conditions in almost every field of medicine. In 2002, the US FDA approved botulinum toxin subtype A (Botox™) for the cosmetic treatment of frown lines.[2]

DIFFERENT TOXIN FORMULATIONS

The currently available botulinum toxin products which are U.S. Food and Drug Administration approved are onabotulinum toxin (OnaA) (Botox™), abobotulinum toxin (AboA) (Dysport™), incobotulinum toxin (IncoA) (Xeomin™), and prabotulinum toxin (PraA) (Jeuveau™/Nabota™), all of which are serotype A complexes and rimabotulinum toxin which is serotype B. The serotype B or rimabotulinum B is only approved for cervical dystonia. It has been used for spasticity and some randomized placebo-controlled studies have shown it to be as effective for glabellar frown lines as serotype A. The onset of action was shorter, about 2–3 days, however, they lasted only 8 weeks. It has an acidic pH and is not well tolerated by patients.[5,6] It has been shown to be effective in cases where tachyphylaxis to BTX-A products has been observed.[7]

The differences between the BTX-A serotypes are shown in [Table 1].

Table 1: Botulinum toxin subtypes and formulations.[12,28,29]

	ONA	ABO	INCO	PRA	RIMA
Company	Allergan, Inc., USA	Ipsen Biopharm Ltd., UK	Merz Pharma, Germany	Daewoong Pharmaceuticals, Korea	US WorldMeds, USA
Trade name	Botox	Dysport	Xeomin	Jeuveau/Nabota	Myobloc/ NeuroBloc
Molecular weight	900 kD	500–900 kD	150 kD	900 kD	700 kD
Shelf life	24–36 months	24 months	36 months	36 months	24 months
Dosage form	Spray-dried powder	Freeze-dried powder	Freeze-dried powder	Vacuum-dried powder	Sterile solution
Excipients	HSA, NaCl	HSA, lactose	HSA, sucrose	HSA, NaCl	HSA, NaCl, sodium succinate, SWI
pH	7.4	7.4	7.4	Not disclosed	5.6
Storage temperature	2–8°C	2–8°C	2–8°C	2–8°C	2–8°C
Recommended volume of reconstitution	10 ml maximum	1 ml maximum	8 ml maximum	2.5 ml std dilution	0.5–2 ml
Mechanism of action	Cleaves SNAP 25	Cleaves SNAP 25	Cleaves SNAP 25	Cleaves SNAP 25	Cleaves Synaptobrevin
US FDA indications	Moderate-to-severe frown, glabellar. and lateral canthal lines	Moderate-to-severe glabellar lines	Moderate-to-severe glabellar lines	Moderate-to-severe glabellar lines	Cervical dystonia

HSA: Human serum albumin, NaCl: Sodium chloride, SWI: Sterile water for injection, Std: Standard, ONA: Onabotulinum toxin, ABO: Abobotulinum toxin, INCO: Incobotulinum toxin, PRA: Prabotulinum toxin, RIMA: Rimabotulinum toxin

The activity of the formulations is measured in units, with onabotulinum measured in Botox unit (bU), abobotulinum in Speywood unit (sU), and incobotulinum in unit (U). One unit is the quantity that is lethal to 50% of 18–20 g female Swiss-Webster mice by an intraperitoneal route of injection. These units are, however, not equivalent due to different murine assay designs.[7] The Dysport assay is more sensitive and requires 68% less toxin per sU to kill 50% mice than one bU.[7] Therefore, a Speywood unit contains less active toxin than one Botox unit. This translates into the fact that the units of all toxins are not equal and dose adjustments need to be made. A meta-analysis using the Cochrane Review as a methodology concluded that a 1:3: OnaA: AboA ratio was closest to achieving bioequivalence.[8]

OnaA and IncoA have similar bioequivalence in a 1:1 dose ratio according to head-to-head clinical trials,[9] but a consensus review suggests a conversion ratio of 1:2.5: Inco: Ona/Abo for glabellar frown lines.[10]

PHARMACOLOGICAL ASPECTS OF BOTULINUM TOXIN

Botulinum toxin used for commercial purposes is a purified and diluted protein which is an exotoxin produced by *C. botulinum*.[4] It has recently been found that non-clostridial bacterium, *Weissella oryzae* has also been found to produce botulinum toxin.[11] Toxin is produced by the bacterium more rapidly at higher temperatures.

The polypeptide chain consists of a heavy chain H, which is a metalloprotease and a light chain L, which are roughly 100 kD and 50 kD, respectively, in molecular weight. The H chain has two domains, the N-terminal which is the translocation domain which helps in the release of the L chain and the C-terminal which is the receptor-binding domain helping in endocytosis into the nerve cell.[12] The H and L chains are linked by a bisulfide bond. This bond that keeps the two chains together is important to maintain the potency of the product before its onset of action.

There are seven antigenically distinct toxins produced by the bacterium, A, B, C (C1 and C2), D, E, F, and G.

The estimated lethal dose of LD50 of botulinum toxin in humans is 3000 U for a 70 kg adult. The biological potency is defined by a unit of the toxin. However, due to an incomplete knowledge of the dose–response relationship, the ceiling dose of 360 U given 12 weeks apart is the recommendation.

Botulinum toxin does not cross the blood–brain barrier and hence does not affect the brain. Toxic doses can form four forms of botulisms, food borne, animal, infant, and wound. Irrespective of the source or route of entry, the symptoms are acute muscle weakness with difficulty in swallowing, speech, blurred vision, progressive flaccid paralysis, and respiratory muscle paralysis.[13]

There are gaps in our understanding of how the toxin is eliminated from the body. After binding irreversibly to the synaptic nerve receptors, the toxin is postulated to be metabolized over months.[14]

The therapeutic formulations of botulinum toxin consist of the core protein, accessory neurotoxin-associated proteins (NAPs), and excipients. The excipients are albumin, sucrose, gelatin, lactose, dextran for stabilization, and buffer systems for pH stabilization. The presence of complexing proteins does not affect the diffusion properties or the therapeutic effects of the product formulation.[15]

The botulinum toxin subtype A in both onabotulinum A and abobotulinum A consists of NAPs. These are protective catalytically inactive proteins which consist of glutenin and non-toxic non-hemagglutinin proteins. Progenitor complexes of 300 kDa, 500 kDa, and 900 kDa have been isolated, but all of them have the 150 kDa core protein.[7,16] These accessory proteins dissociate from the core molecule when the toxin is injected. The dissociation occurs at a physiologic pH and it has been suggested that it is immediate.[16] IncoA is free of accessory proteins and their absence does not affect the stability of the product. The shelf life of OnaA and AboA toxin is 2–3 years versus 3–4 years of IncoA toxin. Incobotulinum does not need refrigeration and can be stored at room temperature. The lack of NAPs also reduces the antigenic potential of the product.[17]

MECHANISM OF ACTION

To describe it in a nutshell, botulinum toxin prevents the release and action of the neurotransmitter acetylcholine, thus preventing the contraction of muscles. It acts on four sites, the neuromuscular junction, autonomic ganglia, post-ganglionic parasympathetic, and post-ganglionic sympathetic nerve endings that release acetylcholine.[2] The steps involved in bringing about the action of botulinum toxin A are as follows [Figure 1]:

1. The toxin which is injected intramuscularly moves toward the nerve ending due to the fluid environment
2. The carboxy (C) terminal of the H chain attaches to the polysialoganglioside receptor on the presynaptic nerve membrane forming a receptor complex.
   BoNT/A binds to a synaptic vesicle (SV2) protein receptor and BoNT/B binds to the synaptotagmin receptor.[12]
3. The entire core molecule of botulinum toxin is internalized into the cytoplasm of the nerve cell by endocytosis by the means of the ATP pump.
4. The cleaving of the H and L chains then occur due to dissociation of the disulfide bond and the zinc protease of L chain cleaves the synaptosomal-associated protein of 25 kDa (SNAP25) docking protein and prevents the action of the soluble N-ethylmaleimide-sensitive factor attachment protein receptor (SNARE), which is a complex of proteins.
5. Normally, the acetylcholine vesicle docks at the nerve terminal in the synaptic cleft which is aided by the SNARE complex consisting of proteins SNAP25, syntaxin and synaptobrevin or VAMP. The acetylcholine is then released into the synaptic cleft and then attaches itself to the receptor on the muscle to bring about its contraction. Inhibition of the SNARE complex prevents the docking of the acetylcholine vesicle and hence its effect on the muscle, bringing about temporary muscle paralysis.

Botulinum toxin also exerts indirect effects on the central nervous system such as reflex inhibition, normalizing reciprocal inhibition, intracortical inhibition, and

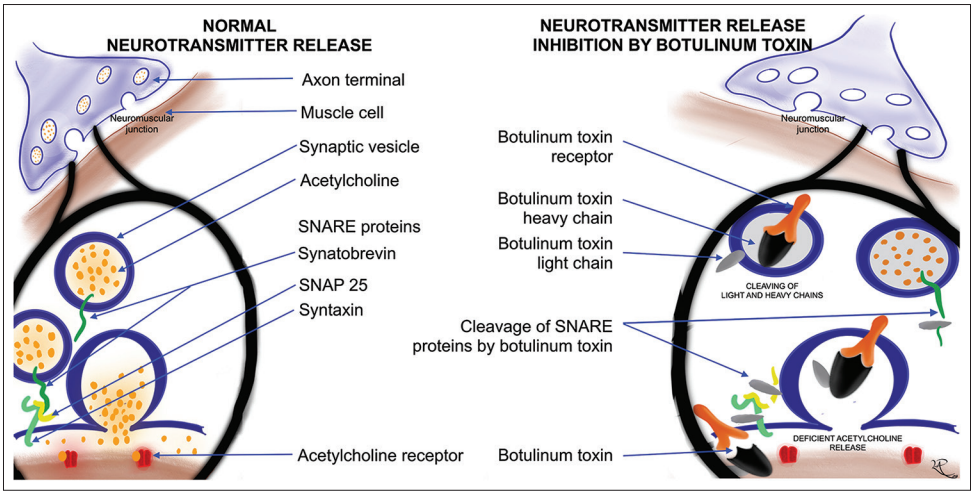

Figure 1: Botulinum toxin: Mechanism of action.

somatosensory evoked potentials. These effects occur by retrograde axonal transport not passive diffusion. This is the reason it exerts nociceptor effects and improvement in spastic gait.[18,19]

Botulinum toxin is effective for wrinkling due to muscle contraction and not those occurring due to soft-tissue volume loss or photoaging.[20] Hyperactive nerve terminals take up the toxin preferentially and it is shown that stimulating the nerve terminals increases the rapidity of toxin poisoning.[21] The effect is reversible and temporary, and the acetylcholine synthesis and storage are not affected by injections of the toxin. Recovery occurs by either metabolism of the botulinum toxin or due to sprouting of new neurons or the development of new extra-junctional acetylcholine receptors.[20]

RECONSTITUTION, DILUTION, AND STORAGE OF INJECTABLE TOXIN

The summary of product characteristics of Botox and Dysport says that the vial should be reconstituted with 2.5 ml of preservative-free saline. 1.5 ml is also a recommended reconstitution volume for Dysport. There is less pain and equal efficacy when the vials are reconstituted with preserved saline according to a randomized, placebo-controlled study. This is probably due to the anesthetic effect of benzyl alcohol which is added as a bacteriostatic product since the pH of the two solutions is similar.[22] Since the double-stranded protein of botulinum toxin is affected by physical influences and Brownian movement,[18] care must be taken not to agitate the toxin during reconstitution. While introducing the saline, the bevel of the reconstitution needle should be directed toward the wall of the vial to prevent the formation of bubbles. The vial should be gently rotated and not shaken or inverted. Some studies, however, say that this does not alter the potency.[23] It has been reported by Almedia *et al.* that despite the vial being shaken during reconstitution process and the presence of foaming, the botulinum toxin maintains it potency.[24] Kazim and Black carried out a double-blind, randomized study with a vigorous reconstitution of OnaA by a Vortex Touch Model 232 at maximum speed for 30 s, supported this.[25] The 2004 consensus panel has also proposed that the fragility of OnaA is not as much as recommended by the summary of product characteristics.[26]

The optimal doses of dilution of botulinum toxin vary greatly. However, dilution remains an important parameter in predicting side effects due to diffusion. Unintended weakness of a muscle due to extra dilution and hence more diffusion must be kept in mind. The dose of 2.5 ml of saline appears to be the most common for OnaA.[27]

OnaA, AboA, and PraA must be stored at 2–8°C. IncoA can be stored at 25°C.[28,29]

The manufacturers of the Botox™ recommend the usage of the vial within 4 h of reconstitution. Other manufacturers recommend using reconstituted BT-A drugs within 8 h (Abo) or 24 h (Ona, Inco).[18] Hexsel *et al.* have published that there is no loss of efficacy of reconstituted OnaA even if reconstituted with preservative-free saline for 6 weeks if stored at 4°C.[23] It has been reported that there is no change in potency of OnaA even if it is refrigerated or refrozen for up to 1 week or for up to 2 weeks after reconstitution.[30,31]

The size of needle plays a key role in loss of volume/units of product. The dead space in a 30 G needle results in loss of 0.03 mL of the toxin, and hence, it is recommended to use no dead space needles.[15]

SPECIAL DILUTIONS

Deviations from the standard dilutions are done to allow for increased or decreased diffusion of the product. Increase in the volume is directly related to more diffusion and field of effect provided other variables are kept constant. Larger volumes may lead to less efficacy.[7] Certain areas where a precise injection is preferred, a higher concentration of toxin is used to prevent inadvertent diffusion into unwanted muscles, a more concentrated dilution of the toxin is preferred. This, however, must be balanced with the right dose, making sure not to overdose the muscle. The lower eyelid is one such area. More dilute formulations are preferred if the muscle activity is to be retained and only dermal effects are needed such as tightening of pores or for giving a glow. Higher volumes are also associated with more pain of injection.[32]

Albumin is present as an excipient in concentrations much higher than the toxin itself. It helps to block the protein-binding sites, thus preventing the toxin from binding to them. Increased dilutions lower the efficacy of the albumin to block the sites, thus leading to lower efficacy of the toxin.

The U.S. Food and Drug Administration Product Labeling recommends diluting it with 0.9% preservative-free saline, with a concentration of 4 U/0.1 ml amounting to a concentration of 20 U in 0.5 ml for glabellar and forehead lines and 24 U in 0.6 ml for lateral canthal lines. It recommends the vials to be stored at 2–8°C for up to 24 months before reconstitution and at 2–8°C with administration within 24 h after reconstitution.[29] However, different consensus groups and panels deviate from these recommendations and use preserved saline, dilution concentrations ranging from 10 to 100 U/ml and administration up to 4–6 weeks after reconstitution.[33] Dysport™ (AboA) is diluted to make a dose of 120–500 U/ml with 0.6–2.5 ml of saline according to the package insert. Myobloc™ (BTX-B) can be diluted up to 6 times.[34]

The dose equivalence of OnaA: IncoA is close to 1:1 and that of OnaA: AboA is 1:3 to 1:2.5.[15]

A special mention about microbotox is worthy here. Microbotox is a technique developed by Dr. Wu, a plastic surgeon who reported the technique and coined the term in 2000 and 2001, respectively.[35] This technique makes use of diluted forms of onabotulinum A (Botox) using multiple microdroplets injected intradermally or just below the dermis in order to target the sebaceous and sweat glands. The dilution recommended by the author for the forehead, lower face, and thinner necks is 20 U/ml, while for thicker necks 28 U/ml. The dilution of 20 U/ml is made by taking the standard dilution of Botox™ which amounts to 0.5ml (20 U) and drawing 0.5 ml of saline to make 1 ml.

CONCLUSION

Knowing the product helps to deliver precise and optimum clinical results. The reader should be aware of how to vary the botulinum toxin preparations dilutions according to their different indications. Hence, a thorough knowledge about the product and their dosages ensures best results with least complications.

Declaration of patient consent

Patient's consent not required as there are no patients in this study.

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Nil.

Conflicts of interest

There are no conflicts of interest.

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