

Therapeutic and Cosmetic uses of Botulinum Toxin

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KEY WORDS

Botulinum toxin, *Clostridium botulinum*.

INTRODUCTION

From times unknown man has greatly been benefited from uncovering and utilizing the chemicals from the natural world. Living organisms, such as plants, animals, microorganisms, offer a huge source of pharmaceutically useful medicine and toxins. Depending upon their source, the toxins are categorized as phytotoxins, mycotoxins and zootoxins including venoms and bacterial toxins. Botulinum toxin is neurotoxic protein produced by the gram-positive, rod shaped, spore forming, strictly anaerobic bacterium *Clostridium botulinum*. These bacteria are widely distributed in soil and water (Dowell, 1984). Botulinum toxin is one of the most acutely toxic naturally occurring substances in the world with a lethal dose of about 200-300 pg/kg (100g could kill every human on earth. Botulinum toxin is odorless and tasteless, and shares many properties with the other bacterial toxins such as tetanospasmin and diphtheria toxin (Davis, 1993). Thousands of people in the world each year continue to be poisoned with botulinum toxin food-borne, infantile, or wound botulism but the neurotoxin is now sufficiently understood to allow

it to be used as medicinal agent to paralyze specific muscles, giving temporary symptomatic relief from variety of neurologic disorders and for certain cosmetic purposes in minute doses. (Davis, 1993). The clostridia produce more protein toxins than any other bacterial genus and are a rich reservoir of toxins for research and medicinal uses. Research is underway to use these clostridial exotoxins or their toxin domains for drug delivery, prevention of food poisoning, and the treatment of cancer and other diseases. The remarkable success of botulinum toxin as a therapeutic agent has created a new field of investigation in microbiology.

BOTULINUM TOXIN

It constitutes a family of serologically closely related 7 neurotoxins i.e. A, B, C1, D, E, F, and G. The primary structure of most serotypes has been determined. Each toxin has a inhibitory chain (light chain) and binding chain (heavy chain) and the two chains are held together with a disulfide bond. These chains have three major types of domains, the binding and translocation domains present on heavy chain and the catalytic domain present on the light chain. Toxin types A, B, E and F are the main toxins that affect humans (Dowell, 1984) and toxin types C and D affects birds and mammals (Davis, 1993). For therapeutic purposes botulinum toxin A is preferred because of its long duration of action and ease of production. Botulinum toxin type A also has several advantages including relatively rare systemic side effects, lack of tissue destruction, graded therapeutic response by dosage adjustment and, above all, high patient acceptance (Dressler et.al, 2000). The type A toxin is produced as a single chain polypeptide with a molecular weight

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of 150000 Da and later on it is transformed into its active structure by protease which causes nicking of single chain polypeptide to di-chain polypeptide of about 100000 Da and 50000 Da molecular weight (Bandyopadhyay, et al.1987).

Botulinum toxin B (BTX-B) is preferred to type A toxin for the treatment of cervical dystonia. BoNT/B is stable in solution at an acidic pH and is available as a solution containing 5000 units/mL.

MECHANISM OF ACTION

At neuromuscular (N-M) junction release of acetylcholine (Ach) is mediated by assembly of synaptic fusion complex that allows membrane of synaptic vesicle to fuse with neuronal cell membrane. Synaptic fusion complex is a set of SNARE (soluble N-ethylmaleimide-sensitive factor attachment protein receptor) proteins, which include synaptobrevin, SNAP-25 (synaptosomal-associated protein of 25 kd), syntaxin. After membrane fusion, acetylcholine is released into synaptic cleft and then bound by receptors on the muscle cell.

When there is exposure to botulinum toxin, it produces its action by involving several steps: systemic absorption, binding to the nerve terminal, internalization and synaptic poisoning. The toxin first reaches the lymphatic channels and then the blood stream either by absorption through the upper gastrointestinal tract (food borne and infantile botulism) or through tissue absorption (wound botulism) (Bonventre, 1979). Then the toxin circulates in the blood until it reaches cholinergic synapses in the peripheral nervous system. The toxin appears not to cross the blood brain barrier, so the cholinergic synapses of central nervous system are not involved (Sugiyama, 1980). The toxin binds with the help of binding domain to cholinergic neuronal cell membrane at nerve terminal and enters neuron by endocytosis (internalization). The light chain of botulinum toxin crosses the membrane of the endocytic vesicle and enters the cytoplasm of the pre synaptic terminal (Davis, 1993). Then it cleaves specific sites on SNARE proteins thus

preventing complete assembly of synaptic fusion complex which in turn blocks docking, fusion and acetylcholine release. Blockage of acetylcholine release in turn blocks activation of muscarinic and nicotinic receptors due to which there will be decreased/no secretion in case of exocrine glands and paresis due to chemical denervation in case of a muscle.

Botulinum toxins types B, D, F, and G cleave synaptobrevin; types A, C, and E cleave SNAP-25; and type C cleaves syntaxin.

HISTORY AND FDA (FOOD AND DRUG ADMINISTRATION) APPROVALS

In 1944, Edward Schantz cultured *Clostridium botulinum* and isolated the toxin, and, in 1949, Burgen's group discovered that botulinum toxin blocks neuromuscular transmission thus blocks the release of acetylcholine at N-effector junctions. By 1973, Alan B Scott, MD, of Smith-Kettlewell Institute used botulinum toxin type A (BTX-A) in monkey experiments, and, in 1980, he officially used BTX-A for the first time in humans to treat strabismus. In December 1989, BTX-A (BOTOX) was approved by the US Food and Drug Administration (FDA) for the treatment of strabismus, blepharospasm, and hemifacial spasm in patients over 12 years old. (Dressler, 2000) observed that the effects of botulinum toxin are fully reversible.

Botulinum Toxin Type B (BTX-B) received FDA approval for treatment of cervical dystonia on December 21, 2000. On April 15, 2002, the FDA announced the approval of botulinum toxin type A to temporarily improve the appearance of moderate-to-severe frown lines between the eyebrows (glabellar lines). BTX-A has also been approved for the treatment of excessive underarm sweating. The acceptance of BTX-A use for the treatment of spasticity and muscle pain disorders is growing, with approvals pending in many European countries and studies on headaches (including migraine), prostatic symptoms, asthma, obesity and many other possible indications are ongoing. Botox is the only FDA-approved botulinum toxin A, but a potential competitor,

Dysport, has an FDA approval application pending.

THERAPEUTIC USES

As the mechanism of action of the botulinum toxin was better understood, it was recognized that the toxin could be used selectively to paralyze muscles. Researchers discovered in the 1950s that injecting minute quantities of botulinum toxin type A in overactive muscles resulted in decreased muscle activity by blocking the release of acetylcholine at the neuromuscular junction, thereby rendering the muscle unable to contract for a period of 4-6 months. At that time, the botulinum toxin is often re-administered to the same muscles. Repeated muscle injection may provide relief for shorter periods than the initial administration. It is not yet clear if botulinum toxin can be re-administered indefinitely or whether the effectiveness eventually wears off. Clinical effect of the serotype A and B toxins begins within 24-48 hours, peaks at 2-3 weeks and lasts for 3-4 months (Brin, 2002). FDA has recommended uses of botulinum toxins for:

1) Migraine Headaches: Botulinum toxin in headache prophylaxis was found by Serendipity whereby patients receiving BoNT/A for frown lines reported reduced frequency of their headaches. BoNT/A at the doses range between 50-100 U is injected in the tender muscles of the frontal, temporal and cervical regions for the relief from tension-type and chronic daily headache. Studies have proven its efficacy in migraine prophylaxis (Silberstein et al., 2000). Though the exact mechanism is unknown, it has been postulated that BoNT/A reduces the afferent volley of pain impulses. The exact doses are still being worked out and it is best to begin with smaller doses and, if necessary, gradually increases the dose.

2) Dystonia: In general, more than 90% of treated cases of blepharospasm and laryngeal dystonia show a satisfactory result. Type B toxin, which has been carefully studied to date only in cervical dystonia, shows similar results as type A toxins. More than 75% of patients with cervical dystonia

and jaw-closing oromandibular dystonias benefit significantly from type A toxins. The response in upper limb dystonias is less.

a) Benign Essential Blepharospasm: Dystonic hyperactivity in the orbicularis oculi muscles causes blepharospasm. Orbicularis oculi, corrugator supercilii and procerus are targeted with botulinum toxin at the dose rate of 25 U for each side. Complications, namely ptosis, dryness of eyes, lateral rectus palsy, facial muscle paralysis and hematoma formation, are transient and reversible (Brin, 1998). Considerable improvement occurs in the quality of life measures in patients with blepharospasm.

b) Meige's syndrome: Patients have dystonia of both upper and lower halves of face. The lower face muscles have a very narrow therapeutic window and thus, have substantially higher chances of functional impairment. The dose used varies between 50 to 100 units.

c) Oromandibular Dystonia: In jaw closure dystonia, masseters are commonly targeted bilaterally by BoNT/A at the dose rate of 30U for each side. In jaw opening dystonia, lateral pterygoids together with anterior belly of omohyoid are targeted but the response is variable. It is advisable to avoid injecting tongue muscles in lingual dystonia as a weak tongue can choke a patient, requiring intubation (Brin, 1998). Oromandibular dystonias are difficult to treat without causing dysphagia and should only be done by well-trained and experienced clinicians with adequate experience in use of neurotoxins.

d) Cervical dystonias: Botulinum toxin is the treatment of choice for cervical dystonias and they significantly improve the quality of life measures (Brin, 1998). Identification of the muscles responsible for torticollis, laterocollis, anterocollis and retrocollis is essential. Muscles are targeted specifically after observing the pattern of shift, tilt and rotation of the neck and through EMG assessment. Approximately 20-60 U are needed for a muscle. Anterocollis with head protrusion has a poor response. Side effects include reduced head control and dysphagia. Injections into the sternocleidomastoid and

scalenes have a higher risk of dysphagia. Dysphagia can be minimized by avoiding bilateral sternocleidomastoid injections, targeting its insertion and ensuring that the muscle bulk is not penetrated. Patients in whom dysphagia might pose increased risk for aspiration pneumonia should be carefully assessed prior to injection. The effect of injections lasts for around 3-4 months.

e) Pharyngolaryngeal dystonias: Botulinum toxin therapy is the treatment of choice for both abductor and adductor forms of pharyngolaryngeal dystonias. Amongst all the indications for BoNT/A therapy, spasmodic dysphonia shows the highest success rate (Dressler, 2000). Botulinum toxin type A is injected either per orally under laryngoscopic guidance or transcutaneously through the cricothyroid membrane under EMG guidance. The vocalis muscle is targeted in the adductor form and posterior cricoarytenoid muscle in the abductor form. Approximately 2.5-10 U are used and the effect begins within 2-3 days and lasts for 2-9 months. Complications include transient dysphagia, weak cough, hoarseness and hypophonia. Dyspnea and stridor can occur after treating the abductor form (Brin, 1998) and (Dressler, 2000). Quality of life measures improve significantly after BoNT/A injections.

f) Writer's cramp and occupational cramps: Dystonic hyperactivity of forearm, hand and, occasionally, proximal arm muscles while writing occurs in writer's cramp. It is the most common occupational cramp. Response to BoNT/A is better if dystonia is limited to isolated muscles and if the initial hyperactive muscle trigger can be identified. Limitations to therapy include narrow therapeutic window of the wrist and finger flexors, large requirement for BoNT/A due to large number of muscles involved and difficulty to distinguish dystonic action from physiologic and compensatory action (Brin, 1998 and Dressler, 2000).

g) Non-action induced limb dystonias: Botulinum toxin type A is useful in pain reduction and improvement in function.

3) Hemifacial Spasms: Unilateral, involuntary, recurrent twitches of the eyelids and other muscles of face characterize hemifacial spasms. Periocular muscles, risorius, depressor anguli oris, depressor labii inferioris, zygomaticus and mentalis are targeted. Doses ranging from 25-50 U are used. Therapy with BoNT/A has a high success rate and the effect is longer than for blepharospasm. Presently, BoNT is the first line treatment for hemifacial spasms and only those with a poor response may need surgical decompression of the facial nerve (Jankovic et al., 1997).

4) Strabismus or crossed eyes

5) Exocrine gland hyperactivity

a) Focal hyperhidrosis: It is defined as excessive sweating of the palms, soles, axilla or face. The iodine-starch test delineates areas of hyperhidrosis and 0.5-0.8 U/cm² BoNT/A are injected intradermally. Approximately 30-80 U are used at 15-25 sites. The benefits last for 3-4 months and increased doses may extend this up to a year or more (Naumann et al., 1999).

b) Relative sialorrhoea: Botulinum toxin type A injection into the parotid gland is effective for controlling drooling in conditions such as Parkinson's disease, motor neuron disease and bulbar/pseudobulbar palsy without causing xerostomia (Dressler, 2000).

c) Frey's syndrome: Areas of skin are targeted that show gustatory sweating due to aberrant innervation of facial nerve secretomotor fibers to sweat glands following parotidectomy (Dressler, 2000).

d) Crocodile tears syndrome: Lacrimal glands are targeted in gustatory lacrimation due to aberrant innervation of facial nerve secretomotor fibers (Dressler, 2000).

Other uses of botulinum toxin type A that are widely known but not specifically approved by FDA include treatment of:

A) Prostate Hyperplasia: BTX- A injection induces prostate apoptosis in dogs and relieves BOO (Bladder outlet obstruction) due to benign prostatic hyperplasia (BPH) in humans. Intraprostate BTX-A injection may be a

promising, reversible and alternative treatment for refractory BOO due to BPH. Furthermore, previous studies in the rats have shown that intraprostatic injection of BTX-A induces selective denervation and subsequent atrophy of the glands.

B) Smooth Muscle Disorder: Direct injection of botulinum toxin is disclosed as an effective, safe and simple method of treatment for disorders of gastrointestinal muscle or smooth muscles elsewhere in the body, with results that appear to be sustained for several months. Muscle disorders which are suitable for such treatment include achalasia, isolated disorders of the lower esophageal sphincter, gastroparesis, hypertrophic pyloric stenosis, sphincter of Oddi dysfunction, short-segment Hirschsprung's, anal fissure, hemorrhoids, proctalgia fugax, irritable bowel syndrome, disorders of the upper esophageal sphincter, vasospastic disorders, and disorders of uterine and bladder spasm. Devices suitable for delivering this therapy are also disclosed.

C) Overactive Bladder Syndrome with or without incontinence.

D) Spastic Disorders associated with injury or disease of the central nervous system including trauma, stroke, multiple sclerosis, or cerebral palsy.

E) Anal Fissure

F) Diabetic neuropathy

G) Wound healing

H) Excessive salivation

I) Parkinson Disease

J) Depression

COSMETIC USES OF BOTULINUM TOXIN A (BTX-A)

The cosmetic effect of BTX-A was initially described by the Carruthers, a dermatologist/ophthalmologist husband and wife team working in Vancouver, Canada, although the effect had been observed by a number of independent groups. Cosmetically desirable effects of Botox were quickly discovered thereafter when the frown lines between the eyebrows were observed to soften following

treatment for eye muscle disorders, leading to clinical trials and subsequent FDA approval for cosmetic use in April 2002. As of 2006, Botox injection is the most common cosmetic operation in the United States.

1) Hyperkinetic Facial Lines: The main application of botulinum toxin in facial plastic surgery is in the effacement of dynamic or hyperkinetic facial lines i.e. Frown/glabellar lines, squint lines /eye crows feet lines, forehead lines and the muscle bands on platysma muscles often visible on the neck, commonly known as "turkey neck" or platysma banding. The granting of US Food and Drug Administration approval for the use of Botulinum Toxin type A in the treatment of glabellar lines for people with age 18–65 years marks a major milestone for the more widespread usage of this product in cosmetic settings. The use of botulinum toxin in treatment of hyperkinetic conditions is well established and enjoys an excellent safety profile (Batniji, 2004). Either local injection or creams are available for local use and typically, no anesthetic is necessary. The "muscle relaxant" effect lasts about three to four months and can be repeated as needed.

2) Eyebrow Lifting: A study has been carried out on 22 patients desiring a cosmetic enhancement and injection of botulinum toxin A (7-10 units) directed to brow depressor muscle (lateral orbicularis oculi) bilaterally. No patients withdrew for adverse effects. All patients were evaluated 2 weeks after treatment. The average brow elevation from the mid-pupil observed after selected injection of brow depressors with botulinum toxin A was 1.02 mm. The average brow elevation from the lateral canthus observed after selected injection of brow depressors with botulinum toxin A was 4.83 mm. Significant temporal brow elevation occurs as the result of paralysis of brow depressors by using botulinum toxin A injection. This procedure may be considered an alternative to surgical brow elevation.

3) Frontalis muscle hyperactivity.

4) Dimpling of the chin from overactive muscles.

5) Raise drooping of the corners of the mouth.

- 6) Fine lines around the lips.
- 7) Fine wrinkles under the eye.

TREATMENT FAILURE

Treatment failure can be either primary, where failure occurs in a BoNT-naïve patient, or secondary, where failure occurs after an initial successful use. Treatment failure may result from misplaced toxin, sub-optimal dosing, or administration of toxin that has been inactivated by improper storage or handling. Antibodies to the toxin are presumed to be responsible for most remaining cases of resistance. Antibodies against the toxin have developed in patients receiving large doses of the toxin, and these patients do not benefit from repeated toxin injections. Treatment failure for antigenicity is assessed when there is either absent or reduced response to BoNT. It is assessed clinically using the Frontalis type A antibody test (FTAT) wherein 15-20 U of BoNT/A are injected into two sites of the muscle and ability to raise the brow at 2 weeks is studied. If eyebrows can be raised, it indicates resistance (Brin, 1998). Persistence of motor activity with ability to raise the eyebrow during electromyography (EMG) of injected muscles gives electrophysiological evidence of resistance. Antibodies are also detectable using ELISA, sphere-linked immunodiagnostic assay, western blot assay and combined fluorescein- and enzyme-linked assays (Brin, 1998). Unfortunately, correlation between clinical resistance and detection of antibodies by assays, especially ELISA, has not been established. Mouse neutralization assay /mouse protection assay is considered the gold-standard assay, whereby injected mice will not die if antibodies are present in the test serum, though the meaning of the test in clinical practice is controversial. Early reports indicated that neutralizing antibodies occurred in 3-10% of cervical dystonia patients treated with BoNT/A. The more recent preparations (available after 1997) have lower protein content, and are believed not to produce neutralizing antibodies possibly due to lower protein load.

Patients with resistance to BoNT/A may benefit from botulinum toxins B or F (commercially BoNT/F is not available presently), as their immunogenicity profiles are different (Dressler, 2000). Care must be taken when the toxin is administered because it can diffuse from the inoculation site through tissue to paralyze neuromuscular junctions of adjacent muscles, causing unwanted side effects such as muscle weakness and dysphagia. Immune responses to BoNT/A and presumably other BoNT preparations can be minimized by using the least required dose and avoiding frequent repeat injections. Current labeling in most countries suggests 12-week separation between doses of BoNT.

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