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BOTULINUM TOXIN

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ABSTRACT

Botoulium being toxin can be used to treat various diseases. BOTOX is mainly well known for cosmetic use, but it has been also can be used to treat various diseases like migraine, cervical dystonia.

INTRODUCTION

TOXIN

International Committee of the Red Cross review of the Biological Weapons Convention, "Toxins are poisonous products of organisms; unlike biological agents, and they are inanimate and not capable of reproducing themselves."

Toxins may be classified as

1) **EXOTOXIN**, being excreted by an organism, and

2) **ENDOTOXIN** that is released mainly when bacteria are lysed.

Biotoxin

The term "biotoxin" is sometimes used to explicitly confirm the biological origin.

Biotoxin in nature have two primary functions:

- Predation i.e. killing the prey and the eventual absorption of the prey's (e.g. spider, snake, scorpion, jellyfish, wasp)
- Defense i.e. strategy of defending against attack (e.g. bee, ant, termite, honeybee, wasp, poison dart frog)[1]

Although they are toxic they posses many useful applications.

BOTULINUM TOXIN

Clostridium botulinum is a bacteria that causes botulism.

Botulinum Toxin A, commonly referred to as Botox®, Dysport®, or Xeomin®, is an artificially produced neuromuscular paralyzing agent.

Botulinum Toxin B, commonly referred to as Myobloc® or Neurobloc®, is an artificially produced neuromuscular paralyzing agent.

History of Botox

1950s	Scientists discover that botulinum toxin can reduce muscle spasms.
1960s/1970s	Studies explore botulinum toxin as a treatment for strabismus (crossed eyes)
1988	Allergan researches other medical uses of botulinum toxin.
1989	Allergan introduces BOTOX, the first botulinum toxin approved by the FDA to treat blepharospasm (eyelid spasms) and strabismus.
2000	FDA approves BOTOX® therapy for cervical dystonia to reduce the severity of abnormal head position and neck pain.
2002	FDA approves BOTOX® Cosmetic (onabotulinumtoxinA), the same formulation as BOTOX®, with dosing specific to moderate to severe frown lines

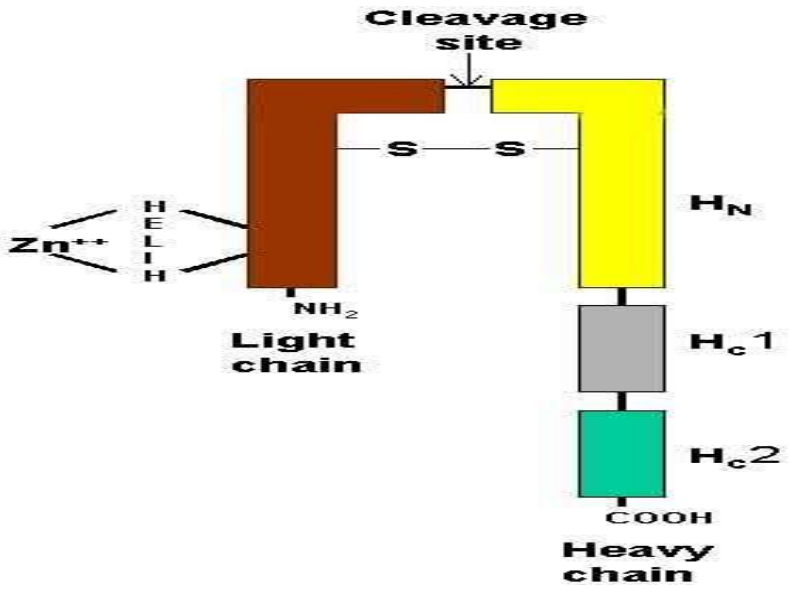
between the brows.

2004 FDA approves BOTOX® for severe underarm sweating when topical medicines don't work well enough.

2010 FDA approves BOTOX® therapy for increased muscle stiffness in elbow, wrist, and finger muscles with upper limb spasticity.[2]

BOTOX® is the first medicine to be studied and then approved by the FDA specifically for the prevention of headaches in adults with Chronic Migraine who have 15 or more days each month with headache lasting 4 or more hours each day in people 18 years or older.

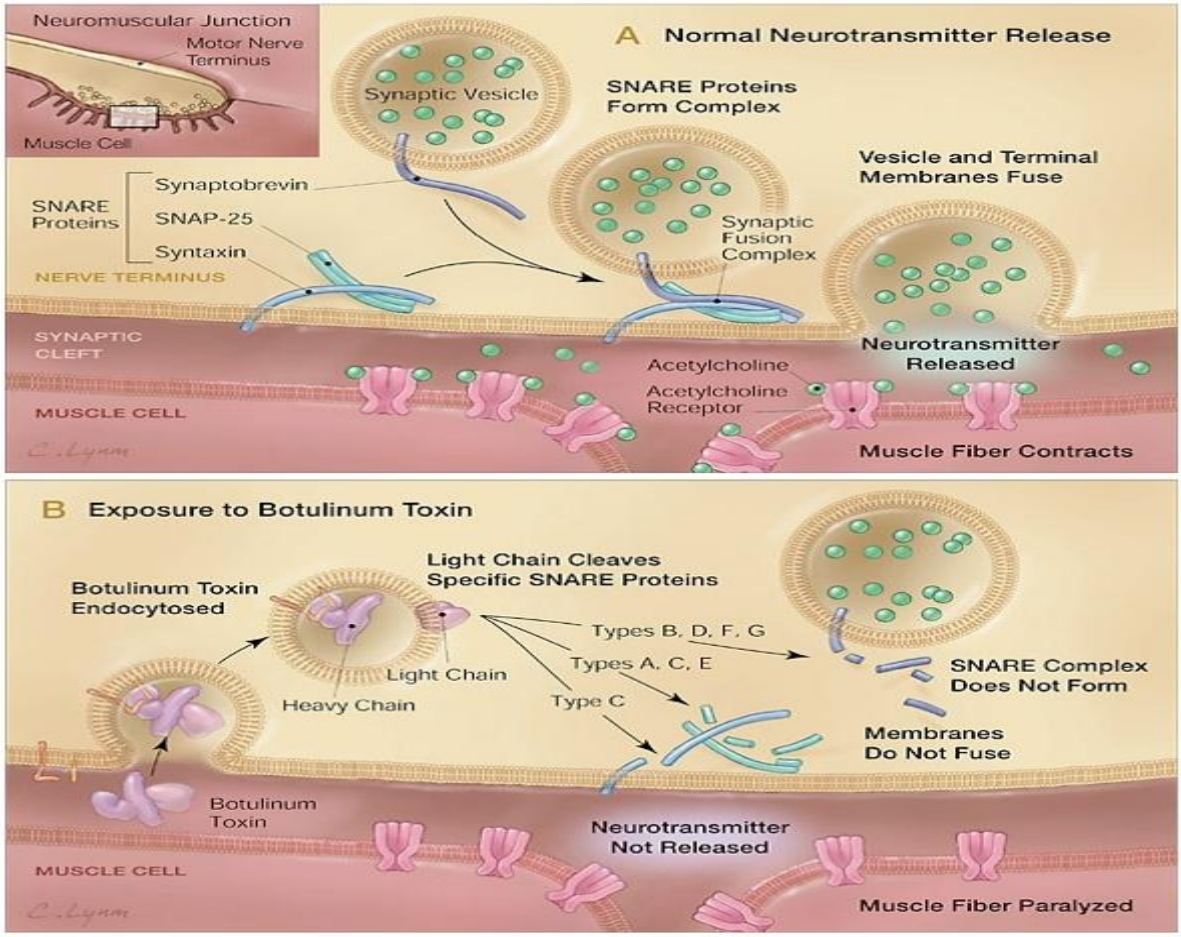
Botulinum toxin structure



BOTULIUM TOXIN

Botulinum toxin consists of a 2 chain polypeptide linked by a disulfide bond as demonstrated in this picture. The larger polypeptide, called the heavy chain, is identical for all 7 toxin types. The smaller polypeptide, called the light chain, varies for each toxin subtype majorly interferes with release of acetyl choline. It produces seven distinct neurotoxins that are neuromuscular paralyzing agents. The toxins are called botulinum toxin type A, B, C1, D, E, F, and G. When either botulinum toxin is injected into muscle the heavy chain binds to the nerve ending.

MECHANISM OF ACTION



Phase I – Nerve-Muscle Communication is blocked

BOTOX® blocks the transmission of overactive nerve impulses to the targeted muscle by selectively preventing the release of the neurotransmitter acetylcholine (ACh) at the neuromuscular junction, temporarily preventing muscle contraction this is primarily a local effect. In cervical dystonia, BOTOX® may also prevent the release of pain-stimulating neuropeptides in peripheral nerves.

A) Binding:

The heavy chain portion of the active ingredient in BOTOX® (onabotulinumtoxinA) neurotoxin binds to the cell membrane of the motor nerve via an unidentified high-affinity "acceptor" molecule. This high-affinity binding action allows for efficient uptake of BOTOX® by the motor nerve and facilitates selective, targeted treatment at the injection site.

B) Internalizing:

After binding, the BOTOX® protein molecule passes through the cell membrane of the motor nerve and into its cytoplasm via a process called endocytosis. It is here that the enzymatic component (light chain) of the BOTOX® protein molecule is activated.

C) Blocking:

Inside the motor nerve, the light chain of the BOTOX® (onabotulinumtoxinA) protein molecule cleaves apart a protein (called SNAP25) that enables vesicles which store the neurotransmitter acetylcholine to attach to the cell membrane. Cleaving SNAP25 prevents these vesicles from fusing with the membrane and prevents the release of acetylcholine into the neuromuscular junction (the space between the motor nerve and the muscle). Thus, nerve impulses that control muscle contractions are blocked decreasing muscle activity. Cleaving SNAP25 also blocks release of neuropeptides involved in the transmission of painful sensations including substance P, glutamate and calcitonin gene-related peptide, or CGRP), theoretically reducing (pain sensitization of peripheral nerves. This may be how BOTOX® reduces the neck pain associated with cervical dystonia.

Phase II – Nerve-Muscle Communication is restored

The effect of BOTOX® is generally temporary. Previous nerve impulse activity and associated muscle contractions resume over the course of a few to several months, depending on the individual patient and the indication for which they are being treated.

A) Nerve Sprouting:

New nerve endings sprout and connect to the muscle after the original nerve ending is blocked, renewing the ability of the nerve to cause muscle contractions.

B) Original Nerve Connection Re-established:

Eventually, the new nerve sprouts retract and the original nerve ending regains its function, suggesting that treatment with BOTOX® (onabotulinumtoxinA) neurotoxin does not permanently alter the neuromuscular junction.

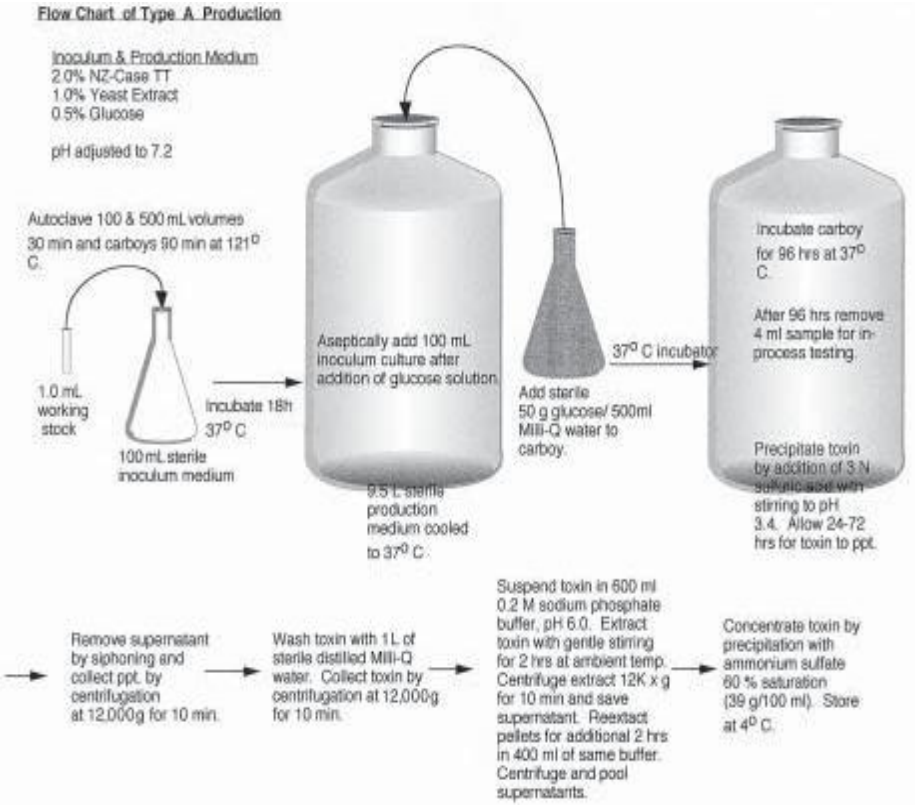
Preparation

Cultures of *Clostridium botulinum*: naturally-occurring cases of botulism arise from food spoiling in the absence of oxygen. The commonest source historically has been spoiled meat, notably sausage (the species name derives from the Latin *botulus* meaning "sausage") and poultry (especially duck), and improperly sterilized canned foods. Botulinum toxin (abbreviated either as BTX or BoNT) is produced by *Clostridium botulinum*, a gram-

positive anaerobic bacterium. BoNT-A is prepared by laboratory fermentation of *C botulinum* cultures. Crude botulinum toxin is a protein with a molecular weight of about 190,000 Dalton.

The fermentation medium consists of 2.0% casein hydrolylsate and 1.5% yeast extract plus an appropriate concentration of glucose. The maximum toxin concentration was attained within 48 h under the following fermentation conditions: an initial glucose concentration of 0.5 or 1.0%, a temperature of 35 degrees C, nitrogen overlay at a rate of 5 liters/min, and an agitation rate of 50 rpm.

Fig. 2. Schematic for toxin production.



Purification of Botulinum Type A Neurotoxin

Recovery of neurotoxin from the complex is typically 10–13%. Because crystalline toxin is one of the more stable forms, only the required amount of neurotoxin (which is more labile than the crystals) is usually purified as needed.

1. The required amount of crystals is dissolve in 0.02 *M* sodium phosphate buffer, pH 7.9, for 1–2 h and dialyze for 18 h (10× with three buffer changes) to remove ammonium sulfate.

2. Pack and wash DEAE-Sephadex A-50 in a column sufficient in size to bind all the complex

3. Centrifuge (12,000*g* for 10 min at 10°C) sample and load onto column. Wash column with at least 50 mL of starting buffer or until A280 is less than 0.01.

4. Set flow rate at 30 mL/h and begin collecting fractions.

5. Neurotoxin is eluted with a linear gradient of sodium chloride made of running buffer plus running buffer containing 0.3 *M* sodium chloride. Toxin elutes at ca. 0.15 *M* chloride ion. The volume of eluant is dependent on column size but is typically 4–5× the volume of the gel volume.

6. Read A280 of fractions and pool first peak eluted in the sodium chloride gradient

Storage

Purified neurotoxin is not as stable as the toxin complex. Precipitated neurotoxin will show degradation within a month when analyzed by SDSPAGE.

Diluted toxin solutions can be stored for years at 20°C by the addition of stabilizing protein excipients and adjustment of pH and ionic strength. Solutions of toxin in 50% glycerol stored at –20 to –70°C are stable for years.

Unopened vials of BOTOX® should be stored in a refrigerator (2° to 8°C) for up to 24 months. Do not use after the expiration date on the vial. Administer BOTOX® within 4 hours of reconstitution; during this period reconstituted BOTOX® should be stored in a refrigerator (2° to 8°C). Reconstituted BOTOX® should be clear, colorless and free of particulate matter.

Precipitation

The toxin solution is made to 60% saturation with ammonium sulfate and store at 4°C.Storage of Standard Toxin Solutions

1. Dilute filter-sterilized toxin (0.2 μm filter) to desired concentration in sterile 0.05*M* sodium acetate buffer + 3% bovine serum albumin + 2% gelatin, pH 4.2.

2. Aliquot toxin solution to desired volumes and store at 4–25°C. Do not freeze these samples to avoid toxin inactivation.

APPLICATIONS OF BOTOX

The application may be classified as cosmetical application and Therapeutic application.

Cosmetic use

The lines that appear between your brows actually result from muscle movement and the passage of time.

Two muscles are largely responsible for frown lines between the brows. When those muscles contract, they draw the brows together. As skin becomes less elastic over time, repeated frowning can result in those moderate to severe lines between your brows.

BOTOX® Cosmetic works by blocking nerve impulses, which reduces the movement of those muscles. With less movement, the skin surface gradually smooth's out, and the moderate to severe frown lines between your brows begin to fade.

It is a nonsurgical, physician-administered treatment that can temporarily reduce moderate to severe frown lines between the brows in patients 18 to 65 years of age.

During treatment, very low doses of BOTOX® Cosmetic are administered via a few tiny injections directly into the muscles that cause those stubborn lines between the brows.

The results is expect within days after your treatment. The appearance of the area between your brows may continue to improve for up to a week. The effects of Botox become apparent 2-5 days after injection and generally last for 4-6 months.

By day 30 in a patient clinical study, 89% of patients judged the severity of their frown lines between their brows as moderate or better. Visible results can last up to four months.

Botulinum Toxin (BOTOX) Treatment for Squint

It can also be used to treat some kinds of squint (strabismus). In a squint where one eye turns out (an exotropia), it can be given to the muscle pulling the eye outwards (the lateral rectus) and this may allow the eye to come straighter. Similarly, in a squint where an eye turns in (esotropia), it can be given to the muscle pulling the eye in (the medial rectus) and when this muscle weakens after the treatment, it may allow the eyes to become straighter. It is only very rarely used for squints where one eye points up or down.

Botox® has been used to treat certain types of squints in the UK since the 1980s.

The Botox® is given to the muscle using a very fine needle which is slid under the white skin of the eyeball (the conjunctiva).

BOTOX for Treatment of Migraine Headaches

Migraine is one of the common causes of recurrent headache. According to World Federation of Neurology,

"Migraine is a familial disorder characterized by recurrent attacks of headache widely variable in intensity, frequency and duration. Attacks are commonly unilateral and are usually associated with anorexia, nausea and vomiting". Numerous agents, encompassing various classes, are used for treatment of migraine. Viewed collectively, they offer limited effectiveness and poor tolerability due to adverse events.

BOTOX weakens or paralyses muscles (depending on dose) by preventing the release of acetylcholine, a signal that the nerves need to cause muscle contraction. In preclinical models, BOTOX has been shown to inhibit the release of nociceptive mediators, such as glutamate, substance P, and calcitonin gene-related peptide, from nociceptive fibers'.

Several open-label and small controlled trials suggest that BOTOX may be effective in the prophylactic treatment of headache. The purpose of study was to evaluate the correlation between pericranial BOTOX administration and alleviation of migraine headache symptoms.

Chronic migraine

OnabotulinumtoxinA (trade name Botox) received FDA approval for treatment of chronic migraines on October 15, 2010. The toxin is injected into the head and neck to treat these chronic headaches. Approval followed evidence presented to the agency from two studies funded by Allergan, Inc. showing a very slight improvement in incidence of chronic migraines for migraine sufferers undergoing the Botox treatment. BOTOX was found to be a safe and effective therapy for both acute and prophylactic treatment of migraine headaches.

How Botox Is Used to Treat Migraines

Botox to treat chronic migraines is given at intervals of about 12 weeks as multiple injections around the head and neck to try to dull future headache symptoms, the FDA says in a statement.

Allergan Inc., the maker of Botox, says in a statement that the FDA's approval applies to people with chronic migraine, which it defines as a "distinct and severe neurological disorder characterized by patients who have a history of migraine and suffer from headaches on 15 or more days per month with headaches lasting four hours a day or longer."

When treating chronic migraine, 31 Botox injections are given into seven specific head and neck sites.

Botox, when injected at labeled doses in recommended areas, is expected to produce results lasting up to three months depending on the individual patient.

Recommended total dose 155 units, as0.1 ml (5 units) injections per each side divided across 7 head/neck muscles. Intramuscularly 0.5 inch needle.

Botox as a prophylactic treatment option for patients with chronic migraine have been assessed in the Phase III Research Evaluating Migraine Prophylaxis Therapy (PREEMPT) programme, the largest comprehensive clinical trial programme in chronic migraine to date[21]. OnabotulinumtoxinA resulted in significant improvements compared with placebo in multiple headache symptom measures, and significantly reduced headache-related disability and improved functioning, vitality, and overall health-related quality of life. Repeat treatments with onabotulinumtoxinA were safe and well tolerated.[26]

Botox as treatment for SWEATING

Indication BOTOX® (onbotulinumtoxinA) is injected into the skin to treat the symptoms of severe underarm sweating (severe primary axillary hyperhidrosis) when medicines used on the skin (topical) do not work well enough in people 18 years and older. It is not known whether BOTOX® is safe or effective for severe sweating anywhere other than your armpits. There has not been a confirmed serious case of spread of toxin effect away from the injection site when BOTOX® has been used at the recommended dose to treat severe underarm sweating.

While treating patients with hemi facial spasm at Southend Hospital in England in 1993, Khalaf Bushara and David Park were the first to show botulinum toxin injections inhibit sweating. This was the first demonstration of nonmuscular use of BTX-A. Bushara further showed the efficacy of botulinum toxin in treating hyperhidrosis (excessive sweating). BTX-A was later approved for the treatment of excessive underarm sweating. This is technically known as severe primary axillary hyperhidrosis - excessive underarm sweating with an unknown cause which cannot be managed by topical agents.

Botox has ability to temporarily block the secretion of acetylcholine that is responsible for turning on the body's sweat gland. By blocking or interrupting Botox turns off the sweating at area where it is injected.

Botulinum toxin does not cure hyperhidrosis; the symptoms will go away gradually usually within a week and return gradually. Follow up injections are required to maintain dryness. These repeat injections may be necessary varying from seven to seventeen months.

Cervical dystonia

Cervical dystonia is also known as spasmodic torticollis. Cervical dystonia is a focal dystonia that affects the neck and sometimes the shoulders. Symptoms include involuntary contracting of the neck muscles, causing abnormal movements and awkward posture of the head and neck. The movements may be sustained (tonic), jerky (clonic), or a combination. Cervical dystonia may result in considerable pain and discomfort.

Botulinum toxin therapy for cervical dystonia

The most likely primary mechanism of action is the reduction of muscle force during the dystonic muscle contractions. Injections of the drug into extensor digitorum brevis reduce the evoked motor response of the muscle and its mean rectified voltage during maximal voluntary effort by 60%, an effect that should improve symptoms. Botulinum toxin interrupts the vicious cycle of untreated cervical dystonia: isometric muscle contraction and work resulting in muscle hypertrophy resulting in greater muscle contraction and work. Botulinum toxin may prevent the release of substances from nerve endings. Because it is known that pain fibers release a variety of chemical mediators, it is possible that Botulinum toxin may be able to reduce the discomfort of cervical dystonia through a nonspecific antisecretory effect as well. Because of its structure, botulinum toxin is unable to affect any significant entry into the central nervous system.

No more than 50 Units per site should be administered. An appropriately sized needle (e.g., 25-30 gauge) may be used for superficial muscles, and a longer 22 gauge needle may be used for deeper musculature.

Future aspect

Use in Parkinson's, cerebral palsy, chronic pain.

Researchers at the Medical Research Council's Laboratory of Molecular Biology has open up ways to develop new forms of Clostridium botulinum neurotoxin, commonly known as Botox, which may be used as long-term painkillers.

Davletov's and his team has synthetically break down Botox molecules into two separate building blocks, which is able to produce them separately and safely, and then they "clip" them back together again, which results in formation of Botox-like molecule, but would not have unwanted toxic effects.

May be used in weight loss

Researchers investigated whether it might aid patients with major depressive disorder who had not responded to antidepressant medications. Enhancement of cognition, mood and pro-social behavior and blunting of unwanted memories.

Side effect

The effect of botulinum toxin may affect areas of the body away from the injection site and cause symptoms of a serious condition called botulism. The symptoms of botulism include:

- Loss of strength and muscle weakness all over the body
- double vision
- Blurred vision and drooping eyelids
- Hoarseness or change or loss of voice (dysphonia)
- Trouble saying words clearly (dysarthria)

- Loss of bladder control
- Trouble breathing
- Trouble swallowing

These symptoms can happen hours, days, to weeks after you receive an injection of **BOTOX.**

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