Botulinum toxin

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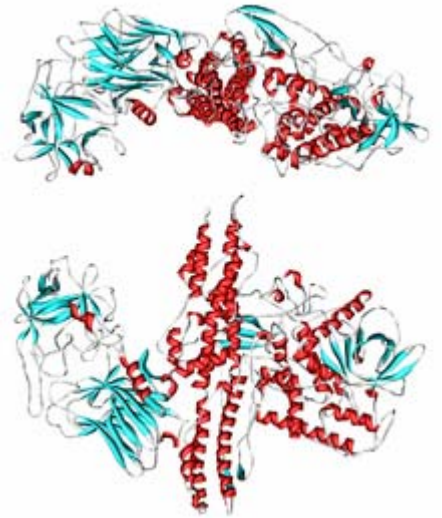


Botulinum toxin is a protein and neurotoxin produced by the bacterium *Clostridium botulinum*. Botulinum toxin can cause botulism, a serious and life-threatening illness in humans and animals.[1][2] When introduced intravenously in monkeys, type A (Botox Cosmetic) of the toxin exhibits an LD_{50} of 40–56 ng, type C1 around 32 ng, type D 3200 ng, and type E 88 ng[*citation needed*]; these are some of the most potent neurotoxins known.[3] Popularly known by one of its trade names, **Botox**, it is used for various cosmetic and medical procedures. Botulinum can be absorbed from eyes, mucous membranes, respiratory tract or non-intact skin.[4]

History

Justinus Kerner described botulinum toxin as a "sausage poison" and "fatty poison",[5] because the bacterium that produces the toxin often caused poisoning by growing in improperly handled or prepared meat products. It was Kerner, a physician, who first conceived a possible therapeutic use of botulinum toxin and coined the name botulism (from Latin *botulus* meaning "sausage"). In 1897, Emile van Ermengem found the producer of the botulin toxin was a bacterium, which he named *Clostridium botulinum.* [6] In 1928, P. Tessmer Snipe and Hermann Sommer for the first time purified the toxin.[7] In 1949, Arnold Burgen's group discovered, through an elegant experiment, that botulinum toxin blocks neuromuscular transmission through decreased acetylcholine release.[8]

Therapeutic research

In the late 1960s Alan Scott, M.D., a San Francisco ophthalmologist, and Edward Schantz were the first to work on a standardized botulinum toxin preparation for therapeutic purposes.[9] By 1973, Scott (now at 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 "crossed eyes", a condition in which the eyes are not properly aligned with each other, and "uncontrollable blinking" (blepharospasm). In 1993, Pasricha and colleagues showed that botulinum toxin could be used for the treatment of achalasia, a spasm of the lower esophageal sphincter.[10] In 1994 Bushara showed that botulinum toxin injections inhibit sweating.[11] This was the first demonstration of non-muscular use of BTX-A in humans.

Blepharospasm and strabismus

In the early 1980s, university-based ophthalmologists in the U.S.A. and Canada further refined the use of botulinum toxin as a therapeutic agent. By 1985, a scientific protocol of injection sites and dosage had been empirically determined for treatment of blepharospasm and strabismus.[12] Side effects were deemed to be rare, mild and treatable.[13] The beneficial effects of the injection lasted only 4–6 months. Thus, blepharospasm patients required re-injection two or three times a year.

In 1986, Scott's micro-manufacturer and distributor of Botox was no longer able to supply the drug because of an inability to obtain product liability insurance. Patients became desperate as supplies of Botox were gradually consumed, forcing him to abandon patients who would have been due for their next injection. For a period of four months, American blepharospasm patients had to arrange to have their injections performed by participating doctors at Canadian eye centers until the liability issues could be resolved.[14]

The global botox market is forecast to reach $2.9 billion by 2018 at a CAGR of 14%. The entire global market for facial aesthetics is forecast to reach $4.7 billion in 2018, of which the US is expected to contribute over $2 billion.In December 1989, Botox, manufactured by Allergan, Inc., was approved by the U.S. Food and Drug Administration (FDA) for the treatment of strabismus, blepharospasm, and hemifacial spasm in patients over 12 years old.[15]

Cosmetic

The cosmetic effect of BTX-A on wrinkles was originally documented by a plastic surgeon from Sacramento, California, Dr. Richard Clark, and published in the journal *Plastic and Reconstructive Surgery* in 1989.[16] Canadian husband and wife ophthalmologist and dermatologist physicians Carruthers JD and Carruthers JA were the first to publish a study on BTX-A for the treatment of glabellar frown lines in 1992.[17] Similar effects had reportedly been observed by a number of independent groups (Brin, and the Columbia University group). After formal trials, on April 12, 2002, the FDA announced regulatory approval of botulinum toxin type A (Botox Cosmetic) to temporarily improve the appearance of moderate-to-severe frown lines between the eyebrows (glabellar lines).[18] Subsequently, cosmetic use of botulinum toxin type A has become widespread with many celebrities viewing it as less intrusive and/or artificial than other types of plastic surgery.[19] The results of cosmetic procedures vary but can last

up to eight months.[20] The U.S. Food and Drug Administration approved an alternative product-safety testing method in response to increasing public concern that LD50 testing was required for each batch sold in the market.[21] [22]

Muscle spasms

The acceptance of BTX-A use for the treatment of muscle pain disorders is growing, with approvals pending in many European countries. The efficacy of BTX-A in treating a variety of other medical conditions (including prostatic dysfunction, asthma, and others) is an area of continued study.

Upper motor neuron syndrome

BTX-A is now a common treatment for muscles affected by the upper motor neuron syndrome such as cerebral palsy, for muscles with an impaired ability to effectively lengthen. Muscles affected by the Upper Motor Neuron Syndrome frequently are limited by weakness, loss of reciprocal inhibition, decreased movement control and hypertonicity (including spasticity). Joint motion may be restricted by severe muscle imbalance related to the Upper Motor Neuron Syndrome, when some muscles are markedly hypertonic, and lack effective active lengthening. Injecting an overactive muscle to decrease its level of contraction can allow improved reciprocal motion, and so improved ability to move and exercise. In June 2009, its use for treating hypertonic muscles helped an Australian man to walk again. He had required a wheelchair for mobility following a stroke 20 years prior.[23]

Sweating

While treating patients with hemifacial spasm at Southend Hospital in England in 1993, Khalaf Bushara and David Park were the first to show that botulinum toxin injections inhibit sweating.[11] This was the first demonstration of non-muscular 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.

Cervical dystonia

Botulinum Toxin Type B (BTX-B) received FDA approval for treatment of cervical dystonia on December 21, 2000. Trade names for BTX-B are Myobloc in the United States, and Neurobloc in the European Union.[*citation needed*]

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.[24][25]

Since then, several randomized control trials have shown Botulinum Toxin Type A to improve headache symptoms and quailty of life when used prophylactically for patients with chronic migraine[26] who exhibit headache characteristics consistent with: pressure perceived from outside source, shorter total duration of chronic migraines (<30 years), "detoxification" of patients with co-existing chronic daily headache due to medication overuse, no current history of other preventative headache medications.[27]

Denaturing

Botulinum toxin is denatured at temperatures greater than 80 °C (176 °F).[28]

Sources

Botulism toxins are produced by these bacteria: *Clostridium botulinum*, *C. butyricum*, *C. baratii* and *C. argentinense*.[29] Foodborne botulism can be transmitted through food that has not been heated correctly prior to being canned or food that was not cooked correctly from a can. Most infant botulism cases cannot be prevented because the bacteria that cause this disease are in soil and dust. The bacteria can be found inside homes on floors, carpet, and countertops even after cleaning. Honey can contain the bacteria that cause infant botulism, so children less than twelve months old should not be fed honey. Honey is safe for persons one year of age and older.[30]

Chemical overview and lethality

There are seven serologically distinct toxin types, designated A through G. Additionally, six of the seven toxin types have subtypes with five subtypes of BoNT A having been described. The toxin is a two-chain polypeptide with a 100-kDa heavy chain joined by a disulfide bond to a 50-kDa light chain. This light chain is an enzyme (a protease) that attacks one of the fusion proteins (SNAP-25, syntaxin or synaptobrevin) at a neuromuscular junction, preventing vesicles from anchoring to the membrane to release acetylcholine. By inhibiting acetylcholine release, the toxin interferes with nerve impulses and causes flaccid (sagging) paralysis of muscles in botulism, as opposed to the spastic paralysis seen in tetanus.

There are over 70 structures of this toxin deposited in the PDB which reveal the tertiary structure of this class of toxins, as shown above.

It is the most acutely toxic substance known, with a median lethal dose of about 1 ng/kg when introduced intravenously[3] and 3 ng/kg when inhaled.[31] This means that, depending on the method of introduction into the body, a mere 90–270 nanograms of botulinum toxin could be enough to kill an average 90 kg (200 lb) person, and four

kilograms of the toxin, if evenly distributed, would be more than enough to kill the entire human population of the world.

The potency of botulinum toxin is well illustrated by this sentence from the 2011 Allergan annual report: "In 2011, we required less than a gram of raw neurotoxin to supply the world's requirements for 25 indications approved by Government agencies around the world."[32]

Food-borne botulism usually results from ingestion of food that has become contaminated with spores (such as a perforated can) in an anaerobic environment, allowing the spores to germinate and grow. The growing (vegetative) bacteria produce toxin. It is the ingestion of preformed toxin that causes botulism, not the ingestion of the spores or the vegetative bacteria. Infant and wound botulism both result from infection with spores, which subsequently germinate, resulting in production of toxin and the symptoms of botulism.

Proper refrigeration at temperatures below 3 °C (38 °F) retards the growth of *Clostridium botulinum*. The organism is also susceptible to high salt and low pH levels. The toxin itself is rapidly destroyed by heat, such as in thorough cooking.[33] On the other hand, the spores that produce the toxin are heat-tolerant and will survive boiling water for an extended period of time.[34] Fortunately, ingestion of the spores is safe, except in infants, as the highly oxygenated and highly acidic environment of an adult human digestive system prevents the spores from growing and producing the botulinum toxin.[*citation needed*]

Botulinum toxin has been recognized and feared as a potential bioterror weapon.[35]

Medical and cosmetic uses

Although botulinum toxin is a lethal, naturally occurring substance, it can be used as an effective and powerful medication.[36] Researchers discovered in the 1950s that injecting overactive muscles with minute quantities of botulinum toxin type-A would result in decreased muscle activity by blocking the release of acetylcholine from the neuron by preventing the vesicle where the acetylcholine is stored from binding to the membrane where the neurotransmitter can be released. This will effectively weaken the muscle for a period of three to four months.[37]

In cosmetic applications, a Botox injection, consisting of a small dose of botulinum toxin, can be used to prevent development of wrinkles by paralyzing facial muscles.[38] As of 2007, it is the most common cosmetic operation, with 4.6 million procedures in the United States, according to the American Society of Plastic Surgeons. Qualifications for Botox injectors vary by county, state and country. Botox cosmetic providers include dermatologists, plastic surgeons, aesthetic spa physicians, dentists, nurse practitioners, nurses and physician assistants. The wrinkle-preventing effect of Botox normally lasts for approximately three to four months,[38][39] but can last up to six months.[39]

In addition to its cosmetic applications, Botox is currently used in the treatment of spasms and dystonias, by weakening involved muscles, for the 60–70 day effective period of the drug.[40] The main conditions treated with botulinum toxin are:

- Cervical dystonia (spasmodic torticollis) (a neuromuscular disorder involving the head and neck)[41]
- Blepharospasm (excessive blinking)[42]
- Severe primary axillary hyperhidrosis (excessive sweating)[43]
- Strabismus (Squints)
- Achalasia (failure of the lower oesophageal sphincter to relax)
- Local intradermal injection of BTX-A is helpful in chronic focal neuropathies. The analgesic effects are not dependent on changes in muscle tone.[44]
- Migraine and other headache disorders, although the evidence is conflicting in this indication[45]
- Excessive sweating is a condition for the treatment of which FDA has approved the use of Botox.[46]

Other uses of botulinum toxin type A that are widely known but not specifically approved by the U.S. Food and Drug Administration (off-label uses) include treatment of:

- Idiopathic and neurogenic detrusor overactivity,[47]
- Pediatric incontinence,[48] incontinence due to overactive bladder,[49] and incontinence due to neurogenic bladder.[50]
- Anal fissure[51]
- vaginismus To reduce the spasm of the vaginal muscles.[52]
- Movement disorders associated with injury or disease of the central nervous system including trauma, stroke, multiple sclerosis, Parkinson's disease, or cerebral palsy
- Focal dystonias affecting the limbs, face, jaw, or vocal cords
- TMJ pain disorders
- Diabetic neuropathy
- Wound healing
- Excessive salivation
- Vocal cord dysfunction (VCD) including spasmodic dysphonia and tremor
- Reduction of the Masseter muscle for decreasing the apparent size of the lower jaw
- Painful bladder syndrome,[47]
- Detrusor sphincter dyssynergia and benign prostatic hyperplasia,[47]

Treatment and prevention of chronic headache[53] and chronic musculoskeletal pain[54] are emerging uses for botulinum toxin type A. In addition, there is evidence that Botox may aid in weight loss by increasing the gastric emptying time.[55]

Links to deaths

In September 2005, a paper published in the *Journal of American Academy of Dermatology* reported from the FDA saying that use of Botox has resulted in 28 deaths between 1989 and 2003, though none were attributed to cosmetic use.[56]

On February 8, 2008, the FDA announced that Botox has "been linked in some cases to adverse reactions, including respiratory failure and death, following treatment of a variety of conditions using a wide range of doses," due to its ability to spread to areas distant from the site of the injection.[57] In April 2009, the FDA updated its mandatory boxed warning cautioning that the effects of the botulinum toxin may spread from the area of injection to other areas of the body, causing symptoms similar to those of botulism.[58]

In January 2009, the Canadian government warned that botox can have the adverse effect of spreading to other parts of the body, which could cause muscle weakness, swallowing difficulties, pneumonia, speech disorders and breathing problems.[59][60]

Several cases of death have been linked to the use of other chemicals as substitutes for Botox,[61] one of the causes of death listed on the Spike TV show, *1000 Ways to Die*.

Side effects

Side effects, which are generally minor and temporary,[38] can be predicted from the mode of action (muscle paralysis) and chemical structure (protein) of the molecule, resulting broadly speaking in two major areas of side effects: paralysis of the wrong muscle group and allergic reaction. Bruising at the site of injection is a side effect not of the toxin, but rather the mode of administration. In cosmetic use, this can result in inappropriate facial expression such as drooping eyelid,[38] double vision,[38] uneven smile, or loss of the ability to close eyes. This will wear off in around six weeks. Bruising is prevented by the clinician applying pressure to the injection site, but may still occur, and will last around 7–11 days. When injecting the masseter muscle of the jaw, loss of muscle function will result in a loss or reduction of power to chew solid foods.[56] All cosmetic treatments are of limited duration, and can be as short a period as six weeks, but usually the effective period lasts from two to three months. At the extremely low doses used medicinally, botulinum toxin has a very low degree of human and animal toxicity.

Other adverse events from cosmetic use include headaches, dysphagia, flu-like syndromes, blurred vision, dry mouth, fatigue, allergic reactions and swelling or redness at the injection site.[56][62]

There has been a petition by Public Citizen to the FDA requesting regulatory action concerning the possible spread of botulinum toxin (Botox, Myobloc) from the site of injection to other parts of the body.[63]

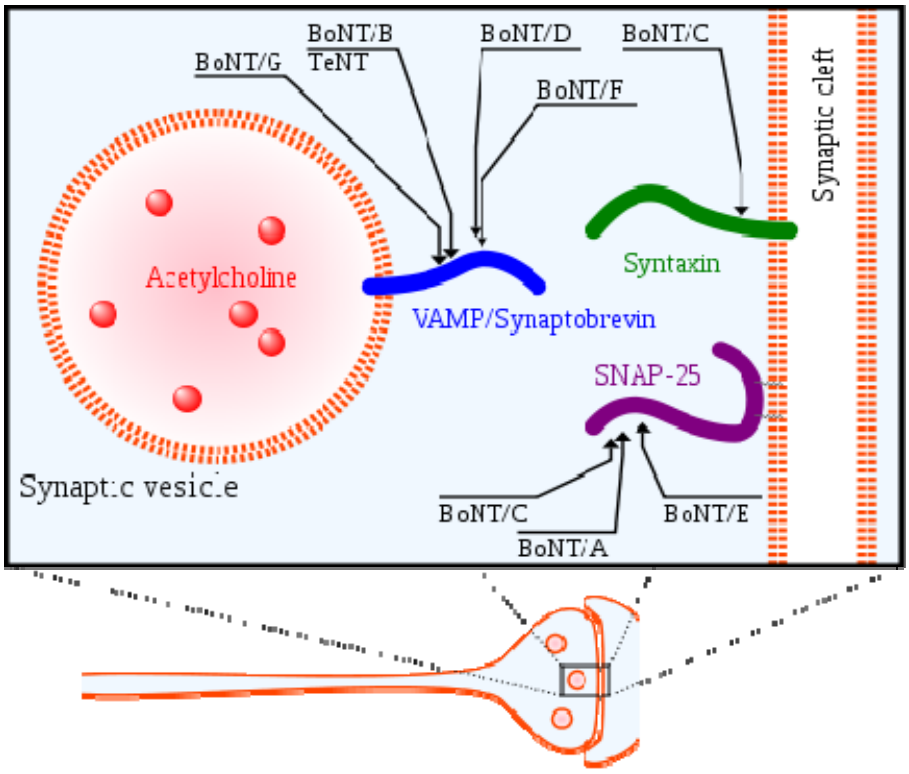
Individuals who are pregnant, have egg allergies or a neuromuscular disorder are advised to avoid Botox.[38]

As published in Forbes and originally published in the journal Social Psychology and Personality Science, Botox takes away or dampens the emotional feelings in a particular situation. That may be due to less interaction between facial muscle movement and brain. According to David Neal, a psychology professor at the University of Southern California, “if muscular signals from the face to the brain are dampened, you’re less able to read emotions.”[64]

One way botox might affect emotional feelings is by dampening the relay of signals from the face to the amygdala and brainstem centers for autonomic arousal.[65]

The mental effects of botox may extend beyond emotional feelings to the ability to understand language about emotions. An experimental study suggests that cosmetic use of botulinum toxin for treatment of glabellar lines affects human cognition. As reported in the L.A. Times,[66] Havas and colleagues (Havas, Glenberg, Gutowski, Lucarelli, & Davidson, 2010 [67]) asked subjects to read emotional (angry, sad, happy) sentences before and two weeks after botox injections in the corrugator supercilii muscle used in frowning. Reading times for angry and sad sentences were longer after botox injection than before injection, while reading times for happy sentences were unchanged. This finding suggests that facial muscle paralysis has a selective effect in human cognition, and shows that botox hinders the ability to understand language. According to the lead researcher in this study, "botox causes a kind of mild, temporary, cognitive blindness to information in the world, social information about the emotions of other people."

Biochemical mechanism of toxicity



Target molecules of botulinum (BoNT) and tetanus (TeNT) toxins inside the axon terminal.[68]

The heavy chain of the toxin is particularly important for targeting the toxin to specific types of axon terminals. The toxin must get inside the axon terminals in order to cause paralysis. Following the attachment of the toxin heavy chain to proteins on the surface of axon terminals, the toxin can be taken into neurons by endocytosis. The light chain is able to cleave endocytotic vesicles and reach the cytoplasm. The light chain of the toxin has protease activity. The type A toxin proteolytically degrades the SNAP-25 protein, a type of SNARE protein. The SNAP-25 protein is required for vesicle fusion that releases neurotransmitters from the axon endings (in particular Acetylcholine).[69] Botulinum toxin specifically cleaves these SNAREs, and so prevents neuro-secretory vesicles from docking/fusing with the nerve synapse plasma membrane and releasing their neurotransmitters.

Though it affects the nervous system, common nerve agent treatments (namely the injection of atropine and pralidoxime) will *increase* mortality by enhancing botulin toxin's mechanism of toxicity[*citation needed*]. Attacks involving botulinum toxin are distinguishable from those involving nerve agent in that NBC detection equipment (such as M-8 paper or the ICAM) will not indicate a "positive" when a sample of the agent is tested. Furthermore, botulism symptoms develop relatively slowly, over several days compared to nerve agent effects, which can be instantaneous.

Treatment of botulinum poisoning

If the symptoms of botulism are diagnosed early, an equine antitoxin, use of enemas, and extracorporeal removal of the gut contents can be used to treat the food-borne illness. Wound infections can be treated surgically. Information regarding methods of safe canning, and public education about the disease are methods of prevention. Tests to detect botulism include a brain scan, nerve conduction test, and a tensilon test for myasthenia gravis in order to differentiate botulism from other diseases that manifest in the same way. Electromyography (EMG) can be utilized to differentiate myasthenia gravis and Guillain-Barré syndrome, diseases that botulism often mimics. Toxicity testing of serum specimens, wound tissue cultures, and toxicity testing, and stool specimen cultures are the best methods for identifying botulism. Laboratory tests of the patient's serum or stool, which are then injected into mice are also indicative of botulism.[70] But the faster way to detect botulinum toxin in people is using the mass spectrometry technology because it reduces testing time to three or four hours and at the same time it can identify the seven types of the toxin.[71]

The case fatality rate for botulinum poisoning between 1950 and 1996 was 15.5%, down from approximately 60% over the previous 50 years.[72] Death is generally secondary to respiratory failure due to paralysis of the respiratory muscles, so treatment consists of antitoxin administration and artificial ventilation until the neurotoxins are excreted or metabolised. If initiated on time these treatments are quite effective, although antisera can not affect BoNT polypeptides that have already entered cells.[73] Occasionally, functional recovery may take several weeks to months or more.

There are two primary Botulinum Antitoxins available for treatment of botulism.

- Trivalent (A,B,E) Botulinum Antitoxin is derived from equine sources utilizing whole antibodies (Fab & Fc portions). This antitoxin is available from the local health department via the CDC in the USA.

- The second antitoxin is Heptavalent (A,B,C,D,E,F,G) Botulinum Antitoxin, which is derived from "despeciated" equine IgG antibodies, which have had the Fc portion cleaved off leaving the F(ab')2 portions. This is a less immunogenic antitoxin that is effective against all known strains of botulism where not contraindicated. This is available from the United States Army. On June 1, 2006 the United States Department of Health and Human Services awarded a $363 million contract with Cangene Corporation for 200,000 doses of Heptavalent Botulinum Antitoxin over five years for delivery into the Strategic National Stockpile beginning in 2007.[74]

Manufacturers

In the United States, Botox is manufactured by Allergan, Inc. for both therapeutic and cosmetic use (100Unit). In the United States, Xeomin (manufactured in Germany by Merz) is available for both therapeutic and cosmetic use.

Dysport, a therapeutic formulation of the type A toxin developed and manufactured in Ireland, is licensed for the treatment of focal dystonias and certain cosmetic uses in the US and worldwide in 100, 300 and 500 Units. Lanzhou Institute (China) manufactures a BTX-A product, producing 50U and 100U type A toxin.[75] Neuronox, a BTX-A product, was introduced by Medy-Tox Inc. of South Korea, in 2009.[76] In America, Neuronox is also known as Siax. Merz manufactures the toxin and sells it under the trade name Xeomin. Solstice Neurosciences, LLC, a wholly owned subsidiary of US WorldMeds, LLC sells their product under the names Myobloc or Neurobloc, although it contains Botulinum Toxin Type B, not the common Type A found in Botox.