Review Article

Botulinum Toxin: A Review on Its Transition from a Lethal Poison to a Magical Therapeutic Drug

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ABSTRACT

Botulinum toxin is a neurotoxin produced by *Clostridium botulinum* which causes 'Botulism'. Historically it has been associated with food intoxication and was used as a lethal weapon since ancient times. Identification of *C. botulinum* in the late 19th century by Van Ermengem led to clearer understanding of the action of this toxin and characterization of the microbe's culture requirements. It has come a long way since and is now used therapeutically for a variety of clinical conditions like strabismus, blepharospasm, axillary hyperhidrosis etc. by employing a wide variety of available preparations (Botox®, Dysport® etc). This review highlights the clinical pharmacology of botulinum toxin along with the side effect profile and outlines the shift from using it as a lethal weapon to a promising therapeutic tool.

Keywords: Botulinum toxin, Sausage Poisoning, Botulism, Botox, Clostridium botulinum, Strabismus.

INTRODUCTION

Botulinum toxin is a protein produced by Clostridium botulinum, a gram positive spore forming bacteria.[1] It is a preformed heat labile neurotoxin which inhibits acetylcholine release at the neuromuscular junction and causes a clinical condition known as botulism.[2] Unintentional intoxication with botulinum toxin is very rare. An important source of intoxication is canned foods, either from consumption of food that has not been heated properly before canning or from food that was not properly cooked from the can before consuming. Three important types of botulism are identified, namely food botulism, infant botulism and wound botulism.[3] Honey is the main source of infant botulism. Wound botulism results from direct infection with spores. High fatality rate with botulinum toxin makes it a great concern for people and medical community. Though it is a most potent neuromuscular toxin in nature, lot of research on its clinical applications have emerged and now a variety of commercial preparations are available for treating several conditions. This review covers the important historical events about botulinum toxin as a deadly toxin and its development as an effective therapeutic drug, highlighting the pharmacological aspects and adverse effects.

HISTORIC MILESTONES

Pre therapeutic history

In ancient times, a lack of awareness existed about the causal relationship between eating spoiled food which resulted in a deadly paralytic disease, which was later discovered to be botulism. The history dates back to ancient Indian maharajas, where in formulas were given to them for killing their enemies. The recipe was a tasteless powder taken from blood sausages dried in anaerobic condition which were then added to food products and given to enemies in an invited party or gathering. Since the death is slow and happens few weeks after consumption, it was a widely preferred method. This gives a hint on an intended lethal application of botulinum toxin.[4]

In 1815, J.G. Steinbuch (1770-1818), a health officer in a village of Germany, gave an extensive report of several intoxicated patients who ate liver sausage and peas which took life of three patients. This is considered to be the first ever recorded observation of botulinum toxin. Further, Justinus Kerner 1786-1862), a physician and romantic poet from Germany who was working as a medical officer in a small village, reported a case of lethal food poisoning and published a first monogram in 1820 on ' Sausage Poisoning', titled 'New observation on the lethal poisoning that occurs so frequent in Wurttemberg owing to the consumption of smoked sausages'.[5] Kerner further summarized the case history of 76 patients and gave a complete clinical description which gave him a nickname 'Sausage Kerner' and the sausage poisoning was also known by that time as 'Kerner's disaese'.

In 1869, Muller described in his case report that eating fish causing food poisoning. Muller was the first one to coin the term 'Botulism' in his report. The word 'botulism' is derived from the Latin word '*botulus*' meaning 'sausage'. Hence, the name 'botulism' refers to the poisoning caused due to consumption of sausages and not to the sausage like shape of causative bacillus.[5] Identification of Clostridium botulinum in 1895-6 by the Belgian microbiologist 'Emile Pierre Marie Van Ermengem' was an important step which brought about the basic understanding of botulinum toxin. He isolated the bacterium from ham and the corpses of the victims and used it for animal experiments. Further, he

characterized its culture requirements and described toxin produced by the bacterium. At first, he named the organism what he isolated as '*Bacillus botulinus*'. Van Ermengem published his findings in a German microbiological journal in 1879. The English version of the same was published in 1979.[6] He was the first scientist who associated 'sausage poisoning' with newly discovered ananerobic microorganism.

Various case reports emerged and the understanding evolved that not only meat, fish but also canned beans could cause botulism. Georgian Burke, who worked in Stanford University, found that the causative organism had different strains and the toxins produced were also serologically distinct and he further designated them as type A and type B.[7] Bengston identified type C in the year 1922 in United states, Meyer and Gunnison identified type D and Bier characterized type E in Ukraine by 1936.[8] Type F and type G toxins were identified by Moller aand Scheible in Scandinavia by 1960. Gimenex and Cicarelli found the same in Argentina by 1970. [9,10]

Burgen and his colleagues in 1949 discovered in London that botulinum toxin blocked the release of acetylcholine at neuromuscular junction.[11] In 1976, spores of Clostridium botulinum was discovered in the intestines of babies and in contaminated wounds, which were given specific names such as infant botulism and wound botulism respectively.[12-14.]

Use as a war weapon

In 1920, Hermann Sommer and colleagues from university of California studied the basis of use of botulinum toxin and they were the first to isolate pure botulinum toxin type A as a stable acid precipitation[15] and which was further used in world war I as a biological weapon. [16] United States government focused more on the research in biological war weapons during world war II and botulinum toxin was one of their main focus. Carl Lamanna and James Duff in 1946 pioneered the technique of concentration and crystallization at Fort Detrick labouratory in Maryland. This method was further used by Edward J Schantz to produce the first batch of toxin and from here on the era of using botulinum toxin as a drug had began. [17]

BOTULINUM TOXIN AS A THERAPEUTIC AGENT

The clinical use of botulinum toxin emerged as a search for an effective alternative to surgical realignment of extra ocular muscle for strabismus. Alan B. Scott, an ophthalmologist from United States investigated different compounds by injecting them into the extra ocular muscle to weaken them which were unsuccessful. Further, Scott was introduced to Edward J Schantz, who produced purified botulinum toxin for experimental use. Both worked together and found that in animal experiments botulinum toxin produced long lasting, localized, dose dependant muscle weakness with no systemic toxicity and necrotizing side effects.[18]

In 1977, US Food and drug administration (FDA) on the basis of above results gave permission to test botulinum toxin in humans under an Investigational New Drug (IND) license for the treatment of strabismus. By 1980, Scott published his successful results of the test.[19] Further by 1980s, Scott and colleagues used botulinum toxin injection for treating strabismus, blepharospasm, hemifacial spasm, cervical dystonia and thigh adductor spasm.[20] Tsui and colleagues reported the successful use of botulinum toxin for cervical dystonia[21] and they conducted a double-blind, cross over study and found botulinum toxin was superior to placebo at reducing the symptoms of cervical dystonia[22]. Further, positive results of botulinum toxin in many conditions emerged, such as in spasmodic dystonia[23], oromandibular dystonia[24], dystonia of hand[25] and limb spasticity [26].

Finally by 1989, the US FDA licensed the manufacturing facility to produce botulinum toxin for therapeutic use. The FDA identified this as an orphan drug for treatment of strabismus, hemifacial spasm and blepharospasm.[27] The first batch of botulinum toxin type A manufactured by Scott and Schantz was named Oculinum®. Later by 1991, the manufacturing facility and license were turned over to Allergan and got a new name Botox®.

The clinical application for Botox® expanded from 1990s. Botox was approved by FDA for glabellar rhytides in 2002 and for primary axillary hyperhidrosis in 2002. At present there are a number of off label use for botulinum toxin such as in tremor, spasticity, over active bladder, anal fissure, achalasia, various pain disorders including headache.[28,29] The most recent and well known indication of botulinum toxin in public is use of botox for wrinkles and various cosmetic uses.

Currently available botulinum toxin drugs

- Botox® – Allergan Inc, Irvine, United States.
- Dysport® – Ipsen Ltd, Slough, Berks, United Kingdom.
- NeuroBloc® / Myobloc® – Solstic Neuroscience Inc., Malvern, United States.
- Xeomin® – Merz pharmaceuticals, Frankfut, Germany.
- Hegli® – Lanzhov Institute of biological products, China.
- CBTX-A® / Prosigne® – South America and Asian market.
- Neuronox® – Medy-Tox, Ochang, South Korea.

Botulinum toxin drugs and year of registrations

- Botox – 1989
- Dysport – 1991
- Hengli – 1993
- NeuroBloc / Myobloc – 2000
- Xeomin – 2005

BASIC PHARMACOLOGY OF BOTULINUM TOXIN

Botulinum toxins are exotoxins produced by Clostridium botulinum, an anaerobic gram positive sporulating organism. Different serotypes produce different toxins. Botulinum toxin is the most potent toxic substance in nature. There are seven serotypes of botulinum toxins, types A-G. Botulism is caused by serotypes A,B,E,F and potentially G.

Structurally the botulinum toxin is made up of two basic pillars, one being the botulinum toxin component and the other being the added excipients (Figure 1)

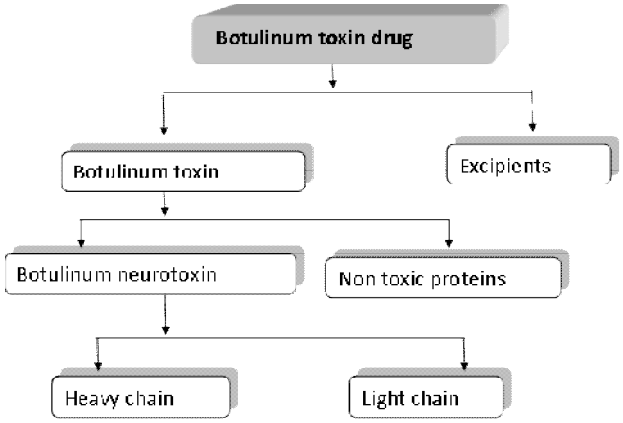

Figure 1: basic structure of botulinum toxin

The excipients used are for two main reasons, for stabilisation and pH calibration. Lactose, sucrose and serum albumin are used for stabilisation where as buffer system is used for pH calibration. Botulinum neurotoxin and non toxic proteins combine to form botulinum toxin component. Botulinum neurotoxin have a heavy amino acid chain and a light amino acid chain. Heavy chain has a molecular weight of 100 Kilodaltons (KDa) and light chain has a molecular weight of 50 KDa. Both this chains is connected by a disulfide bond. Different preparations have different total weights of these complexes, ranging from 300-900 Kilodaltons. The total complex weight may be a factor determining diffusion of the toxin from the site where it is injected. Botulinum neurotoxin serotype used is the main differentiating factor in therapeutic application. Till now only type A and type B are commercially available. Type C and type F have been tried in humans on an experimental basis only.

The biological activity of botulinum toxin drug preparation is measured with 'mouse units' (MU). One MU describes the amount of botulinum toxin which would kill 50 % of botulinum toxin intoxicated mouse population. Different brands have different MU. For example 1MU of Botox is approximately equals to 3 MU of Dysport.[30] Botox and Xeomin have identical MU.[31]

MECHANISM OF ACTION

Botulinum toxin gets bound selectively to glycoprotein on cholinergic nerve terminal when injected into a specific tissue. The light chain cleaves the different proteins of transport cascade such as SNARE (soluble N ethyl maleimide sensitive factor attached protein receptor) proteins which transports acetylcholine vesicle to synaptic cleft from intracellular space.[32] Different type of botulinum toxin targets specific type of SNARE protein for example Botulinum toxin A (BT-A), BT-B and BT-C binds to SNAP 25 where as BT-B, BT-D and BT-F targets vesicle associated membrane proteins (VAMP). [33-36]

'Sprouting' is the process by which the neurons form a new synapse by replacing the original ones which are blocked by botulinum toxin.[37,38] This is only a temporary recovery process and eventually the original synapse gets regenerated.[39] This explains the fact that the action of botulinum toxin is only transient since it interrupts the synaptic transmission only temperorarily.

The botulinum toxins action has a dose –effect correlation.[40] It has a direct action on striated muscle and it also acts upon the muscle spindle organ by reducing its centripetal information traffic.[41] After almost completely binding to the cholinergic nerve terminal, a very little quantity of botulinum toxin can enter blood circulation which can be detected by increased neuromuscular jitter in muscles far away from the site of injection.[42-44] This systemic spread differs with different botulinum toxin subtypes. BT-A gets detected systemically only in very high doses but BT-B has a very high systemic spread than BT-A and hence autonomic adverse effects occur even with a small dose.[45]

The main action of botulinum toxin is blockade of acetylcholine secretion, but it is also found from animal studies that it blocks transmitters involved in pain perception, transmission and processing such as substance P[46-48], glutamate[49,50], Calcitonin gene-related peptide[51] and nor adrenalin[52].

BOTULINUM TOXIN DRUGS PROPERTIES

Different preparations of botulinum toxin drug have different pharmacological properties and this is expressed in the below mentioned table1.[53-55]

ADVERSE EFFECT PROFILE

Over all, the botulinum toxin derived drugs have good adverse effect profiles.[56] The adverse effects can be divided into three major categories such as obligate, local and systemic.

- Obligate - inborn effects caused by therapeutic principle itself.
- Local - caused by diffusion of botulinum toxin from the target tissue into adjacent tissue.
- Systemic - adverse effects in tissues distant from the injection site and based upon botulinum toxin transport with in the blood circulation.

Table 1: Comparison of different botulinum toxin drugs

Properties		Preparations			
		Botox	Dysport	XEOMIN	NEUROBLOCK
1.	Formulation	Powder	Powder	Powder	Ready to use solution
2.	Storage condition	< 8°C	< 8°C	< 25°C	< 8°C
3.	Self life	36 months	24 months	36 months	24 months
4.	Botulinum toxin type	A	A	A	B
5.	SNARE target	SNAP25	SNAP25	SNAP25	VAMP
6.	Purification process	Precipitation and chromatography	Precipitation and chromatography	Precipitation and chromatography	Precipitation and chromatography
7.	pH value	7.4	7.4	7.4	5.6
8.	Stabilization	Vacuum drying	Freeze drying	Vacuum drying	pH reduction
9.	Excipients	Human serum albumin, NaCl	Human serum albumin, lactose	Human serum albumin, sucrose	Human serum albumin, disodium succinate, NaCl, H_2O, hydrochloric acid
10.	Biological activity	100 MU-A/vial	500 MU-I/ vial	100 MU-M/vial	1.0-2.5/10 k MU-E/ vial

MU-A: Mouse unit in the Allergan mouse lethality assay; MU-I: Mouse unit in the Ipsen mouse lethality assay; MU-M: Mouse unit in the Merz mouse lethality assay; MU-E: Mouse unit in the Solstice mouse lethality assay

The adverse effect occurs within a typical time window after application, starting after one week and lasting about one to two weeks. The duration and severity depends on the dose used. The estimated human median lethal dose (LD 50) of botulinum toxin is 1.3-2.1 ng/ kg when given in intravenous or intra muscular administration and 10-13 ng/kg when it is inhaled.[57]

Transport of botulinum toxin through the blood brain barrier is not possible due to its molecular size. Hence, central nervous system adverse effects are not much common and not much reported. In cosmetic use, it results in inappropriate facial expression, like double vision, uneven smile, drooping of eyelids, inability to close eyes, difficulty in chewing food, headache, dysphasia, flu like symptoms, blurring of vision, dry mouth, fatigue, allergic reactions, swelling and redness at the injection site.[58, 59]

The use of botulinum toxin during pregnancy and in lactating mother is usually contraindicated as a precautionary measure until enough evidence are collected regarding its safety is time tested in these special groups. It should be used with caution in patients with pre-existing pareses such as amyotrophic lateral sclerosis, myopathies and motor neuromuscular transmission such as myasthenia gravis and lambert-Eaton syndrome.[60] As a very rare event, botulinum toxin can trigger acute autoimmune brachial plexopathies.[61] Other effects include dampening of the emotional expressions, feelings and could also affect human cognition.[62]

Cote TR and coworkers reported to FDA that Botox injection resulted in 28 deaths (1989 to 2003).[58] With this evidence in 2008, the FDA announced 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.[63] In 2009, the FDA mandated a boxed warning stating that botulinum toxin may spread to other areas of body from the site of injection and it could cause symptoms similar to botulism.[64]

CLINICAL USE OF BOTULINUM TOXIN

At present botulinum toxin is used as a magical therapeutic agent for various disorders such as in cervical dystonia, hemifacial spasm, blepharospasm, ophthalmological applications, spasticity, hyperhidrosis and cosmetic uses.

Cervical Dystonia

Cervical dystonia is also known as spasmodic torticollis. It presents with abnormal head and neck posture due to tonic involuntary contraction in a set of cervical muscles. Botulinum toxin injection is the most effective treatment for cervical dystonia. Its use was first explained by Tsui and colleagues.[21,22] Further, many clinical studies have resulted in establishing its use in cervical dystonia.[65,66,67]

Hemifacial Spasm

Hemifacial spasm is an involuntary, irregular, clonic or tonic movement of the facial muscles innervated by the seventh cranial nerve on one side of the face. Mainly results from vascular compression of the facial nerve. Various clinical studies have proven the effect of botulinum toxin in effective management of hemifacial spasm.[68,69,70,71]

Blepharospasm

Blepharospasm is an adult onset focal dystonia. Patients presents with forceful eye closure, difficulty in opening and closing the eyes due to contraction of periocular muscles. Botulinum toxin is injected in the orbital or preseptal portion of the orbicularis oculi muscle for treating this condition. Various clinical studied have

proven the effect of botulinum toxin in blepharospasm. [72,73,74]

Ophthalmological Applications

Strabismus which is also called as heterotrophia or squint, occurs due to lack of coordination between extraocular muscles and results in improper alignment of eyes. Alan B. Scott was the first ophthalmologist who successfully used botulinum toxin for effective treatment of strabismus.[18-20] Further, botulinum toxin have been tried in various ophthalmological conditions such as in essential blepharospasm, hemifacial spasm . It has gained importance in Grave's disease to treat double vision[75]; to reduce oscillation and improve vision in nystagmus.

Spasticity

Spasticity is characterized by increased muscle tone, exaggerated tendon reflex, repetitive stretch reflex called as clonus, extension in great toe and flexion at ankle, knee and hip. It is mainly a upper motor neuron lesion due to stroke, multiple sclerosis or cerebral palcy. Various studies proved the effect of reduction of muscle tone after botulinum toxin therapy in limbs[76-78] and also improvement in the muscle functions [79,80].

Hyperhidrosis

Hyperhidrosis is excessive sweating or sweating more than physiological needs. It can be generalized, regional and localized/focal types. Botulinum toxin is mainly used for focal hyperhydrosis. When it is injected intradermally it blocks the release of acetylcholine from sympathetic nerve fiber that stimulate sweat glands and results in localized, long term and reversible abolishment of sweating [81]. Many clinical trials have proved the positive effect of botulinum toxin in primary axillary hyperhidrosis,[82-85] and in primary hyperhidrosis of other sites, such as scalp, forehead [86] and sole.

Cosmetic Uses

Peoples need to look younger and more beautiful grew more in the present generation. Exploration for a less invasive procedure grew more. Ultimately botulinum toxin gained popularity for variety of cosmetic procedures. The main target of botulinum toxin in cosmetic use is the muscles of facial expression. Botulinum toxin will temporarily weaken hyperfunctional muscles, thereby improving or eliminating the overlying skin creases.[87] it is used in treatment of glabellar lines, horizontal forehead lines[88], wrinkles correction, brow lift, nasal scrunch, rejuvenation of mouth, mandibular contouring and so on.

Other Uses

Number of clinical studies is being conducted for gaining scientific evidence for use of botulinum toxin in various disorders. Botulinum toxin is a potential agent for achalasia, dysphagia, obesity, annal fissure, spastic infantile cerebral palsy, over active bladder, chronic pelvic pain, benign prostatic hyperplasia, musculoskeletal pain and headache.

CONCLUSION

Botulinum toxin drugs are a group of highly potent drugs with specific mechanism of action. They are not at the end of their development cycle, but rather at the starting phase. Judicious use of botulinum toxin and imparting knowledge about its various clinical applications to the physicians will ensure that it will be an important treatment option for improving quality of life of patients.

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