

An Overview: Botulinum Toxins and their Therapeutics usages

Kanaan AL-Tameemi^{1*}, Hiba Serdani², Hiba Mohammad³, Raiaan Kabakli³

¹Department of Microbiology, Faculty of Pharmacy, Al Andalus University for Medical Sciences, Tartous, Syria

²Department of basic science, Al Andalus University for Medical Sciences, Tartous, Syria

³Department of Basic Sciences, Al Andalus University for Medical Sciences, Tartous, Syria

*Corresponding author: E-Mail ID: d_knaan@yahoo.com

ABSTRACT

Botulinum toxin (BoNT) is one of the most toxic and well-known biological compounds in the world which resulting from *Clostridium botulinum* (which causes botulism). They are anaerobic, gram-positive, microbes that form widespread spores in nature (soil, water, plant, even invertebrate gut). Botulinum has 7 known and distinct serotypes (A, B, C1, D, E, F, G). Botulinum inhibits neurotransmission and releasing of acetylcholine in neuromuscular contact points to prevent skeletal muscle contraction, causing paralysis in the body.

The knowledge of botulinum doses and an understanding of functional anatomy of the muscles is essential for the safe and effective use of botulinum in clinical treatments.

KEY WORDS: Botulinum toxin, Botox, serotypes, contraction.

1. INTRODUCTION

Botulism may have occurred in humans for thousands of years ago, but has only recently been recognized. In the late 18th century, an outbreak of "sausage poisoning" in southern Germany leads to an early investigation into a potential causative agent. This research got Justin Kerner's attention (1786-1862) who was a German poet and medical officer in the region.

He then conducted a study on botulism between 1817 and 1822 (Erbguth, 2004; Kerner, 1817, 1822). He concluded that the toxin works to prevent the transmission of signals to the peripheral and sympathetic nerves and to avoid Sensation transporting. Kerner was been interested in the therapeutic uses of such toxins, and he conducted many animal experiments, but he was unable to isolate the toxin (Erbguth, 2000).

In 1985, microbiologist Emile Pierre van Ermengem discovered that the bacillus (later called *Clostridium botulinum*) was the causative pathogen (Van Ermengem, 1897), to the outbreak of toxicity with smoked pork.

Although it was originally thought that it occurs only because of meat and fish products, the spread of canned beans in 1904 led to the isolation of a different breed that produced a serologically distinct toxin (Leuchs, 1920).

Again, in 1905 the increasing interest in public health contributed in conclusion that *Clostridium* produced a neurotoxin (Tchitchikine, 1905), Georgina Burke categorized the toxins with their serotypes by naming them A and B in 1919 (Burke, 1919). During World War II, Edward J. Schantz (Fig.1) purified and crystallized the toxin as a potential biological weapon in the US army.



Figure.1. Edward Schantz

In the 1970s Scott conducted his experiments on monkeys (Scott, 1973) and humans (Scott, 1981) for strabismus and blepharism treatment. Other clinical experiments have begun to evaluate the usefulness of botulinum toxin (BoNT) in treating other focal dystonia, torticollis and meigs's syndrome.

In 1989 The successful using of botulinum in the treatment of strabismus and blepharism treatment prompted the US Food and Drug Administration (FDA) to accept the first group of botulinum toxin type (BoNT-A) "Oculinum" as the only drug that renamed in 2004 by Allergan to Botox® (Ting, 2004). In 1984A similar and competitive efforts by Porton International in the UK market the BoNT-A drug called Dysport®. The BoNT-B (B) was produced under the name Myobloc™ in the United States. United and NeuroBloc® in Europe (Lew, 2000). In 2005, Merz produced free BoNT-A of complex non-toxic proteins and called it Xeomin® (Benecke, 2005). Due to ongoing interesting in botulinum, other serotypes are also under investigation for future therapeutic benefits.

Morphology And Viability Characteristics: *Clostridium botulinum* infects humans and animals causing botulisms. The bacilli may be single cell (Fig.2) or pairs cells (Fig.3), they are Gram-positive, Non-capsulated bacterium, ciliated, obligate anaerobe, their Dimensions range in length from 4-8 microns and about 1 micron wide, with a

rounded end, they move slowly by 4-8 flagella. The spore is elliptical that located before the end of the bacterium, and its diameter greater than the diameter of the bacterium (Figure.4).

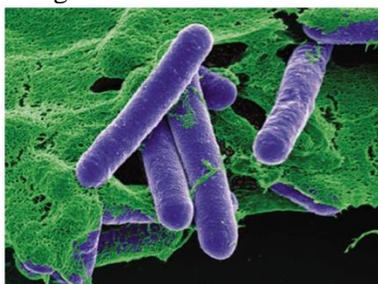


Figure.2. single cell of *Clostridium botulinum*

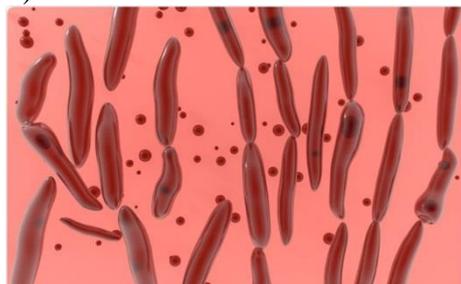


Figure.3. Pairs cell of *Clostridium botulinum*

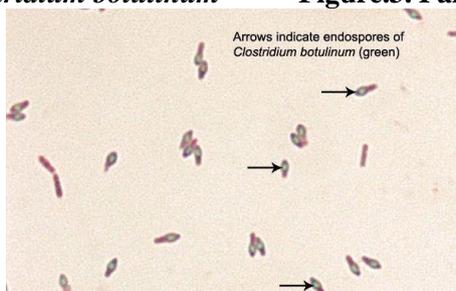


Figure.4. Endospores of *Clostridium botulinum*

The viability of bacterium is weak and dies at 60°C in 30 minutes, 80°C in 15 minutes and 100°C in 10 minutes. It's appropriate pH is 3 so it is resistant to the digestive enzymes.

As we mentioned earlier that *Clostridium botulinum* is obligate Anaerobe which grows on the normal culture media at a temperature of 26-28°C and in the alkaline medium pH = 8, it grows on blood agar and causes hemolysis of type β (complete dissolution) (Figure.5).

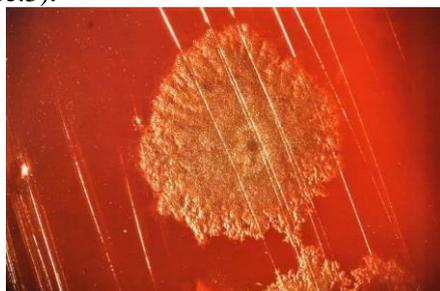


Figure.5. *Clostridium botulinum* grows on Blood agar causing hemolysis of type β

These bacteria can turn out from the normal form of the bacterium to a special form called spore that very resistant to the various factors that usually lead to killing these spores such as high temperature, dehydration and disinfectant chemicals.

It is worth mentioning that Bacteria themselves are non-pathogenic but they produce botulinum toxins which are responsible for the clinical symptoms of botulism. The appropriate temperature for toxin synthesis is about 16°C, while the best temperature is between 25°C and 38°C.

The toxin contains 7 types A to G. Four of these types can cause human botulism, A, B, E and sometimes F. The types C, D and F cause diseases in other mammals, birds and fish. Type A toxin is one of the weapon of mass destruction, only 200g of A toxin is enough to kill billions of people.

Mechanism of Toxin Action: Botulinum neurotoxins are produced by several strains of *Clostridium* bacteria, mainly *Clostridium botulinum*. There are 7 known serotypes of botulinum neurotoxin (A-G), including BTA and BTB, which are commercially produced for clinical uses. Although all of these serotypes prevent the acetylcholine releasing from nerve endings, their intracellular target proteins, their working properties, and their energies vary considerably.

Botulinum is produced as proteins (150 kDa) which are subsequently modified after translation to give a heavy chain (100 kDa) and a light chain (50 kDa) bound by a disulfide bridge. Figure.6.

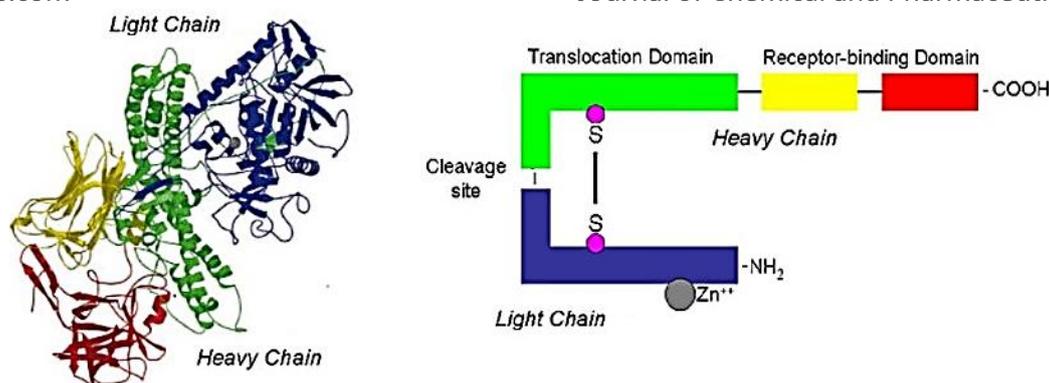


Figure.6. Structure of Botulinum toxin

The heavy chain C binds to the presynaptic neurons at the Neuromuscular junction, where it combines with specific receptors on the cell membrane, thereby facilitating the entry of the light chain into the cell cytoplasm. The light chain (L) interacts with different proteins in nerve terminals such as synaptosomal associated protein (SNAP25), syntaxin and vesicle-associated membrane protein, so it prevents acetylcholine fusion with the cell membrane therefore releasing it out of the neuron, this leads to paralysis of the target muscles within a few hours of botulinum injection. The maximum effect of botulinum toxin in muscle paralysis occurs after four to seven days of injection, the effect lasts about three months and maybe more, here the injection is repeated again.

People may lose the response to botulinum toxin after repeated injections. The cause is not clearly known but may be due to the development of antibodies to the toxic and non-toxic components of botulinum. Figure.7. (Yidi, 2017; Iqbal Multani, 2019; Kukreja, 2015).

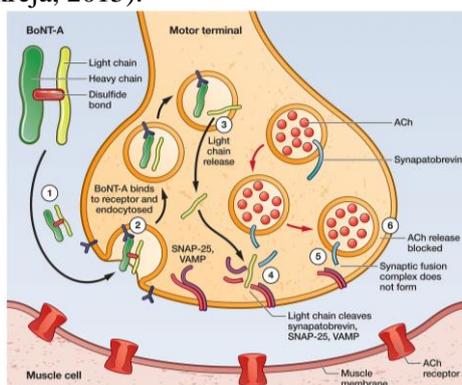


Figure.7. Mechanism of toxin action

Some clinical usages of Botulinum:

Strabismus: Strabismus is a visual problem in which the eyes are not aligned correctly. This is due to problems in muscles or nerves around the eye. Doctors were able to use Botulinum toxin type A in strabismus therapy where Toxin type A paralyzes or reduces the contraction of the extraocular muscle that is pulling the eye out of alignment (Rowe, 2017; Debora Mayumi Sugano, 2013)

Many studies were conducted on strabismus have and were showed the effectiveness of botulinum because of its ease of use, and its low complications.

Glabella: Botulinum type A or as known "Botox" is useful in the temporary improvement of moderate and severe glabellar lines cases Figure 8, also it is used in the therapy of forehead lines and Crow's feet (Barbano, 2006; Nayyar, 2014). Figure.9.



Figure.8. Glabellar lines before and after Botox using **Crow's feet before and after Botox using**
Gummy smile: This is a cosmetic problem related to hyperactivity of the upper lip muscle; doctors inject botox in small doses and carefully to reduce muscle contraction of the upper lip (Meunier, 2002). (Figure 9)



Figure.9. The effect of Botox on Gummy smile

Teeth implantation: Excess hard work that is applied to masticatory muscles can prevent the multiplication of transplanted cells when teeth implantation or callus break (Yilmaz Caner, 2017; Mijiritsky, 2016).

BTA has been used mainly in the treatment of temporomandibular joint disorders, hyperactivity of the masticatory muscles and for cosmetic purposes as reducing facial wrinkles (Kyung-Soo Park, 2016; Pushpalatha Govindaraju, 2016).

Axillary Hyperhidrosis: In 2001, the UK and Canada approved the use of Botox to axillary hyperhidrosis (excessive sweating) and palm hyperhidrosis therapy. Botulinum toxin A prevents the releasing of acetylcholine which mediates sympathetic neurotransmission in the sweat glands. The problem when using this mechanism is the pain at the injection sites. The use of botulinum toxin A in lidocaine solution has been suggested to reduce pain associated with injection (Jankovic, 2015; Tsui, 1985; Melissa, 2012).

Chronic Migraine: The migraine pain pathway is believed to originate in the brainstem, cerebral cortex and bridge, followed by intracranial vasodilation and rapid neurogenic inflammation in the perivascular area. Studies in humans have found sensory/pain fibres that extend from the inside of the cranial and pass through the intracranial dura mater, cranial sutures, extracranial periosteum and craniofacial muscles.

Jakubowski et al conducted studies in neuropsychological response by using BoNT-A and they found that the patient's response to BoNT-A was associated with headache type, with patients with imploding or ocular headaches having a response rate of 94% and 100%, respectively, while patients People with exploding headache had a low response rate of 19%. They attribute this difference to the view that the explosive head is mediated by intracranial innervation, whereas ocular and internal headaches may include extracranial innervation (Kosaras, 2009; Schueler, 2013; Jakubowski, 2006).

In 2010, the US Food and Drug Administration approved the use of BoNT / A for the treatment of chronic migraine.

Bladder detrusor overactivity: One of the most common uses of BoNT / A is the treatment of urinary incontinence syndrome. Several experiments which are conducted in more than 600 patients showed a marked improvement in incontinence in most patients with hypersecretory neuronal hyperactivity (Frucht, 2009; Tsui, 1993; Cole, 1995). Also, it is used to reduce the urinary infections such as pyelonephritis, orchitis and prostatitis which is commonly observed in NDO patients (Kruisdijk, 2007). The average duration of the effect is about a year and adverse side effects are rare. Clinical trials on hyperactivity that caused by neurological conditions such as multiple sclerosis or spinal cord injury have shown a significant reduction in the incontinence cases in patients who treated with BOTOX. In 2012, the US Food and Drug Administration approved BOTOX for injection in incontinence cases that due to hypersecretion associated with neurological problems as spinal cord injuries and MS in patients with an inadequate or intolerant response to anticholinergic drugs.

Sialorrhea: Sialorrhea or excessive salivation is a common manifestation of various neurological disorders. Sialorrhea is associated with common cerebral palsy, Parkinson's disease and amyotrophic lateral sclerosis (Campos, 2003; Van der Salm, 2014). BoNT / A can reduce the salivation excess. The determination of effective therapeutic doses and the good form of use are still needed for the best results.

Chronic Musculoskeletal Pain: Chronic musculoskeletal pain is due to the occurrence of musculoskeletal disorders in different parts of the human body. BoNT-A has been used successfully in the cases of spasmodic torticollis, limb dystonia, and spasticity. Researchers have become interested in the possibility of using it in the treatment of many markets of chronic pain. There is evidence supporting the use of BoNT-A for pelvic pain, plantar fasciitis, temporomandibular joint dysfunction that associated with facial pain, chronic lower back pain, carpal tunnel syndrome, joint pain (Singer, 2007; Eisa, 2007).

Botulinum toxins in cancer therapy: Recent studies have shown the possibility of botulinum toxin using in the treatment of cancer, it opens the tumour vessels briefly allowing more effective controlling of cancer cells than radiation and chemotherapy. The action of Clostridium toxin is done directly on the tumour and cancer cells rather

than the toxic effect on whole-body cells (Ansiaux, 2007). Also, it is known that radiotherapy and chemotherapy can induce contractures and muscle spasms causing pain in muscle spasms of the head and the neck musculature. here the treatment with BoNT-A injections may be helpful (Reginald Ansiaux, 2007).

2. CONCLUSION

The use of botulinum toxins developed the medical field, especially types A and B which have been used to treat many of neurological disorders based on their ability in intervening in a wide range of physiological functions making it a multiple treatment options, but more research is needed to improve their medical uses.

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