BOTULINUM TOXIN - HISTORICAL OVERVIEW

Boris Kyosev

Department of Prosthetic Dental Medicine, Faculty of Dental Medicine, Medical University of Varna

ABSTRACT

Cases of food poisoning with botulinum toxin have been reported as far back as Roman times. Emperor Leo VI of Byzantium prohibited the manufacturing of blood sausages with a law due to cases attributed to poisoning with Atropa belladonna. It was later proven that atropine does not have the effects described in the cases during his reign. It was not until the 19th century that scientists began detailed studies of the rising number of cases in which the patients reported nausea, vomiting, relaxation of muscles and inability to breathe properly. Such "outbreaks" of cases were reported in southern Germany and Belgium in short succession, which led medical scientists to believe they were connected. The toxin was first identified by Justinus Kerner - a German medical officer in Wuertemberg, as "fatty acids" or "fat toxin". He failed to point the source of the toxin, however, he recognized the potential for medical use. Later in the 19th century Emile Pierre van Ermengem, a Belgian scientist, successfully identified the source of the "fatty acids". It was later called Botulinum toxin after the Latin word for sausages "botulus". Since then this substance, produced by C. botulinum, has been studied in detail by scientists around the world and many uses have been found. It is utilized in the treatment of neurological conditions, posttraumatic conditions, muscle spasms, irregular sweating, chronic pain, skin wrinkles, etc. The military have found use for it as well - the potency of the toxin can be effectively used in closed environments.

Keywords: *botulinum toxin, Botox, Clostridium botulinum, therapeutic use, history, historical overview*

WHAT IS THE BOTULINUM TOXIN?

Botulinum toxin is produced by the bacterium *Clostridium botulinum*. It is among the most poisonous substances currently known to man.

The structure of botulinum toxin type A consists of dual polypeptide chains (heavy 100 kda and light 50 kda) connected via disulfide bond. The toxin is secreted from the bacterium as a single chain, then it is activated when divided into two chains by its own protease enzyme. The heavy chain is responsible for the attachment of the toxin to the target cell and the light is the active factor, blocking the release of neurotransmitter (acetylcholine) (5).

There are seven subtypes of botulinum toxin currently known to scientists - type A, B, C, D, e, F and G. Only type A botulinum toxin has received approval for medical use by the US Federal Drug Administration so far (7).

Intoxication occurs as a result of either ingesting the toxin in spoilt food or it getting into the bloodstream through a wound. The lethal dose for humans is estimated to be 1.3-2.1 ng/kg intravenously, 10-13 ng/kg if inhaled and 1000 ng/kg when ingested through the mouth.

The condition, caused by intoxication with botulinum toxin is known as botulism. The symptoms include high temperature, nausea, relaxation of facial muscles (mask-like facial expression), inability to breathe and lack of muscle strength in general (8).

In infants, *C. botulinum* can grow inside the intestinal tract, producing botulinum toxin and can poison the patient. This condition is known as "floppy baby syndrome". In severe cases, the toxin can block parts of the respiratory system or the heart, which results in death. The syndrome can be mistaken with other conditions such as Guillain-Barre syndrome, myasthenia gravis, and stroke. Tests like brain scanning and spinal fluid examination can help in diagnosing the correct condition. With proper diagnosing and treatment the damage to the nerves heals within weeks to several months (9).

Exposure to low temperatures (below 3°C) slows down the growth of *C. botulinum*. The bacterium is also susceptible to high oxygen, high salt, and acidic

pH. The botulinum toxin is destroyed by heat (cooking at more than 85°C for more than five minutes). The spores that produce the toxin are heat-resistant and will survive at around 100°C for extended periods of time.

Currently there are two types of botulinum antitoxins available. Trivalent (A, B, E) botulinum antitoxin is derived from animal sources (equines) using antibodies. The other substance is heptavalent (A, B, C, D, E, F, G) botulinum antitoxin, which is from the same source, but modified to be less immunogenic (6).

DISCOVERY

J. Kerner, painting by A. Bruckmann

Botulinum toxin was discovered in 1820 by Justinus Kerner (1,2) in Germany after a series of food poisoning cases with similar symptomatic complex. The name of the toxin was given from the Latin term *botulus*, which means sausage, since all the cases were reported as food poisoning from eating sausages. After numerous experiments, conducted on animals and on himself, Kerner concluded that the new toxin blocks the transmission of motor neuron signals without inhibiting sensory signals. However, the blockage was temporary and the severity was proportional to the amount of poisonous food ingested. He therefore concluded that botulinum toxin might have medicinal use (2).

Emile Pierre van Ermengem also conducted a research on this new toxin and in 1895 identified the

Emile Pierre van Ermengem

bacteria responsible for its production - Clostridium botulinum. It is an anaerobic, rod-shaped spore-forming bacterium, producing one of the most potent neurotoxic substances known to man (3).

20th Century

In the beginning of the 20th century Carl Meier, a veterinarian working in San Francisco (USA) developed methods both for growing and eliminating *C. botulinum*. The results from his research were used in the fast developing canning industry in the USA at that time.

WWII

After the beginning of World War II the United States Army searched for ways to weaponize the botulinum toxin. Fort Detrick became the base for research and a research team, headed by Carl Lamanna and James Duff, (4) was formed. They developed a method for concentrating and crystalizing botulinum toxin, which was later used by Eduard Shantz for commercial production of cosmetic products.

Modern Day Use

During the late 1960s Scott, an ophthalmologic surgeon in San Francisco, who developed a technique for precision injection of ocular muscles, talked to Shantz. The reason for Scott's interest in Shanz's research was another doctor's (Daniel Drachmann) successful attempt at paralyzing a chicken's legs. Scott considered the toxin used in those experiments as a viable injectable agent against eye muscle spasticity (Drachman, 1971; Scott, 2004).

Scott experimented on monkeys' muscles with different substances, including alcohol, enzyme

blockers, snake neurotoxins. He tried botulinum toxin as well, intrigued by Drachmann's discovery. Scott noted that a very small quantity of the toxin (picograms) induced prolonged paralysis in the target muscles alone, without any noticeable side effects (15).

Human experiments on healthy volunteers, as well as strabismus patients, began in the late 1970s.

- involuntary contraction of the bladder muscles and anal sphincter (which can lead to the exacerbation of anal fissures)
- excessive armpit sweating with unknown cause (13)
- migraines pain (2000 William Binder) (12)
- lower limb spasticity in pediatric patients 2 years and older

Table 1. Historical steps in the discovery and development of botulinum toxin

18th century – the first documented endemic outbreaks of food-borne botulism called "sausage poisoning" in Europe	
1817–1822	Justinus Kerner and botulinum toxin: Preliminary animal experiments, systematic descriptions of its clinical effects; theoretical considerations of its possible therapeutic use
1895–1897	Emile Pierre van Ermengem: Discovery of the neurotoxin-producing pathogen Clostridium botulinum
1910	J. Leuchs: Discovery of a second serologically distinct botulinum toxin serotype (type B)
1920–1930	H. Sommer: Purification of botulinum toxin
1946	C. Lamanna and J. Duff: Techniques of toxin concentration and crystallization
1949	A. Burgen: Description of the toxin's action on acetylcholine release at the neuromuscular junction
1970s	Description of wound and infant botulism
1941–1972	Edward Schantz: Production of a batch of toxin at Fort Detrick (USA)
1968	Contact between Alan Scott and Edward Schantz; search for therapeutic agents (e.g. botulinum toxin) to relax eye muscles
1973	Alan Scott: Publication of animal experiments with injections of botulinum toxin into eye muscles
1977–1980	Alan Scott: Treatment of strabismus patients with botulinum toxin; first publications of application in humans
1989	Approval of Alan Scott's type A toxin batch as "Oculinum" in the USA; later named "Botox"
1881–1988	Development of a type A toxin preparation in the UK; later called "Dysport"
1990s	Discovery of the molecular action of botulinum toxin (Schiavo, Montecucco, Dolly)
2000–2001	Approval of a therapeutic type B preparation in the USA and Europe (Myobloc, Neurobloc)
2005	Approval of a type A preparation in Germany (Xeomin)

(16) The success of this new treatment method lead to the approval of the toxin by the FDA under the name of "Oculinum" (1989), which was later changed to Botox+ (17).

Since late 1980s and early 90s botulinum toxin type A (Botox) has successfully been used for the treatment of the following conditions:

- skin wrinkles such as crow's feet, for example (11)
- high upper lip smile
- horizontal wrinkles on the forehead
- conditions caused by facial and neck muscle spasticity: blepharospasm, strabismus, dystonia, spasmic dysphonia, achalasia, etc. (10)
- muscle spasticity after stroke, after brain or spinal cord injuries (14)

Economics

Since 2018, botulinum toxin injections have been the most widely spread cosmetic operations. Medical personnel, authorized for the performance of such operation, varies by countries and includes plastic surgeons, aesthetic spa physicians, dermatologists, dentists, nurses etc.

The market for botulinum toxin products, used for cosmetic applications, was expected to reach approximately $3 billion by 2018.

Bioterrorism

The toxin, produced by *C. botulinum* can potentially by used in terrorist attacks. This is due to the fact, that it can be absorbed through the eyes, mucous membranes, non-intact skin and respiratory tract (19).

A Japanese terrorist group produced botulinum toxin and used it as aerosol in downtown Tokyo during a 1990s terrorist attack, but fortunately caused no fatalities (20).

As far as detection is concerned, current NBC detection equipment (ICAM or detection paper) will not test positive when used to test botulinum toxin-containing samples. To confirm the presence of botulinum toxin in the human body, the toxin can be quantitated by immunoassay of human biological fluids.

PRECAUTIONS

With all the opportunities for treatment botulinum toxin A provides, precautions must be taken because of all the possible side effects:

- temporary localized paralysis (1 week to 4 months after toxin application)
- difficulty swallowing after application of botulinum toxin in the neck area
- fever and nausea
- headache
- allergic reactions
- bruising at the injection site; it is not a direct side effect of the botulinum toxin itself, but rather the way of injecting it; it is recommended to apply pressure to the injection site; if bruising occurs, it will most often last 7-11 days
- unwanted effects from therapeutic use are much more diverse than those from cosmetic use; they can vary both in time and intensity and be more dangerous to the patient's health; they can occur as a result of critical muscle paralysis and include heart attack, arrhythmia, seizures, respiratory arrest, and death.

Usually, botulinum toxin can be administered into the wrong muscle group or spread from the injection site, thus temporarily paralyzing adjacent muscles or muscle groups.

It is important to strictly follow the dosage protocol and to apply it after proper training and knowledge of use is acquired.

Address for correspondence:
Boris Kyosev
Faculty of Dental Medicine
Medical University of Varna
84 Tzar Osvoboditel Blvd
9002 Varna
Bulgaria
e-mail: boris.kyosev@mu-varna.bg

REFERENCES

1. Нели Ерменлиева, „Ботулинов невротоксин- от отровата до лекарството“
2. Burgen AS, Dickens F, Zatman LJ (August 1949). „The action of botulinum toxin on the neuro-muscular junction“. The Journal of Physiology. 109 (1–2): 10–24
3. Li B, Peet NP, Butler MM, Burnett JC, Moir DT, Bowlin TL (December 2010). „Small molecule inhibitors as countermeasures for botulinum neurotoxin intoxication“. Molecules. 16 (1): 202–20. doi:10.3390/molecules16010202. PMC 6259422. PMID 21193845.
4. Shukla HD, Sharma SK (2005). „Clostridium botulinum: a bug with beauty and weapon“. Critical Reviews in Microbiology. 31 (1): 11–18. doi:10.1080/10408410590912952. PMID 15839401
5. „Kinds of Botulism“. Centers for Disease Control and Prevention. Retrieved 4 October 2016.
6. Barash JR, Arnon SS (January 2014). „A novel strain of Clostridium botulinum that produces type B and type H botulinum toxins“. The Journal of Infectious Diseases. 209 (2): 183–91. doi:10.1093/infdis/jit449. PMID 24106296.
7. Kerner J (1820). Neue Beobachtungen über die in Württemberg so häufig vorfallenden tödlichen Vergiftungen durch den Genuss geräucherter Würste. Tübingen: Osiander.
8. Erbguth FJ, Naumann M (November 1999). „Historical aspects of botulinum toxin: Justinus Kerner (1786–1862) and the „sausage poison““. Neurology. 53 (8): 1850–53.
9. Snipe PT, Sommer H (August 1928). „Studies on Botulinus Toxin: 3. Acid Precipitation of Botulinus Toxin“. The Journal of Infectious Diseases. 43 (2): 152–60.
10. Lamanna C, McElroy OE, Eklund HW (May 1946). „The purification and crystallization of Clostridium botulinum type A toxin“. Science. 103 (2681): 613–14.
11. Drachman, 1971; Scott, 2004
12. Scott A (2004) Development of botulinum toxin therapy. Dermatol Clin 22: 131–133
13. Scott AB (1979) Botulinum Toxin injection to correct strabism. Trans Am Ophtalmol Soc 79:924-927
14. Ting PT, Freiman A (2004) The story of Clostridium botulinum: from food poisoning to Botox. Clin Med 4: 258-261
15. Clark RP, Berris CE (August 1989). “Botulinum toxin: a treatment for facial asymmetry caused by facial nerve paralysis”. Plastic and Reconstructive Surgery. 84 (2): 353–55. doi:10.1097/01.prs.000020
16. Scott AB, Rosenbaum A, Collins CC (December 1973). „Pharmacologic weakening of extraocular muscles“. Investigative Ophthalmology. 12 (12): 924–27. PMID 4203467.
17. Charles PD (November 2004). „Botulinum neurotoxin serotype A: a clinical update on non-cosmetic uses“. American Journal of Health-System Pharmacy. 61 (22 Suppl 6): S11-23. doi:10.1093/ajhp/61.suppl_6.S11. PMID 15598005.
18. Eisenach JH, Atkinson JL, Fealey RD (May 2005). „Hyperhidrosis: evolving therapies for a well-established phenomenon“. Mayo Clinic Proceedings. 80 (5): 657–66. doi:10.4065/80.5.657. PMID 15887434.
19. Binder WJ, Brin MF, Blitzer A, Schoenrock LD, Pogoda JM (December 2000). „Botulinum toxin type A (BOTOX) for treatment of migraine headaches: an open-label study“. Otolaryngology–Head and Neck Surgery. 123 (6): 669–76. doi:10.1067/mhn.2000.110960. PMID 11112955.
20. Clostridium botulinum – Public Health Agency of Canada. Phac-aspc.gc.ca (19 April 2011). Retrieved on 6 May 2012.
21. Arnon SS, Schechter R, Inglesby TV, Henderson DA, Bartlett JG, Ascher MS, et al. (Working Group on Civilian Biodefense) (February 2001). „Botulinum toxin as a biological weapon: medical and public health management“. JAMA. 285 (8): 1059–70. doi:10.1001/jama.285.8.1059. PMID 11209178.