CAMBRIDGE

Cambridge University Press
978-1-107-02535-6 - Manual of Botulinum Toxin Therapy: Second Edition
Daniel Truong, Mark Hallett, Christopher Zachary and Dirk Dressler
Excerpt
More information

Chapter 1

The pretherapeutic history of botulinum neurotoxin

Frank J. Erbguth

Unintended intoxication with botulinum neurotoxin (botulism) occurs only rarely, but its high fatality rate makes it a great concern for the general public and the medical community. In the USA, an average of 110 cases of botulism are reported each year. Of these, approximately 25% are food borne, 72% are infant botulism and the rest are wound botulism. Outbreaks of food-borne botulism involving two or more persons occur most years and are usually caused by eating contaminated home-canned foods.

Botulism in ancient times

Botulinum neurotoxin poisoning probably has afflicted humankind through the mists of time. As long as humans have preserved and stored food, some of the chosen conditions would be optimal for the presence and growth of the toxin-producing pathogen *Clostridium botulinum*: for example, the storage of ham in barrels of brine, poorly dried and stored herring, trout packed to ferment in willow baskets, sturgeon roe not yet salted and piled in heaps on old horsehides, lightly smoked fish or ham in poorly heated smoking chambers and insufficiently boiled blood sausages.

However, in ancient times there was no general knowledge about the causal relationship between the consumption of spoiled food and a subsequent fatal paralytic disease, nowadays recognized as botulism. Only some historical sources reflect a potential understanding of the life-threatening effects of consuming food intoxicated with botulinum neurotoxin. Louis Smith, for example, reported in his textbook on botulism a dietary edict announced in the tenth century by Emperor Leo VI of Byzantium (886–911), in which manufacturing of blood sausages was forbidden (Smith, 1977). This edict may have its origin in the recognition of some circumstances connected with cases of food poisoning. Also, some ancient formulae suggested by shamans to Indian maharajas for the killing of personal enemies give hint of an intended lethal application of botulinum neurotoxin: a tasteless powder extracted from blood sausages dried under anaerobic conditions should be added to the enemies' food at an invited banquet. Because the consumer's death occurred after he or she had left the murderer's place, with a latency of some days, the host was probably not suspected (Erbguth, 2008).

Botulism outbreaks in Germany in the eighteenth and nineteenth centuries

Accurate descriptions of botulism emerge in the German literature from two centuries ago when the consumption of improperly preserved or stored meat and blood sausages gave rise to many deaths throughout the kingdom of Württemberg in southwestern Germany. This area near the city of Stuttgart developed as the regional focus of botulinum toxin investigations in the eighteenth and nineteenth centuries. In 1793, 13 people were involved in the first well-recorded outbreak of botulism in the small southwest German village of Wildbad; six died. Based on the observed mydriasis in all affected victims, the first official medical speculation was that the outbreak was caused by an atropine (*Atropa belladonna*) intoxication. However, in the controversial scientific discussion, the term "sausage poison" was introduced by the exponents of the opinion that the fatal disease in Wildbad was caused by the consumption of "Blunzen," a popular local food from cooked pork stomach filled with blood and spices.

The number of cases of suspected sausage poisoning in southwestern Germany increased rapidly at the end of the eighteenth century. Poverty followed the

Manual of Botulinum Toxin Therapy, 2nd edition, ed. Daniel Truong, Mark Hallett, Christopher Zachary and Dirk Dressler. Published by Cambridge University Press. © Cambridge University Press 2013.

© in this web service Cambridge University Press www.cambridge.org

CAMBRIDGE

Cambridge University Press
978-1-107-02535-6 - Manual of Botulinum Toxin Therapy: Second Edition
Daniel Truong, Mark Hallett, Christopher Zachary and Dirk Dressler
Excerpt
More information

devastating Napoleonic Wars (1795–1813) and led to the neglect of sanitary measures in rural food production (Grüsser, 1986). In July 1802, the Royal Government of Württemberg in Stuttgart issued a public warning about the "harmful consumption of smoked blood-sausages." In August 1811, the medical section of the Department of Internal Affairs of the Kingdom of Württemberg, on Stuttgart again, addressed the problem of "sausage poisoning," considering it to be caused by hydrocyanic acid, known at that time as "prussic acid." However, the members of the nearby Medical Faculty of the University of Tübingen disputed that prussic acid could be the toxic agent in sausages, suspecting a biological poison. One of the important medical professors of the University of Tübingen, Johann Heinrich Ferdinand Autenrieth (1772–1835), asked the government to collect the reports of general practitioners and health officers on cases of food poisoning for systematic scientific analyses. After Autenrieth had studied these reports, he issued a list of symptoms of the so-called "sausage poisoning" and added a comment, in which he blamed the housewives for the poisoning because they did not dunk the sausages long enough in boiling water while trying to prevent the sausages from bursting (Grüsser, 1998). The list of symptoms was distributed by a public announcement and contained characteristic features of food-borne botulism such as gastrointestinal problems, double vision, mydriasis and muscle paralysis.

In 1815, a health officer in the village of Herrenberg, J. G. Steinbuch (1770–1818), sent the case reports of seven intoxicated patients who had eaten liver sausage and peas to Professor Autenrieth. Three of the patients had died and the autopsies had been carried out by Steinbuch himself (Steinbuch, 1817).

Justinus Kerner's observations and publications on botulinum toxin 1817–1822

Contemporaneously with Steinbuch, the 29-year-old physician and Romantic poet Justinus Kerner (1786–1862) (Fig. 1.1), then medical officer in a small village, also reported a lethal food poisoning. Autenrieth considered the two reports from Steinbuch and Kerner as accurate and important observations and decided to publish them both in 1817 in the *Tübinger Blätter für Naturwissenschaften und Arzneykunde* [*Tübinger Papers for Natural Sciences and Pharmacology*] (Kerner, 1817; Steinbuch, 1817).

Fig. 1.1 Justinus Kerner; photograph of 1855.

Kerner again disputed that an inorganic agent such as hydrocyanic acid could be the toxic agent in the sausages, suspecting a biological poison instead. After he had observed further cases, Kerner published a first monograph in 1820 on "sausage poisoning" in which he summarized the case histories of 76 patients and gave a complete clinical description of what we now recognize as botulism. The monograph was entitled "*Neue Beobachtungen über die in Württemberg so häufig vorfallenden tödlichen Vergiftungen durch den Genuss geräucherter Würste* [*New Observations on the Lethal Poisoning that occurs so frequently in Württemberg Owing to the Consumption of Smoked Sausages*] (Kerner, 1820). Kerner compared the various recipes and ingredients of all sausages that had produced intoxication and found that among the ingredients of blood, liver, meat, brain, fat, salt, pepper, coriander, pimento, ginger and bread the only common ones were fat and salt. Because salt was probably known to be "innocent," Kerner concluded that the toxic change in the sausage

© in this web service Cambridge University Press www.cambridge.org

CAMBRIDGE

Cambridge University Press
978-1-107-02535-6 - Manual of Botulinum Toxin Therapy: Second Edition
Daniel Truong, Mark Hallett, Christopher Zachary and Dirk Dressler
Excerpt
More information

must take place in the fat and, therefore, called the suspected substance "sausage poison," "fat poison" or "fatty acid." Later Kerner speculated about the similarity of the "fat poison" to other known poisons, such as atropine, scopolamine, nicotine and snake venom, which led him to the conclusion that the fat poison was probably a biological poison (Erbguth, 2004).

In 1822, Kerner published 155 case reports including autopsy studies of patients with botulism and developed hypotheses on the "sausage poison" in a second monograph *Das Fettgift oder die Fettsäure und ihre Wirkungen auf den thierischen Organismus, ein Beytrag zur Untersuchung des in verdorbenen Würsten giftig wirkenden Stoffes* [*The Fat Poison or the Fatty Acid and its Effects on the Animal Body System, a Contribution to the Examination of the Substance Responsible for the Toxicity of Bad Sausages*] (Kerner, 1822) (Fig. 1.2). The monograph


Das

Fettgift

oder

die Fettsäure

und

ihre Wirkungen auf den thierischen Organismus,

ein Beytrag

zur Untersuchung des in verdorbenen Würsten giftig wirkenden Stoffes,

von

Dr. Justinus Kerner,

Oberamtsarzt zu Weinsperg.

Stuttgart und Tübingen,
in der J. G. Cotta'schen Buchhandlung.
1822.

Fig. 1.2 Title of Justinus Kerner's second monograph on sausage poisoning, 1822.

contained an accurate description of all muscle symptoms and clinical details of the entire range of autonomic disturbances occurring in botulism, such as mydriasis, decrease of lacrimation and secretion from the salivary glands, and gastrointestinal and bladder paralysis. Kerner also experimented on various animals (birds, cats, rabbits, frogs, flies, locusts, snails) by feeding them with extracts from bad sausages and finally carried out high-risk experiments on himself. After he had tasted some drops of a sausage extract he reported: "...some drops of the acid brought onto the tongue cause great drying out of the palate and the pharynx" (Erbguth and Naumann, 1999).

Kerner deduced from the clinical symptoms and his experimental observations that the toxin acts by interrupting the motor and autonomic nervous signal transmission (Erbguth, 1996). He concluded: "The nerve conduction is brought by the toxin into a condition in which its influence on the chemical process of life is interrupted. The capacity of nerve conduction is interrupted by the toxin in the same way as in an electrical conductor by rust" (Kerner, 1820). Finally, Kerner tried in vain to produce an artificial "sausage poison."

In summary, Kerner's hypotheses concerning "sausage poison" were that (1) the toxin developed in bad sausages under anaerobic conditions, (2) the toxin acts on the motor nerves and the autonomic nervous system, and (3) the toxin is strong and lethal even in small doses (Erbguth and Naumann, 1999).

In Chapter 8 of the 1822 monograph, Kerner speculated about using the "toxic fatty acid" botulinum toxin for therapeutic purposes. He concluded that small doses would be beneficial in conditions with pathological hyperexcitability of the nervous system (Erbguth, 2004). Kerner wrote: "The fatty acid or zoonic acid administered in such doses, that its action could be restricted to the sphere of the sympathetic nervous system only, could be of benefit in the many diseases which originate from hyperexcitation of this system" and "by analogy it can be expected that in outbreaks of sweat, perhaps also in mucous hypersecretion, the fatty acid will be of therapeutic value." The term "sympathetic nervous system" as used during the Romantic period, encompassed nervous functions in general. "Sympathetic overactivity" then was thought to be the cause of many internal, neurological and psychiatric diseases. Kerner favored the "Veitstanz" (St. Vitus dance – probably identical

© in this web service Cambridge University Press www.cambridge.org

CAMBRIDGE

Cambridge University Press
978-1-107-02535-6 - Manual of Botulinum Toxin Therapy: Second Edition
Daniel Truong, Mark Hallett, Christopher Zachary and Dirk Dressler
Excerpt
More information

with chorea minor) with its "overexcited nervous ganglia" to be a promising indication for the therapeutic use of the toxic fatty acid. Likewise, he considered other diseases with assumed nervous overactivity to be potential candidates for the toxin treatment: hypersecretion of body fluids, sweat or mucus; ulcers from malignant diseases; skin alterations after burning; delusions; rabies; plague; consumption from lung tuberculosis; and yellow-fever. However, Kerner conceded self-critically that all the possible indications mentioned were only hypothetical and wrote: "What is said here about the fatty acid as a therapeutic drug belongs to the realm of hypothesis and may be confirmed or disproved by observations in the future" (Erbguth, 1998).

Justinus Kerner also advanced the idea of a gastric tube, suggested by the Scottish physician Alexander Monro in 1811, and adapted it for the nutrition of patients with botulism; he wrote: "if dysphagia occurs, softly prepared food and fluids should be brought into the stomach by a flexible tube made from resin." He considered all characteristics of modern nasogastric tube application: the use of a guide wire with a cork at the tip and the lubrication of the tube with oil.

Botulism research after Kerner

After his publications on food-borne botulism, Kerner was well known to the German public and amongst his contemporaries as an expert on sausage poisoning, as well as for his melancholy poetry. Many of his poems were set to music by the great German Romantic composer Robert Schumann (1810–56), who had to quit his piano career because of the development of a pianist's focal finger dystonia. Kerner's poem *The Wanderer in the Sawmill* was the favourite poem of the twentieth century poet Franz Kafka (in full in Appendix 1.1). The nickname "Sausage Kerner" was commonly used and "sausage poisoning" was known as "Kerner's disease." Further publications in the nineteenth century by various authors (e.g. Müller, 1869) increased the number of reported cases of "sausage poisoning," describing the fact that the food poisoning occurred after the consumption not only of meat but also of fish. However, these reports added nothing substantial to Kerner's early observations. The term "botulism" (from the Latin *botulus*, sausage) appeared at first in Müller's reports and was subsequently used. Therefore, "botulism" refers to poisoning caused by sausages and not to the sausage-like shape of the causative bacillus discovered later (Torrens, 1998).

The discovery of *"Bacillus botulinus"* in Belgium

The next and most important scientific step was the identification of *Clostridium botulinum* in 1895–6 by the Belgian microbiologist Emile Pierre Marie van Ermengem of the University of Ghent (Fig. 1.3).

On December 14, 1895, an extraordinary outbreak of botulism occurred amongst the 4000 inhabitants of the small Belgian village of Ellezelles. The musicians of the local brass band "Fanfare Les Amis Réunis" played at the funeral of the 87-year-old Antoine Creteur and as it was the custom gathered to eat in the inn "Le Rustic" (Devriese, 1999). Thirty-four people were together and ate pickled and smoked ham. After the meal, the musicians noticed symptoms such as mydriasis, diplopia, dysphagia and dysarthria, followed by increasing muscle paralysis. Three of them died and ten nearly died. A detailed examination of the ham and an autopsy were ordered and conducted by van Ermengem, who had been appointed Professor of Microbiology at the University of Ghent in 1888 after he had worked in the laboratory of Robert Koch in Berlin in 1883. Van Ermengem isolated the bacterium

Fig. 1.3 Emile Pierre Marie van Ermengem 1851–1922.

© in this web service Cambridge University Press www.cambridge.org

CAMBRIDGE

Cambridge University Press
978-1-107-02535-6 - Manual of Botulinum Toxin Therapy: Second Edition
Daniel Truong, Mark Hallett, Christopher Zachary and Dirk Dressler
Excerpt
More information

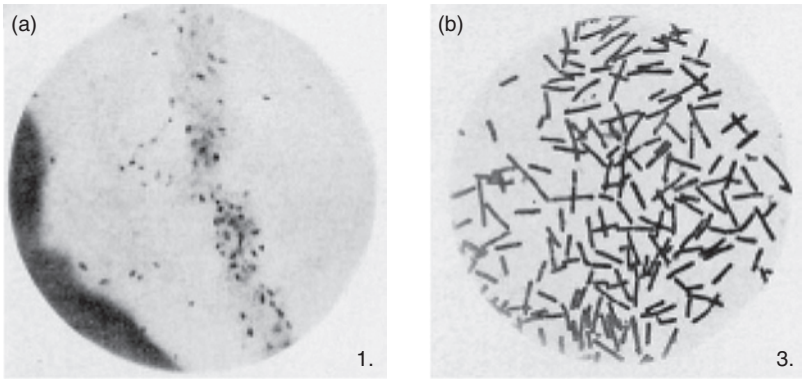

Fig. 1.4 Microscopy of the histological section of the suspect ham at the Ellezelles botulism outbreak. (a) Numerous spores among the muscle fibers (Ziehl ×1000). (b) Culture (gelatine and glucose) of mature rod-shaped forms of "Bacillus botulinus" from the ham on the eighth day (×1000). (From van Ermengem, 1897.)

in the ham and in the corpses of the victims (Fig. 1.4), grew it, used it for animal experiments, characterized its culture requirements, described its toxin, called it "Bacillus botulinus," and published his observations in the German microbiological journal *Zeitschrift für Hygiene und Infektionskrankheiten* [*Journal of Hygiene and Infectious Diseases*] in 1897 (an English translation was published in 1979) (van Ermengem, 1897). The pathogen was later renamed *Clostridium botulinum*. Van Ermengem was the first to correlate "sausage poisoning" with the newly discovered anaerobic microorganism and concluded that "it is highly probable that the poison in the ham was produced by an anaerobic growth of specific micro-organisms during the salting process." Van Ermengem's milestone investigation yielded all the major clinical facts about botulism and botulinum neurotoxin: (1) botulism is an intoxication, not an infection; (2) the toxin is produced in food by a bacterium; (3) the toxin is not produced if the salt concentration in the food is high; (4) after ingestion, the toxin is not inactivated by the normal digestive process; (5) the toxin is susceptible to inactivation by heat; and (6) not all species of animals are equally susceptible.

Botulinum neurotoxin research in the early twentieth century

In 1904, when an outbreak of botulism in the city of Darmstadt, Germany, was caused by canned white beans, the opinion that the only botulinogenic foods were meat or fish had to be revised. The bacteria isolated from the beans by Landmann (1904) and from the Ellezelles ham were compared by Leuchs (Leuchs, 1910) at the Royal Institute of Infectious Diseases in Berlin. He found that the strains differed and the toxins were serologically distinct. The two types of Bacillus botulinus did not receive their present letter designations of serological subtypes until Georgina Burke, who worked at Stanford University, designated them as types A and B (Burke, 1919). Over the next decades, increases in food canning and food-borne botulism went hand in hand (Cherington, 2004). The first documented outbreak of food-borne botulism in the USA was caused by commercially conserved pork and beans and dates from 1906 (Drachmann, 1971; Smith, 1977). Techniques for killing the spores during the canning process were subsequently developed. The correct pH (<4.0), the osmolarity needed to prevent clostridial growth and toxin production, and the requirements for toxin inactivation by heating were defined.

In 1922, type C was identified in the USA by Bengston and in Australia by Seddon; type D and type E were characterized some years later (type D in the USA in 1928 by Meyer and Gunnison; type E in the Ukraine 1936 by Bier) (Kriek and Odendaal, 1994; Geiges, 2002). Type F and type G toxins were identified in 1960 in Scandinavia by Moller and Scheibel and in 1970 in Argentina by Gimenex and Ciccarelli (Gunn, 1979; Geiges, 2002). In 1949, Burgen and his colleagues in London discovered that botulinum toxin blocked the release of acetylcholine at neuromuscular junctions (Burgen *et al.*, 1949). The essential insights into the molecular actions of botulinum toxin were gained by various scientists after 1970 (Dolly *et al.*, 1990; Schiavo *et al.*, 1992, 1993; Dong *et al.*, 2006; Mahrhold *et al.*, 2006), when its use as a therapeutic agent was pioneered by Edward J. Schantz and Alan B. Scott.

Until the last century, botulism was thought to be caused exclusively by food that was contaminated with preformed toxin. This view has changed since the 1950s, as spores of *C. botulinum* were discovered in

© in this web service Cambridge University Press www.cambridge.org

CAMBRIDGE

Cambridge University Press
978-1-107-02535-6 - Manual of Botulinum Toxin Therapy: Second Edition
Daniel Truong, Mark Hallett, Christopher Zachary and Dirk Dressler
Excerpt
More information

contaminated wounds (wound botulism) in the 1950s and in the intestines of babies in 1976 (infant botulism) (Merson and Dowell, 1973; Pickett *et al.*, 1976; Arnon *et al.*, 1977). The number of cases of food-borne and infant botulism has changed little in recent years, but wound botulism has increased because of the use of black-tar heroin, especially in California.

Swords to ploughshares

Before the therapeutic potential of botulinum neurotoxin was discovered, around 1970, its potential use as a weapon was recognized during World War I (Lamb, 2001). The basis for its use as a toxin was investigations by Hermann Sommer and colleagues working at the Hooper Foundation, University of California, San Francisco in the 1920s: the researchers were the first to isolate pure botulinum neurotoxin type A as a stable acid precipitate (Snipe and Sommer, 1928; Schantz, 1994). With the outbreak of World War II, the USA Government began intensive research into biological weapons, including botulinum toxin, particularly in the laboratory at Camp Detrick (later named Fort Detrick) in Maryland. Development of concentration and crystallization techniques at Fort Detrick was pioneered by Carl Lamanna and James Duff in 1946. The methodology was subsequently used by Edward J. Schantz to produce the first batch of toxin, which was the basis for the later clinical product (Lamanna *et al.*, 1946). The entrance of botulinum toxin into the medical therapeutic armamentarium in Europe also came from military laboratories to hospitals: in the UK, botulinum toxin research was conducted in the Porton Down laboratories of the military section of the Centre for Applied Microbiology and Research (CAMR), which later provided British clinicians with a therapeutic formulation of the toxin (Hambleton *et al.*, 1981).

Appendix *The Wanderer in the Sawmill* (Justinus Kerner 1826)

Down yonder in the sawmill
I sat in good repose
and saw the wheels go spinning
and watched the water too.

I saw the shiny saw blade,
as if I had a dream,
which carved a lengthy furrow
into a fir tree trunk.

The fir tree as if living,
in saddest melody,
through all its trembling fibres
sang out these words for me:

At just the proper hour,
o wanderer! you come,
it's you for whom this wounding
invades my heart inside.

It's you, for whom soon will be,
when wanderings cut short,
these boards in earth's deep bosom,
a box for lengthy rest.

Four boards I then saw falling,
my heart was turned to stone,
one word I would have stammered,
the blade went round no more.

References

Arnon SS, Midura TF, Clay SA, Wood RM, Chin J (1977). Infant botulism: epidemiological, clinical and laboratory aspects. *JAMA*, **237**, 1946–51.

Burgen A, Dickens F, Zatman, L (1949). The action of botulinum toxin on the neuromuscular junction. *J Physiol*, **109**, 10–24.

Burke GS (1919). The occurrence of Bacillus botulinus in nature. *J Bacteriol*, **4**, 541–53.

Cherington, M (2004). Botulism: update and review. *Semin Neurol*, **24**, 155–63.

Devriese PP (1999). On the discovery of *Clostridium botulinum*. *J Hist Neurosci*, **8**, 43–50.

Dolly JO, Ashton AC, McInnes, C *et al.* (1990). Clues to the multi-phasic inhibitory action of botulinum neurotoxins on release of transmitters. *J Physiol*, **84**, 237–46.

Dong M, Yeh F, Tepp WH *et al.* (2006). SV2 is the protein receptor for botulinum neurotoxin A. *Science*, **312**, 592–6.

Drachmann DB (1971). Botulinum toxin as a tool for research on the nervous system. In Simpson LL (ed.) *Neuropoisons: Their Pathophysiology Actions*, Vol. 1. New York: Plenum Press, pp. 325–47.

Erbguth, F (1996). Historical note on the therapeutic use of botulinum toxin in neurological disorders. *J Neurol Neurosurg Psychiatry*, **60**, 151.

Erbguth, F (1998). Botulinum toxin, a historical note. *Lancet*, **351**, 1280.

Erbguth FJ (2004). Historical notes on botulism, *Clostridium botulinum*, botulinum toxin, and the idea of the therapeutic use of the toxin. *Mov Disord*, **19**(Suppl 8), S2–6.

© in this web service Cambridge University Press www.cambridge.org

CAMBRIDGE

Cambridge University Press
978-1-107-02535-6 - Manual of Botulinum Toxin Therapy: Second Edition
Daniel Truong, Mark Hallett, Christopher Zachary and Dirk Dressler
Excerpt
More information

Erbguth FJ (2008). From poison to remedy: the chequered history of botulinum toxin. *J Neural Transm*, **115**, 559–65.

Erbguth F, Naumann, M (1999). Historical aspects of botulinum toxin: Justinus Kerner (1786–1862) and the "sausage poison." *Neurology*, **53**, 1850–3.

Geiges ML (2002). The history of botulism. In Kreyden OP, Böne R, Burg G (eds.) *Current Problems in Dermatology*, Vol. 30: *Hyperhidrosis and Botulinum Toxin in Dermatology*. Basel: Karger, pp. 77–93.

Grüsser OJ (1986). Die ersten systematischen Beschreibungen und tierexperimentellen Untersuchungen des Botulismus. Zum 200. Geburtstag von Justinus Kerner am 18. September 1986. *Sudhoffs Arch*, **10**, 167–87.

Grüsser OJ (1998). Der "Wurstkerner". Justinus Kerners Beitrag zur Erforschung des Botulismus. In H. Schott (ed.) *Justinus Kerner als Azt und Seelenforscher*, 2nd edn. Weinsberg: Justinus Kerner Verein, pp. 232–56.

Gunn RA (1979). Botulism: from van Ermengem to the present. A comment. *Rev Infect Dis*, **1**, 720–1.

Hambleton P, Capel B, Bailey N, Tse CK, Dolly O (1981). Production, purification and toxoiding of *Clostridium botulinum* A toxin. In Lewis G (ed.) *Biomedical Aspects of Botulism*. New York: Academic Press, pp. 247–60.

Kerner J (1817). Vergiftung durch verdorbene Würste. *Tübinger Blätter für Naturwissenschaften und Arzneykunde*, **3**, 1–25.

Kerner J (1820). *Neue Beobachtungen über die in Württemberg so häufig vorfallenden tödlichen Vergiftungen durch den Genuss geräucherter Würste. [New Observations on the Lethal Poisoning that occurs so frequently in Württemberg Owing to the Consumption of Smoked Sausages.]* Tübingen: Osiander.

Kerner J (1822). *Das Fettgift oder die Fettsäure und ihre Wirkungen auf den thierischen Organismus, ein Beytrag zur Untersuchung des in verdorbenen Würsten giftig wirkenden Stoffes. [The Fat Poison or the Fatty Acid and its Effects on the Animal Body System, a Contribution to the Examination of the Substance Responsible for the Toxicity of Bad Sausages.]*. Tübingen: Cotta.

Kriek NPJ, Odendaal MW (1994). Botulism. In Coetzer JAW, Thomson GR, Tustin RC (eds.) *Infectious Diseases of Livestock*. Cape Town: Oxford University Press, pp. 1354–71.

Lamanna C, Eklund HW, McElroy OE (1946). Botulinum toxin (type A); including a study of shaking with chloroform as a step in the isolation procedure. *J Bacteriol*, **52**, 1–13.

Lamb A (2001). Biological weapons: the facts not the fiction. *Clin Med*, **1**, 502–4.

Landmann G (1904). Über die Ursache der Darmstädter Bohnenvergiftung. *Hyg Rundschau*, **10**, 449–52.

Leuchs J (1910). Beiträge zur Kenntnis des Toxins und Antitoxins des Bacillus botulinus. *Z Hyg Infektionskrankh*, **65**, 55–84.

Mahrhold S, Rummel A, Bigalke H, Davletov B, Binz T (2006). The synaptic vesicle protein 2C mediates the uptake of botulinum neurotoxin A into phrenic nerves. *FEBS Lett*, **580**, 2011–14.

Merson MH, Dowell J (1973). Epidemiologic, clinical and laboratory aspects of wound botulism. *N Engl J Med*, **289**, 1105–10.

Müller H (1869). *Das Wurstgift*. Deutsche Klinik, pulserial publication, **35**, 321–3, 37, 341–3, 39, 357–9, 40, 365–7, 381–3, 49, 453–5.

Pickett J, Berg B, Chaplin E, Brunstetter-Shafer MA (1976). Syndrome of botulism in infancy: clinical and electrophysiologic study. *N Engl J Med*, **295**, 770–2.

Schantz EJ (1994). Historical perspective. In Jankovic J, Hallett M (eds.) *Therapy with Botulinum Toxin*. New York: Marcel Dekker, pp. xxiii–vi.

Schiavo G, Benfenati F, Poulain B *et al.* (1992). Tetanus and botulinum-B toxins block transmitter release by proteolytic cleavage of synaptobrevin. *Nature*, **359**, 832–5.

Schiavo G, Cantucci A, Das Gupta BR *et al.* (1993). Botulinum neurotoxin serotypes A and E cleave SNAP-25 at distinct COOH-terminal peptide bonds. *FEBS Lett*, **335**, 99–103.

Smith LD (1977). *Botulism. The Organism, its Toxins, the Disease*. Springfield, IL: Charles C Thomas.

Snipe PT, Sommer H (1928). Studies on botulinus toxin. 3. Acid preparation of botulinus toxin. *J Infect Dis*, **43**, 152–60.

Steinbuch JG (1817). Vergiftung durch verdorbene Würste. *Tübinger Blätter für Naturwissenschaften und Arzneykunde*, **3**, 26–52.

Torrens JK (1998). *Clostridium botulinum* was named because of association with "sausage poisoning." *BMJ*, 316, 151.

van Ermengem EP (1897). Über einen neuen anaeroben Bacillus und seine Beziehung zum Botulismus. *Z Hyg Infektionskrankh*, **26**, 1–56 (English version: Van Ermengem EP (1979). A new anaerobic bacillus and its relation to botulism. *Rev Infect Dis*, 1, 701–19).

© in this web service Cambridge University Press www.cambridge.org

CAMBRIDGE

Cambridge University Press
978-1-107-02535-6 - Manual of Botulinum Toxin Therapy: Second Edition
Daniel Truong, Mark Hallett, Christopher Zachary and Dirk Dressler
Excerpt
More information

Chapter 2

Botulinum neurotoxin: history of clinical development

Daniel Truong and Mark Hallett

The clinical development of botulinum neurotoxin began in the late 1960s with the search for an alternative to surgical realignment of strabismus. At that time, surgery of the extraocular muscles was the primary treatment for strabismus, but it was unsatisfactory for some patients because of the variability in results, consequent high reoperation rates and its invasive nature. In an attempt to find an alternative, Alan B. Scott, an ophthalmologist from the Smith–Kettlewell Eye Research Institute in San Francisco, investigated the effects of different compounds injected locally into the extraocular muscles to chemically weaken them. The drugs tested initially proved unreliable, short acting or necrotizing (Scott *et al.*, 1973).

About this time, Scott became aware of Daniel Drachman, a renowned neuroscientist at Johns Hopkins University and his work, in which he had been injecting minute amounts of botulinum neurotoxin directly into the hind limbs of chickens to achieve local denervation (Drachman, 1964). Drachman introduced Scott to Edward Schantz (1908–2005), who was producing purified botulinum neurotoxins for experimental use and generously making them available to the academic community. Schantz himself credits Vernon Brooks with the idea that botulinum neurotoxin might be used for weakening muscle (Schantz, 1994). Brooks worked on the mechanism of action of botulinum toxin for his PhD under the mentorship of Arnold Burgen, who suggested the project to him (Brooks, 2001). Schantz had left the US Army Chemical Corps at Fort Detrick, Maryland, in 1972 to work at the Department of Microbiology and Toxicology, University of Wisconsin in Madison. Using acid precipitation purification techniques worked out at Fort Detrick by Lamanna and Duff, Schantz was able to make purified botulinum toxins.

In extensive animal experiments, low doses of botulinum neurotoxin produced the desired long-lasting, localized, dose-dependent muscle weakening, reportedly without any systemic toxicity and without any necrotizing side effects (Scott *et al.*, 1973). Based on these results, the US Food and Drug Administration (FDA) permitted Scott in 1977 to test botulinum neurotoxin in humans under an Investigational New Drug (IND) license for the treatment of strabismus. These tests proved successful and the results of 67 injections were published in 1980 (Scott, 1980). With this publication, botulinum neurotoxin was established as a novel therapeutic. Scott approached several drug companies to take the drug on and manufacture it. However, he had disclosed the drug in earlier publications and thus could not get it patented. Without this, none of the manufacturers would undertake it. Scott then moved the activity from Smith–Kettlewell, setting up his own company, Oculinum, in Berkeley California. Dennis Honeychurch, a pharmacist, joined him and devised many of the tests for safety, potency, stability, sterility and water retention in the freeze-dried product that were required before botulinum toxin could be registered as a drug by the FDA. In addition to establishing a laboratory for testing and record keeping, a sterile facility for filling and freeze-drying was required. This was found at Adria Labs in Albuquerque, where Scott and Honeychurch went several times a year to fill 8000–10 000 vials.

Some time in the 1960s, Robert Crone, a professor of ophthalmology in Amsterdam, whose interest was in strabismus, was able to get the Porton group to send him dried toxin from the UK, with the idea of use in strabismus. The package was damaged, and dried toxin leaked out – probably enough to kill all of Amsterdam (A. Scott, personal communication)! Crone decided not to pursue it further. By the early 1980s, Scott and

Manual of Botulinum Toxin Therapy, 2nd edition, ed. Daniel Truong, Mark Hallett, Christopher Zachary and Dirk Dressler. Published by Cambridge University Press. © Cambridge University Press 2013.

© in this web service Cambridge University Press www.cambridge.org

CAMBRIDGE

Cambridge University Press
978-1-107-02535-6 - Manual of Botulinum Toxin Therapy: Second Edition
Daniel Truong, Mark Hallett, Christopher Zachary and Dirk Dressler
Excerpt
More information

colleagues had injected botulinum neurotoxin for the treatment of strabismus, blepharospasm, hemifacial spasm, cervical dystonia and thigh adductor spasm (Scott, 1994). Prior to FDA approval, the neurotoxin was made available to a group of investigators for research, including Calne, Dykstra, Fahn, Hallett, Jankovic, Roggenkamper, Tanner and Truong. Stanley Fahn's group at Columbia University reported in 1985 the first double-blind study testing Scott's toxin in improving the symptoms of blepharospasm (Fahn *et al.*, 1985). Also in 1985, Tsui and colleagues reported the successful use of botulinum neurotoxin for treatment of cervical dystonia in 12 patients based on earlier dosage data from Scott's injections (Tsui *et al.*, 1985). This was followed by the first double-blind, crossover study in which botulinum neurotoxin was significantly superior to placebo at reducing the symptoms of cervical dystonia, including pain (Tsui *et al.*, 1986). Therapeutic use of botulinum neurotoxin for the treatment of blepharospasm and hemifacial spasm proceeded along similar lines, with several groups reporting success for these indications by the mid 1980s and documenting the benefits of repeated injections after the effects waned (Frueh *et al.*, 1984; Mauriello, 1985; Scott *et al.*, 1985). Brin *et al.* (1987) reported on use of Scott's toxin to treat multiple dystonias (e.g. cranial, cervical, laryngeal, limb) and related hyperkinetic disorders. Reports of the successful use of botulinum neurotoxin in many conditions of focal muscle overactivity continued, including spasmodic dysphonia (Blitzer *et al.*, 1986; Truong *et al.*, 1991), oromandibular dystonia (Jankovic and Orman, 1987), dystonias of the hand (Cohen *et al.*, 1989) and limb spasticity (Das and Park, 1989). Soon, botulinum neurotoxin was accepted as safe and efficacious for blepharospasm, cervical dystonia and other focal dystonias, and was the treatment of choice for some indications (National Institutes of Health, 1991).

In December 1989, the FDA licensed the manufacturing facilities and batch 79–11 of botulinum neurotoxin type A, manufactured by Scott and Schantz in November 1979. The therapeutic preparation contained 100 mouse units of neurotoxin per vial. A mouse unit was defined as the LD_{50} for Swiss Webster mice. Scott named this drug Oculinum (**ocu** and **lin**ing-up) and it was recognized as an orphan drug for the treatment of strabismus, hemifacial spasm and blepharospasm. According to Scott (personal communication), he asked FDA to approve 88–4, a four times more potent batch for which he had ample data. However, the FDA required the use of 79–11 in the USA because it was used for generating most of the clinical data on which approval was based. Some European regulatory agencies accepted 88–4 with the initial filings. All current neurotoxins have greater specific potency than 79–11 and are equal to or better than 88–4. For about 2 years, Scott's Oculinum Inc. was the licensed manufacturer, with Allergan Inc. (Irvine, CA, USA) the sole distributor. Manufacturing rights and license were acquired by Allergan in late 1991; a different batch of Botox was distributed in 1998. This and subsequent batches of Botox contained less protein per unit, which may have made them less liable to elicit antibodies than the original 79–11.

The name Botox was perhaps first used by Stanley Fahn and Mitchell Brin, who did not think of it as a possible trade name. Around 1985, Scott trademarked the name B-botox for the type B neurotoxin that he studied. Finding type B was inferior to type A, he abandoned it and also the name. "Botox" is a name readily derived from laboratory lingo for toxins, probably newly invented several times prior to Allergan's use (A. Scott, personal communication). The non-proprietary name is now onabotulinumtoxinA.

In 2000, a product containing the botulinum neurotoxin B serotype, NeuroBloc/MyoBloc, was registered with the FDA by Elan Pharmaceuticals (South San Francisco, CA, USA) with the indication of cervical dystonia. MyoBloc is the trade name in the USA and NeuroBloc is the trade name used elsewhere. The name NeuroBloc was coined by Mitchell Brin and MyoBloc by Lloyd Glenn (Elan). The initial research on botulinum toxin B was carried out by Tsui, Truong and O'Brien. MyoBloc was eventually sold to Solstice Neurosciences Inc. (Malvern, PA, USA) and recently to US WorldMeds (Louisville, KY, USA). The non-proprietary name is rimabotulinumtoxinB. Botox was also approved for cervical dystonia in 2000.

In Europe, botulinum neurotoxin was first produced for therapeutic purposes at the Defence Science and Technology Laboratory in Porton Down, UK. When commercialized, manufacturing operations were renamed several times – to Centre of Applied Microbiology and Research (CAMR), Porton Products, Public Health Laboratory Service (PHLS) and Speywood Pharmaceuticals. In 1994, Speywood Pharmaceuticals was acquired by Ipsen (Paris, France). The UK botulinum neurotoxin product was first registered in 1991 as

© in this web service Cambridge University Press www.cambridge.org

CAMBRIDGE

Cambridge University Press
978-1-107-02535-6 - Manual of Botulinum Toxin Therapy: Second Edition
Daniel Truong, Mark Hallett, Christopher Zachary and Dirk Dressler
Excerpt
More information

Dysport (**dys**tonia **Port**on Products; non-proprietary name now abobotulinumtoxinA). It is manufactured for worldwide use by Ipsen (Slough, UK). It was approved in the USA for cervical dystonia and glabellar facial wrinkles in April 2009. It was first used to treat strabismus and blepharospasm in the UK not long after Scott's initial reports (Elston 1985; Elston *et al.*, 1985). The movement disorders group of C. David Marsden at the National Hospital of Neurology and Neurosurgery, London pioneered its use in neurology (Stell *et al.*, 1988). Soon afterwards, Dirk Dressler, a student of Marsden, introduced this product (Dysport) to continental European neurology (Dressler *et al.*, 1989). However, it was Roggenkamper who personally carried botulinum neurotoxin (Oculinum) that he received from Alan Scott to Germany and who initiated investigations in patients with blepharospasm (Roggenkamper, 1986). A flabbergasted German custom officer waved Roggenkamper with his hand-carried botulinum neurotoxin into Germany without even looking as he perceived Roggenkamper's declaration as a joke (Roggenkamper, personal communication). More details about the expansion of botulinum neurotoxin therapy in continental European are described by Homann *et al.* (2002).

Subsequently, another botulinum neurotoxin drug named Xeomin (incobotulinumtoxinA) was marketed by Merz Pharmaceuticals (Frankfurt/M, Germany). It is a botulinum neurotoxin type A preparation with high specific biological activity and, as a consequence, a reduced protein load (Dressler and Benecke, 2006). Structurally, it is free of the complexing botulinum neurotoxin proteins. It is currently approved in most European countries, USA, Canada, some middle and South American countries, as well as several Asian countries. Besides blepharospasm, cervical dystonia and glabellar lines, it is also approved for spasticity and some other indications depending on the country.

An additional source of therapeutic botulinum neurotoxin type A is the Lanzhou Institute of Biological Products (Lanzhou, Gansu Province, China), where the manufacturing expertise comes from Wang Yinchun, a former collaborator of Schantz. Wang used the protocol for acid precipitation of the crystalline toxin from the cultures worked out at the Army Chemical Laboratories at Fort Detrick (A. Scott, personal communication). Its product was registered as Hengli in China in 1993. In some other Asian and South American markets, it is distributed as CBTX-A, Redux or Prosigne. The international marketing is provided by Hugh Source International Ltd (Kowloon, Hong Kong). Registration of this product in the USA and in Europe seems unlikely. Publications about this product are scarce.

In South Korea and some other Asian countries, Neuronox, a botulinum neurotoxin type A drug manufactured by Medy-Tox (Ochang, South Korea), is distributed. Other botulinum neurotoxin drugs are under development at Tokushima University, Tokushima City, Japan and at the Mentor Corporation (Santa Barbara, CA, USA).

Over the years that these other products were developed, the clinical applications for botulinum neurotoxin continued to expand. Botox, which has most indications, was further approved by the FDA for glabellar rhytides in 2002 and for primary axillary hyperhidrosis in 2004. In 2010, Botox was approved for chronic migraine and upper limb spasticity in adults, in 2011 for the treatment of neurogenic detrusor overactivity and in 2013 for overactive bladder. Off-label use by physicians is widespread and includes tremor, anal fissure, achalasia, various conditions of pain and others (Dressler, 2000; Moore and Naumann, 2003; Truong and Jost, 2006). Outside the USA, there are at least 20 indications in 83 countries. Numerous formal therapeutic trials for registration are in progress. The use of Botox for wrinkles has been very popular and is perhaps the indication best known by general public.

These expanded uses were paralleled by increased understanding of the mechanism of action of botulinum neurotoxins from basic research (Lalli *et al.*, 2003). The multistep mechanism of action postulated by Simpson (1979) was verified, and research on botulinum neurotoxin has itself contributed much to the understanding of vesicular neurotransmitter release. It has also been demonstrated that botulinum neurotoxin, which was once believed to exert its activity solely on cholinergic neurons, can, under certain conditions, inhibit the evoked release of several other neurotransmitters (Welch *et al.*, 2000; Durham *et al.*, 2004). These discoveries continue to intrigue basic scientists and clinicians alike, as the therapeutic uses and applications of botulinum neurotoxin appear destined to increase still further in the years to come.

Acknowledgment

Some historical information was provided by Mitchell F. Brin, MD (Allergan, Irvine, CA, USA).

© in this web service Cambridge University Press www.cambridge.org