Botulinum Toxin: From Poison to Pharmaceutical

The History of a Poison That Became Useful to Mankind

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The Poison and Its Effect

Botulinum toxin (BTX) is a neurotoxin produced by Clostridium botulinum under anaerobic conditions. C. botulinum and BTX are best known as the causative agents of ‘botulism’, a serious type of food poisoning. The rod-shaped, gram-positive organism is found ubiquitously in soil and water. BTX is the most potent toxin known to mankind; the LD$_{50}$ in mice after intraperitoneal injection is less than $0.2 \times 10^{-12}$ mol (i.e. 0.2 picomol). Studies on BTX began at the end of the 19th century, but its use in ever widening fields of medicine began in earnest only about 20 years ago.

The Greek term ‘pharmakon’ implies that a drug originates from a poison (potion, remedy). Theophrastus Bombastus von Hohenheim, better known as Paracelsus (1493/94–1541), first described this duality with his dictum ‘alle ding sind gift und nichts on gift; alein die dosis macht das ein ding kein gift ist’ (only the dose makes a remedy poisonous). Precisely this philosophy could be said to describe the slow evolution of this most fearsome poison into a useful drug. From the pioneering work of Dr. Justinus Christian Kerner (fig. 1), who realized that BTX had the potential to be a useful tool in the treatment of various diseases, a considerable amount of research was carried out on the structure and mechanism of the protein. Nowadays, BTX is used in daily practice.

BTX causes degrees of flaccid paralysis by blocking the release of acetylcholine at the nerve terminal (chemodenervation). Because the sweat glands also use acetylcholine as neurotransmitter, BTX also produces anhidrosis.
Fig. 1. Dr. Justinus Christian Kerner was an accomplished romantic poet and official physician in South Germany. Because of his notes, botulism was known as Kerner’s disease in those days.

The prominent clinical features of botulism include multiple bulbar palsies, symmetrical descending flaccid paralysis, a clear sensorium and the absence of fever [1]. Symptoms appear 12–36 h after toxin exposure. They begin with blurred vision, diplopia and dilated pupils, a dry mouth with dysphagia, asthenia, weakness of the upper extremities, constipation, nausea, vomiting and abdominal cramps. These symptoms are followed by increasing muscle weakness, culminating in respiratory failure requiring mechanical ventilation [2].

From Kerner’s Disease to the Detection of Botulinum Toxin

The first recorded case of botulism was in 1735. An epidemic in Southern Germany in 1793 claimed the lives of over half of those patients who became ill after eating smoked but uncooked ham and blood sausages [3]. In 1817, Dr. Justinus Christian Kerner (1786–1862) published in the Tübinger Blätter für Naturwissenschaften und Arzneykunde (fig. 2) a very precise description of the symptoms of patients dying of botulism after the intake of uncooked sausages (see Appendix) [4]. Because of his pioneering work, botulism became known as Kerner’s disease (fig. 3). Interestingly, Kerner was not only the official physician of the district of Baden-Württemberg, Germany, but was also a romantic poet. Because very little was known at that time about infections or microorganisms, Kerner believed a fatty acid to be responsible for the illness [5]. In 1895, 23 of 34 musicians in Ellezelles, Belgium, fell ill after eating a meal of
raw salted ham. Three of the group died of botulism a few days later. Subsequently, Pierre Emile van Ermengem (1851–1932), professor of bacteriology in Ghent, Belgium, found the germ responsible and was able to refute the fatty acid theory of Kerner [6]. Van Ermengem called the germ *Bacillus botulinus* (lat. *botulus* 'sausage*'). When it was discovered that this bacillus belongs to the genus *Clostridium*, it was given the name *Clostridium botulinum*. Incidentally, van Ermengem was a student of Robert Koch (1843–1910), who received the Nobel prize in 1905 for his discovery of the tubercle bacterium.
Fig. 3. The findings of Kerner were confirmed in 1822 by Dr. Weiss who reported 29 patients suffering from botulism under the title ‘Die neuesten Vergiftungen durch verdorbene Würste’ (‘The newest poisonings from spoil sausages’).

From Poison to Pharmaceutical

Before the first World War, Tchitchikine [7] discovered that the toxin of C. botulinum acts as a neurotoxin. For a long period of time, drug research on BTX was restricted to studies in animals. The first quantitative measurement of the toxicity of BTX was made by Burke. He defined the ‘minimal lethal dose’ as the dose of BTX that would kill a 250-gram guinea pig 48 h after intraperitoneal injection [8]. This definition was replaced in 1927 by the so-called LD$_{50}$. 

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which is defined as the amount of toxin which leads to the death of 50% of a population of Swiss Webster mice following intraperitoneal injection [9]. Because of the scale of dilution necessary when using BTX therapeutically, it made more sense to speak in terms of 'units' rather than grams. One unit of BTX was defined as equal to the LD₅₀ (0.2 × 10⁻¹² mol, i.e. 0.2 picomol). BTX's median lethal dose has been determined across several animal species. The sensitivity towards BTX has been found to vary among species. The LD₅₀ in monkeys, for example, is 39 units/kg. Based on these findings in primates, the human LD₅₀ is estimated to lie at approximately 3,000 units (i.e. 0.6 nmol BTX) for a 70-kg adult, if the toxin were injected intraperitoneally.

An important step from poison to medicine was made by Dr. Hermann Sommer in 1920, when he succeeded in purifying the toxin. Further purification work by Carl Lamanna led to the production of the first crystalline form of BTX. This was a crucial step towards the large-scale production of the toxin. During the second World War, several countries had the knowledge and the capacity to produce biological weapons. At US Army Camp Detrick in Maryland, the research of Dr. Edward J. Schantz on the protein structure of BTX laid the groundwork for our present-day experience with this material [10]. Despite this progress, the mechanism of action of BTX remained unclear until 1949. It was Burgen et al. [11], who showed that the block of acetylcholine release by BTX occurred in the presynaptic nerve endings rather than via a postsynaptic blockade of receptors as had previously been believed. In the 1950s, Dr. Vernon Brooks finally suggested a possible therapeutic application of the toxin in the treatment of hyperfunctional muscles.

In the latter part of the 1960s, Dr. Alan Scott, an ophthalmologist at the Smith-Kettlewell Eye Research Institute in San Francisco, searched for a nonsurgical alternative in the treatment of strabismus. His idea of weakening the extraocular muscles with BTX brought him into contact with Dr. Schantz. Scott et al. [12] found that tiny amounts of the toxin injected into the eye muscles of primates with crossed eyes (strabismus) produced muscle movement that was close to normal. These studies on monkeys confirmed the potential of BTX for clinical applications [13]. However, there were a number of practical problems to be overcome before the toxin could be used on a wide-spread basis. The first problem were the antibodies. Schantz realized very quickly that 80% of the potency of the toxin was abolished by antibodies. A second problem was the instability of the protein after dilution. This problem was solved by adding human albumin to the toxin [10]. Scott obtained an Investigational New Drug approval to treat certain disorders with the toxin and did so successfully for years. Deciding BTX deserved wider use, Scott tried to get FDA approval but was unsuccessful on his own. He enlisted the help of a pharmaceutical company to see BTX through FDA approval and to market it for his company, Occulín.
Inc. The pharmaceutical company did not believe BTX would become a major product and was not very enthusiastic about trying to get the drug approved by the FDA. But researchers Steve Carlson and Judy Leon saw the possibilities of using BTX for a range of conditions involving abnormal muscle contraction. BTX was accepted by the FDA for the treatment of strabism, blepharospasm and hemifacial spasm and it went on the market in 1989 under the name ‘oculimum’. Further new applications followed [14]. With a lead time of almost 200 years, Dr. Justinus Christian Kerner’s audacious idea had finally borne fruit!

Other fields of medicine quickly became interested in BTX. It was not long before BTX was used for a wide variety of indications, in particular for the treatment of cramped sphincters in conditions such as anal fissure, vaginism, achalasia and urogenital dystonias [15–18]. Until 1994, BTX was used exclusively to treat muscle disorders. Bushara et al. [19] were the first to suggest a possible indication of BTX in the treatment of hyperhidrosis. They had noticed incidentally that patients treated for hemifacial spasm no longer sweated in the treated area [19]. BTX has now taken its place in the dermatological arsenal, where it is used to treat focal hyperhidrosis [20–23] and hyperfunctional facial lines [24].

Appendix

‘On the 12th of February 1815, a 44-year-old farmer, who was no stranger to alcohol, bought a liver sausage (Leberwurst) and ate it that evening with disgust because it was mouldy, acidic and stank. On the following day, he felt dizzy and suffered from double vision. On the second day, he had difficulties in breathing, could no longer swallow and was extremely hoarse. He called for the surgeon to let his blood. The patient said he “knew he would die and that it was all the fault of that miserable sausage”. On the fifth day, the patient had great difficulties in speaking, despite the fact that he was fully conscious. When the doctor opened his closed eyelids, he found the pupils dilated and the eyes motionless and staring. The patient’s face was reddish, his body hot and the skin dry. His flow of urine had dried up, and the stool did not appear even after an enema. The patient could move his arms, but he said he felt as if they were paralysed. He stumbled when he tried to walk a few steps. In the afternoon of the seventh day, the patient turned pale. He gave a sign that those around him should pray, made some brisk, almost convulsive motions, became peaceful and – stopped breathing! Six days and nineteen hours after eating that miserable sausage.’ [4]

References


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