Defence Science Journal, Vol. 56, No. 2, April 2006, pp. 189-197

SHORT COMMUNICATION

Botulinum Toxin: Bioterror and Biomedicinal Agent

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ABSTRACT

Botulinum toxin is a group of seven homologous, highly poisonous proteins isolated from fermentation of the anaerobic bacterium *Clostridium botulinum*, which naturally occurs in soil and can grow on many meats and vegetables. Botulinum toxin causes neuromuscular disorder called botulism, which is a potentially lethal disease. There are three types of botulism: Food, wound, and infant botulism. It can lead to death unless appropriate therapy is done. Due to the severity and potency of botulinum toxin, its importance as a biological weapon is of major concern to public health officials. Nevertheless, botulinum toxin is also medicament.

Keywords: Botulinum toxin, neurotoxin, *Clostridium botulinum*, biological weapon, terrorism, biological warfare, botulism, neuromuscular disorder

1. INTRODUCTION

Botulinum toxin, a complex protein produced by the anaerobic bacterium *Clostridium botulinum*, is widely recognised as the most potent biological poison. It was earlier known only as a causative agent of a serious and often fatal disease known as botulism, acquired through ingestion of contaminated food.

Botulism is a rare but serious paralytic illness, when the toxin causes paralysis by blocking the presynaptic release of acetylcholine at the neuromuscular junction[1]. Botulinum toxin is a very hazardous natural compound which may be misused as biological weapon as well as the instrument of bioterrorism[2]. Pharmacological properties of botulinum toxin can be utilised in current medicine. Advantage can be taken of its neuromuscular blocking effect to alleviate muscle spasm due to excessive neural activity of central origin, or to weaken a muscle for therapeutic purposes. In therapeutic applications, very small quantities of botulinum toxin type A are injected directly into the selected muscles. Botulinum toxin can be considered as the instrument of terrorism or instrument of biomedicine[3]. Both aspects of botulinum toxin utilisation are discussed. For the past ten years, enormous progress has been made in understanding the action of botulinum toxin at the molecular level, which has opened up the possibility for rational studies of its mechanism of action and active site inhibitor design.

Received 06 November 2005

2. BACTERIUM *CLOSTRIDIUM BOTULINUM*

Clostridium botulinum is the name of a group of bacteria commonly found in soil. These rod-shaped microorganisms grow best in low-oxygen conditions. The bacteria form spores which allow them to survive in a dormant state until exposed to conditions that can support their growth. There are several serologically distinct botulinum toxins, which have slightly different specific effects. These toxins are designated by the letters A through G; only types A, B, E and F cause illness in human beings.

2.1 Botulinum Toxin

All serologically different botulinum toxins are synthesised as a single-chain polypeptides with a molecular weight[5] of approx. 150 kDa. The complete amino acid sequences for the various serotypes are known[6] and regions of sequence homology among the serotypes suggest that all employ similar mechanism of biological action[7]. In the single-chain form, toxins have relatively little potency as neurotoxins. Neurotoxic activation requires a two-step modification in the tertiary structure of the protein[8]. In the first step, the parent chain is cleaved between amino acids 448 and 449. The result is one light chain (amino acids 1-448, approx. 100 kDa) and one heavy chain (amino acids 449-1295, approx. 100 kDa) connected via a disulphide bond. The light chain is associated with one atom of zinc[9]. In this form, the toxin enters the axon terminal. The second activating step, disulphide reduction, occurs only after internalisation by the target cell.

2.1.1 *Mechanism of Botulinum Toxin Action*

The primary target site of botulinum toxin is the cholinergic nerve ending of the neuromuscular junction, where the toxin binds rapidly and prevents the release of acetylcholine. There are three steps in the neurotoxic action of botulinum toxin. First, the toxic protein attaches to the presynaptic nerve membrane. Then, the toxin crosses the presynaptic plasma membrane, following which the toxin inhibits release of vesicle-bounded acetylcholine[10]. Once bound to its receptor, botulinum toxin is productively internalised by receptor-mediated endocytosis. However, before it can act on its intracellular substrate, the toxin must escape the endosomal compartment. During this process, acidification of the endosome results in rearrangement of the toxin and translocation into the cytosol. The final stage of intoxication is inactivation of an intracellular target essential for transmitter release, leading to paralysis. The reason that it is possible to produce sufficient weakness of the muscle to prevent symptomatic spasm, but not completely block voluntary control, may be that more active neuromuscular junctions are more likely to be blocked than the less active junctions. The toxin may also have some action on the central nervous system. Botulinum toxin has been shown to enter the central nervous system in animals by retrograde axonal transport[11].

2.1.2 *Botulinum Toxin Poisoning*

There are the following three main kinds of botulism:

- Foodborne botulism is caused by eating foods that contain the botulism toxin.
- Wound botulism is caused by toxin produced from a wound infected with *Clostridium botulinum*.
- Infant botulism is caused by consuming the spores of the botulinum bacteria, which then grow in the intestines and release toxin.

With foodborne botulism, symptoms begin within 6 hours to 2 weeks (most commonly between 12 h and 36 h) after eating toxin-containing food. Symptoms of botulism include double vision, blurred vision, drooping eyelids, slurred speech, difficulty swallowing, dry mouth, muscle weakness that always descends through the body (first shoulders are affected, then upper arms, lower arms, thighs, calves, etc). Paralysis of breathing muscles can cause a person to stop breathing and die, unless assistance with breathing (mechanical ventilation) is provided. Botulism is not spread from one person to another. Foodborne botulism can occur in all age groups.

Wound botulism, caused by the growth of bacterial cells and release of toxin *in vivo*, is associated

with traumatic wounds and abscesses, and has been reported in drug users such as those injecting heroin or sniffing cocaine. This disease comes from an infection caused by dirty work, dirty skin or dirty drugs. Most patients have used black tar heroin. Initial symptoms are drooping eyelids, blurred or double vision, sore throat or trouble in swallowing. This can lead to complete paralysis and death.

Infant botulism results from a unique infectious disease pathway. Ingested spores of *Clostridium botulinum* germinate, colonise the infant colon, and produce botulinum neurotoxin *in situ*[12,13]. The toxin is subsequently absorbed and carried by the blood stream to peripheral cholinergic synapses, where it binds irreversibly. The light chain is then taken into the cytosol of the neuron, where it blocks the release of acetylcholine by enzymatic cleavage of fusion complex proteins[14]. Clinically, the most important part of the peripheral cholinergic synapses is the neuromuscular junction; the toxin's action results in flaccid paralysis and hypotonia. Preganglionic cholinergic synapses in the autonomic nervous system may also be affected[15].

3. DIAGNOSIS OF BOTULISM

Misdiagnosis of botulism is frequent[16]. It may be confused with stroke, for example, Guillain-Barré syndrome or myasthenia gravis. Several diagnostic tests should therefore be performed to rule out these other syndromes, since waiting for the definitive diagnosis of botulism can take days, and patients need to have treatment immediately. Diagnosis depends on identifying the presence of toxin in blood samples, using some form of antigen-antibody reaction. In the natural disease, the bacterium and/or preformed toxin may be identified in unconsumed food samples.

4. PROPHYLAXIS

Toxoid vaccines against the toxins of types A to F have been produced and evaluated in animal and human studies[17]. Type A toxoid has a product licence in the UK. The present toxoid vaccines require several doses over a period of several weeks to produce protection. Primate studies have also demonstrated passive protection against inhalation or injection of toxin by equine or human immune globulin. The level of protection depends entirely on the stoichiometric relationship between the amount of circulating antibody and the amount of toxin to which an individual may have been exposed.

Vaccination provides a high degree of protection and is commonly administered to laboratory investigators who are at risk of exposure. The current vaccine is pentavalent (A-E) and is available from the Centres for Disease Control and Prevention (CDC Atlanta, USA). The vaccine is administered at 0, 2, and 12 week intervals and requires a booster dose after 1 year to generate long-term protection. A heptavalent vaccine (A-G) is under development by the US Army. In addition, a vaccine made from the recombined binding domain of butulinum toxin-chain (C-fragment) is also under development[18].

5. STABILITY & NEUTRALISATION

Botulinum toxins are rather easily inactivated. In food or drink, heating to 85 °C for more than 5 min is sufficient to detoxify the contaminated food or drinks[19]. In the airborne state, the botulinum toxin is degraded by extremes of temperature or humidity. The rate of decay of aerosolised toxin has been estimated at 1-4 per cent per minute, depending on the weather conditions. Contaminated surfaces should be cleaned with 0.1 per cent hypochlorite solution if these cannot be avoided for the few hours to days that natural degradation would require[20].

6. TREATMENT OF BOTULISM

Emergency hospitalisation is recommended in cases of respiratory problem. The treatment aims to establish a clear airway, aid breathing, give botulinum antitoxin, and provide supportive therapy[21]. If breathing problem starts, intubation (a tube inserted through the nose or mouth into the trachea to provide an airway for oxygen) and mechanical ventilation are given. Intravenous fluids can be given while swallowing difficulties persist. Also, a feeding tube may be inserted in the nose. Antibiotics are often given, but have not been shown to be beneficial always.

Before the development of mechanical ventilators, the respiratory paralysis caused by botulism claimed many more lives than it does today. For example, during 1910-1919, the death rate from botulism was 70 per cent. By the 1980s, the death rate had dropped to 9 per cent, and in 1993, it was less than 2 per cent. But recovery is still slow; assuming the patient receives proper care to ensure continued breathing, recovery occurs only when the affected nerves grow new endings, a process that can take several months, although the length of recovery time varies greatly from case to case. Potassium channel blockers were found to be more effective in antagonising the paralytic action of botulinum toxin. The most promising candidate is 3,4-DAP (diaminopyridine).

7. POTENTIAL BIOLOGICAL WEAPONS FOR MILITARY TERRORISTS

There is a heightened awareness of the threat of biological weapons being used for biological warfare or bioterrorism. Many toxins that may be used biological weapons as such can easily be acquired and mass-produced. Dissemination of aerosols of these biological agents can produce mass casualties. If used by terrorists, these may overwhelm our current public health system.

Some biological agents, such as *Bacillus anthracis* (anthrax) and botulinum toxin, are considered, far more likely than others, to be used as biological weapons[22]. The potential for intentional poisoning with botulinum toxin has come into clearer focus in recent years[23,24]. As many as 17 countries were suspected to include or to be developing biological agents in their offensive weapons[25] programme in 1996, and at present, the number of countries is probably even higher. Botulinum toxin often is included as one of these agents because it is relatively easy to produce and is highly lethal even in small quantities. In August 1995, Iraq revealed that during the Gulf War, 11,200 litre of botulinum toxin was loaded into specially designed SCUD missile war-heads[26]. In addition, before the Aum Shinrikyo used sarin in the 1995 terrorist attack on the Tokyo subway system, the cult had produced botulinum toxin[27].

Research and production, and use of botulinum toxin as a possible biological weapon began at least 65 years ago[28]. The Head of the Japanese Biological Warfare Group (Unit 731) admitted to feeding cultures of *Clostridium botulinum* with lethal effect to prisoners during that country's occupation of Manchuria[29], which began in the 1930s. The US biological weapons program first produced botulinum toxin during World War II. Because of concern that Germany had weaponised botulinum toxin, more than 1 million doses of botulinum toxoid vaccine were made for Allied troops preparing to invade Normandy on D-Day[30].

Although the Biological and Toxin Weapons Convention (1972) prohibits offensive research and production of biological weapons, signatories Iraq and the Soviet Union subsequently produced botulinum toxin for use as a weapon[31]. Botulinum toxin was one of the several agents tested at the Soviet site Aralsk-7 on Vozrozhdeniye island in the Aral sea[32]. A former senior scientist of the Russian civilian bioweapons program reported that the Soviets had attempted splicing the botulinum toxin gene from *Clostridium botulinum* into other bacteria[33]. With the economic difficulties in Russia after the demise of the Soviet Union, some out of the thousands of scientists formerly employed by its bioweapons programme were recruited by the nations attempting to develop biological weapons[34]. Four of the countries listed by the US Govt as state sponsors of terrorism (Iran, Iraq, North Korea, and Syria) have developed, or are believed to be developing, botulinum toxin as a weapon[35,36].

After the 1991 Gulf War, Iraq admitted to the UN Inspection Team to having produced 19,000 litre of concentrated botulinum toxin, of which approx. 10,000 litre were loaded into the military weapons[37]. These 19,000 litre of concentrated toxin are not fully accounted for and constitute approx. 3-times the quantity needed to kill the entire current human population by inhalation of this toxin. In 1990, Iraq deployed specially-designed missiles with a 600 km range; 13 of these were filled with botulinum toxin, 10 with aflatoxin, and 2 with

anthrax spores. Iraq also deployed special 180 kg bombs for immediate use; 100 bombs contained botulinum toxin, 50 bombs contained anthrax spores, and 7 bombs contained aflatoxin[37]. It is noteworthy that Iraq chose to weaponise more botulinum toxin than any other of its known biological agents.

Some contemporary analyses discount the potential of botulinum toxin as a toxin weapon because of constraints in concentrating and stabilising the toxin for aerosol dissemination. But the terrorist use of botulinum toxin in the deliberate contamination of food could produce either a large botulism outbreak from a single meal or episodic, widely separated outbreaks[38]. In the US, the CDC maintains a well-established surveillance system for human botulism based on clinician reporting that would promptly detect such events[39].

8. MEDICINAL USE OF BOTULINUM TOXIN

The therapeutic effects in human beings are primarily due to the blockade of peripheral neuromuscular transmission[40]. Botulinum toxin therapy is invasive but safe and effective for treating strabismus, blepharospasm, hemifacial spasm, adductor spasmodic dysphonia, jaw-closing oromandibular dystonia, and cervical dystonia. It is also used to treat facial tics, swallowing and speech difficulties, migraines, and many other medical conditions. Botulinum toxin is not curative in chronic neurological disorders. The safety of botulinum toxin therapy during pregnancy or lactation, breast feeding, and chronic use during childhood is unknown as well as the long-term effects of chronic treatment. Botulinum toxin should be administered by committed interdisciplinary teams of physicians and related healthcare professionals with appropriate instrumentation. The physician administering this drug should be trained in its use and qualified to manage any complications.

Contraindications of the use of botulinum toxin are allergy to the drug and infection or inflammation at the proposed injection site. Relative contra-indications include diseases of neuromuscular transmission, coagulopathy, and inability of the patient to cooperate.

8.1 Ophthalmic Disorders

Botulinum toxin is effective as an alternative to surgery to regulate the eyes of selected patients with congenital or acquired strabismus[41]. The toxin appears to be more effective in esotropia of small-to-moderate angles than in exotropia, vertical deviations, or large-angle deviations. Botulinum toxin may be indicated in certain ocular conditions where surgery is inappropriate such as acute thyroid ophthalmopathy.

Botulinum toxin may prevent contracture of antagonist muscles in cases of extraocular muscle palsy from which some recovery is expected. In these cases, single binocular vision may be enabled or enhanced during the recovery phase, and late contractures, that would require surgery, may be prevented. Botulinum toxin is also effective in the long-term management of patients with essential blepharospasm, and it has become the treatment of choice. Symptoms are controlled in most patients by injecting the toxin in multiple sites in the periocular and facial muscles[42].

8.2 Neurological Disorders

Botulinum toxin treatment of neurological patients requires proper identification of the pathologically hyperactive muscles, preferably by electromyography. In focal dystonias, most of which respond poorly to conventional therapy, botulinum toxin injection may be the treatment of choice. In more generalised dystonias, however, oral medication may be tried before botulinum toxin. In generalised disorders, botulinum toxin injection is indicated only for the treatment of particularly severe focal abnormalities, because injection of multiple muscle groups would require unacceptably high doses of toxin[43]. Botulinum toxin is an accepted therapy of cervical dystonia (spasmodic torticollis), characterised by asymmetric muscle spasms in the neck and oromandibular dystonia (orofacial dyskinesia, Meige syndrome) consists of continuous, bilateral, asynchronous spasms of muscles of the face, jaw, pharynx, tongue, and, in some severe cases, neck, larynx, and respiratory system[44]. Palliation with repeated injections of botulinum toxin may be appropriate and effective also in hemifacial spasm[45].

8.3 Voice & Speech Disorders

Botulinum toxin therapy for voice and speech disorders requires the involvement of an interdisciplinary team, including an otolaryngologist, a speech-language pathologist, a neurologist, and a physician skilled in regional electromyography. Voice laboratory facilities should be available to assure valid diagnosis and to document and quantify voice and speech function before and following the treatment[46,47].

9. COSMETIC USE OF BOTULINUM TOXIN

Botulinum toxin A has had a lot of publicity recently for its cosmetic use in facial wrinkles[48] and makes beneficial poisoning[49]. Botulinum toxin type A is frequently used to smooth hyperkinetic lines in the periocular and forehead areas of the upper face, but it has been used less frequently for indications in the lower face and neck[50]. Small doses of the toxin are injected into the affected muscles. As happens with botulism, the toxin binds to the nerve endings, blocking the release of the chemical acetylcholine, which would otherwise signal the muscle to contract. The toxin thus paralyses or weakens the injected muscle but leaves the other muscles unaffected. The injections have been shown to be generally safe. But, those wrinkles will come back. The effects of the treatment are temporary and it is possible to repeat it after about half-a-year.

10. CONCLUSIONS

Botulism-causing *Clostridium botulinum* bacteria and their spores are found everywhere. Prevalent in soil and marine sediments worldwide, their spores are often found on the surfaces of fruits and vegetables, and in seafood. The bacteria and spores themselves are harmless; the dangerous substance is the toxin produced by the bacteria when they grow. There are seven varieties of botulinum toxin, designated by the letters A through G. Botulinum toxin is the most poisonous substance known. Once in the body, the toxin binds to nerve endings at the point where the nerves join muscles. This prevents the nerves from signaling the muscles to contract. The result is weakness and paralysis that descends from the cranium down, affecting, among other things, the muscles that regulate breathing. Respiratory failure, secondary to paralysis of the respiratory muscles, can lead to death unless appropriate therapy is promptly initiated. Due to the severity and potency of this neurotoxin, its importance as a biological weapon is of major concern to public health officials. The use of botulinum toxin as a medicinal agent for correcting cholinergic disorders is very promising[51].

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