Botulin Toxin: A Weapon in Terrorism

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Clostridium botulinum, the causative agent of botulism is an anaerobic, spore forming gram-positive bacillus. *C. botulinum* causes three types of botulism; foodborne botulism, wound botulism, and infant botulism. Most strains of the bacterium produce a potent, muscle-paralyzing neurotoxin. 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.

ABBREVIATIONS: BSL = biosafety levels; CDC = Centers for Disease Control and Prevention; kDa = kilodalton; MW = molecular weight; PNS = peripheral nervous system.

INDEX TERMS: bioterrorism; botulism; *Clostridium botulinum;* neurotoxin.

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Focus Continuing Education Credit: see pages 40 to 42 for learning objectives, questions, and application form.

LEARNING OBJECTIVES

See learning objectives #8 through #14 on page 40.

Clostridium botulinum, the causative agent of botulism, is an obligate anaerobic, gram-positive bacillus occurring singly or in pairs. It produces grey-white colonies, which are usually beta-hemolytic on anaerobic blood agar. The organism is motile and lipase positive on egg yolk agar.[1-3] It is ubiquitous in nature found in soil, water, and the environment, and therefore is not considered part of normal human flora. *C. botulinum* produces spores (usually subterminal) that can survive in a dormant state for long periods of time until favorable environmental conditions return.[1,3,4] The bacterium itself is usually harmless except in infants and immunocompromised individuals.[5] Under anaerobic conditions however, the bacterium can produce a lethal neurotoxin, which is released upon cell lyses.[3,5,6] These spores are very resistant to heat and chemicals and can survive for several hours at temperatures of 100 °C. In order to kill the spore, exposure to moist heat at 120 °C is required.[7]

In the U.S., botulism has been associated with improperly home canned foods, such as vegetables, fruits, meat, and fish. There are seven types of toxins associated with *C. botulinum*; A, B, C, D, E, F, and G. Most cases of human poisoning however are associated with types A, B, E, and F.[2,7,8] Less than 200 cases of botulism are reported each year in the U.S.[8,9] Of these cases, approximately 25% are foodborne, 72% are infant botulism, and the rest are wound botulism.[8]

CLINICAL MANIFESTATIONS

Foodborne botulism

This type of botulism results when a person ingests preformed toxin present in contaminated foods, which leads to illness within a few hours to days. Only foodborne botulism is considered a public health emergency since the contaminated food may still be available to the public.[10] Detection of botulinum toxin in epidemiologically implicated foods, with or without isolating the organism, is useful for ascertaining what food was involved in the outbreak.[2]

Signs and symptoms

The onset of symptoms usually begins with cranial nerve involvement. Symptoms include double vision (diplopia) and/or blurred vision, drooping eyelids (ptosis), dizziness, slurred speech (dysarthria), difficulty swallowing (dysphagia), dry mouth, nausea, vomiting, severe constipation or diarrhea, abdominal pain, and muscle weakness that always descends through the body: neck, shoulders upper arms, lower arms, thighs, calves, etc. Paralysis of breathing muscles can result in death, unless assistance with breathing (mechanical ventilation) is provided.[7,8,10,11] The symptoms are due to muscle paralysis caused by the bacterial toxin.

Wound botulism

The rarest form of botulism, wound botulism, occurs when wounds become infected with *C. botulinum* spores that produce the toxin. Within anaerobic conditions the spores germinate, replicate, and secrete the neurotoxin.[12] In the U.S., this type of botulism is becoming common in intravenous drug users, particularly heroin users.[7,8]

Signs and symptoms

Symptoms of wound botulism are virtually the same as foodborne botulism except the individual does not experience gastrointestinal complications. The skin should be carefully examined for wounds. Wound botulism usually occurs after traumatic injury involving contamination with soil, after cesarean delivery, and in chronic intravenous drug abusers.[7]

Infant botulism

Infant botulism occurs in susceptible infants due to ingestion of spores or intestinal colonization by *C. botulinum*. This type of botulism is not considered a public health emergency since the infant is not consuming foods with the toxin but ingesting the spores (which are ubiquitous in nature). The spores grow and produce the neurotoxin in the infant's intestine. A common food source implicated in infant botulism is honey. Therefore, infants and children less than 12 months old should not be fed honey. After age one, the higher acid levels in the baby's stomach and sufficient production of normal flora in the intestinal tract will prevent the germination of these spores.[5,6]

Signs and symptoms

Infants with botulism appear sluggish, feed poorly or stop eating, are constipated, have a weak cry, and poor muscle tone. These symptoms can then be followed by more severe nervous system involvement.[5,8]

INCUBATION PERIODS

Foodborne botulism is typically seen in adults but can occur in all age groups. Symptoms usually occur within 12 to 36 hours after ingestion of the toxin-containing food. Incubation, however may vary from a few hours to several days.[7] Symptoms in wound botulism develop between day 4 and day 14 after exposure while symptoms may be evident between day 3 and day 30 in infant botulism.[5]

PATHOGENESIS

The botulinum toxin

Seven serologically distinct but related neurotoxins (A-G) are produced by the bacterium. *C. botulinum* produces the most potent neurotoxin known to mankind. It is 100,000 times more toxic than sarin nerve gas, the agent used in the terrorist attack on three different Tokyo subway lines in 1995.[12,13] The botulinum toxins are very similar in structure and function to the tetanus toxin, demonstrating 30% to 40% sequence homology, but vary considerably in their clinical effects. *C. botulinum* toxins target cells in the peripheral nervous system, while *Clostridium tetani* toxins target cells located in the central nervous system.[4,14] The toxin is absorbed through the mucous membranes of the respiratory tract or through the walls of the gastrointestinal tract. After entry into the blood stream, the toxin binds irreversibly to the PNS.[3] The botulinum toxin acts primarily by binding to synaptic vesicles of cholinergic nerves and prevents the release of acetylcholine at the neuromuscular junction thereby inhibiting muscle contraction and causing paralysis.[2,4,14]

Mortality rates are 5% to 10% and may reach 15% for foodborne and wound botulism.[15] A steady decline in mortality rates has been observed over the past 50 years due to improvements in intensive care support combined with the rapid administration of antitoxin.

Botulinum toxin is synthesized as a single polypeptide chain with a molecular weight (MW) of approximately 150 kDa. In this form the toxin has a relatively low potency. The toxin is cleaved by a bacterial protease (or possibly by gastric proteases) to produce two chains: a light chain (the A fragment) with a MW of 50 kDa; and a heavy chain (the B fragment), with a MW of 100 kDa. A disulfide bond connects the two chains. The A fragment of the cleaved toxin, on a mw basis, becomes the most potent toxin found in nature.[4,12,14]

CATEGORY AND BIOSAFETY LEVELS

Clostridium botulinum is classified by the CDC as a Category 'A' organism. Due to the fact the toxin is very dangerous to handle, exposure to materials contaminated with the toxin is the laboratory's primary safety concern. BSL-2 practices, equipment, clothing, and facilities are recommended for the handling of all suspected materials.[16] Manipulation of contaminated specimens and materials should be avoided. If avoidance cannot be prevented, then BSL-3 practices should be used.[3] *C. botulinum* is not transmitted person to person. Even if one develops symptoms of botulism, it is not contagious and therefore is not communicable.[11]

SPECIMEN COLLECTION

The CDC has established guidelines for appropriate specimen collection and transport conditions for *C. botulinum.* These guidelines are outlined in Table 1.

DIAGNOSIS

Diagnosis of clinical intoxication known as botulism is based on the patient's history including food history and physical examination. Since symptoms of botulism often mimic other neurological conditions such as Guillain-Barre syndrome, stroke, and myasthenia gravis, the history and physical examination may not be enough to conclude botulinum poisoning.[8,17] Clinical diagnosis requires demonstration of botulinum toxins in the patient's serum or stool samples or direct isolation of the bacterium. Isolation and identification of this organism is by conventional cultural biochemical procedures and the toxin neutralization test.[18] Demonstration of the botulinum toxin in the patient's serum or stool is the most direct way to confirm the diagnosis. The most reliable test of toxin is the mouse neutralization test. The type of botulinum toxin is determined by neutralizing the bioactivity of the toxin by injected type-specific antitoxin into mice, observing any signs indicating botulinum poisoning.[2,8,12] Cultures should also be performed on stool and food specimens of suspected foodborne botulism, wound cultures in suspected wound botulism cases, and stool specimens in suspected cases of infant botulism.

Table 1. CDC guidelines for specimen collection and transport of *Clostridium botulinum*

Specimen	Transport conditions
Enema fluid Collect 20 mL of fluid. Purge with a minimum of sterile, non-bacteriostatic water to minimize dilution of toxin.	4 °C
Food sample Collect 10 to 50 grams. Submit food in original container. Place each container into leakproof, sealed transport device.	4 °C
Nasal swab For aerosolized release, collect nasal swab for *C .botulinum* and serum for toxin testing.	Room temperature
Serum Collect 10 mL of serum.	4 °C
Stool Collect 10 to 50 grams into a sterile, leakproof container.	4 °C
Other In an intentional release, collecting environmental surface samples on swabs may be useful.	4 °C

BIOLOGICAL WEAPON

Bioterrorism can be defined as the intentional use of a biological agent such as microorganisms, toxins, or organic biocides to create fear or illnesses for purposes of intimidation, gaining advantage, or interruption of normal activities.[12] *C. botulinum* poses a major threat due to its extreme potency and lethality. It is the most potent neurotoxin known to mankind. For this reason, it is a potential bioweapon and was one of the first agents to be considered as a biological weapons agent. Researchers in the U.S. investigated the possibility of weaponization of botulinum toxin early during World War II and continued the study for two decades until the offensive research on biological warfare was dismantled.[19] Extremely small quantities can be lethal. A lethal dose for a 70 kg human is estimated to be 0.7 to 0.9 micrograms if inhaled and 70 micrograms if ingested.[20] Botulinum toxin has been developed as an aerosol weapon by several countries however; no human data exists on the effects of inhaling botulinum toxin.[21] It is believed it may resemble the foodborne illness in symptoms and severity. It is estimated that one gram, if aerosolized, can kill 1.5 billion people.[22]

In a bioterrorist attack, inhalation of the toxin or ingestion of the toxin via contaminated food or water is the most likely route of exposure. Since *C. botulinum* is found in soil worldwide, terrorists with the technical capacity to grow cultures of the bacterium, and harvest and purify the toxin could therefore use it as a biological weapon.[21] Individuals who inhale or ingest the toxin may succumb to respiratory paralysis. Contaminating food and/or water with botulinum toxin could cause a devastating event.

IDENTIFICATION OF ISOLATES

Properly collected specimens such as serum, gastric contents or vomitus, feces or return from sterile water enemas, or food samples are to be referred to designated testing laboratories. Prior to the shipment of any botulism-associated specimen, the designated laboratory must be notified and approved by the State Health Epidemiology Office.[23] It is important to inform the state health department laboratory director as to the identity of the suspected biologic agent.

VACCINES AND TREATMENT

Currently there is no vaccine available for botulism that has been tested and proven safe for the public.[5] An antitoxin however is available. The CDC maintains the national botulism antitoxin supply. A physician must contact the CDC through the state health department if treatment with antitoxin is necessary. Fortunately, the CDC can quickly deliver the antitoxin to a physician anywhere in the country. Botulism can be fatal; therefore cases should be considered a medical emergency. Treatment of botulism includes supportive care, especially intensive care and ventilatory support, antimicrobial therapy, surgical debridement for wound botulism, and use of a trivalent antitoxin. Gastric lavage and enema should be attempted in cases with foodborne botulism and suspected food exposure to remove unabsorbed toxin from the intestinal tract.[12] Currently, the antitoxin is not routinely given for treatment of infant botulism.[5]

PREVENTION

It is essential that both home canned and commercially canned foods are prepared properly, using the appropriate heat and pressure. While spores can survive two hours at 100 °C, boiling food at 120 °C will kill the spores. Since the toxin itself is heat-labile, cooking food for 30 minutes at 80 °C protects against botulism.[7] While home-canned food is the most common source for botulism, commercially prepared foods have been implicated in about 10% of the cases. Therefore, any food container that bulges or swells may contain gas produced by *C. botulinum* and should not be opened or tasted.[7]

Although the public can take proper precautions to prevent *C. botulinum* poisoning, an intentional exposure such as in a bioterrorist attack is not as easily avoidable. Unfortunately, if foods and/or the water supply were contaminated, a tremendous number of people would become ill before health officials can take the appropriate steps in trying to contain the source. The use of *C. botulinum* toxin as a biological weapon can cause devastating consequences resulting in many casualties unless the diagnosis is made immediately and treatment is administered promptly.

CONCLUSION

It is ironic *C. botulinum* toxin is considered a bioweapon at this moment in history since it has become the first biological toxin to become licensed for treatment of human disease. Currently in the U.S., botulinum toxin is licensed for treatment of cervical torticollis, strabismus, and blepharospasm associated with dystonia. It is also used 'off label' for a variety of more prevalent conditions that include migraine headache, chronic low back pain, stroke, traumatic brain injury, cerebral palsy, achalasia, various dystonias, and BOTOX treatment.[20]

Although there are certain benefits associated with the use of *Clostridium botulinum* toxin, it poses a serious health threat especially in an intentional attack where thousands of people may be affected. Public health officials, medical staff, and most important, clinical laboratory scientists must be prepared to react, diagnose, confirm laboratory findings, and try to contain the exposure in an actual event. Bioterrorism preparedness plans have been implemented throughout the country in the event of a biological attack. Unfortunately since the devastating events that took place on September 11, 2001, these plans will be in existence indefinitely.

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