Botulinum Toxin

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INTRODUCTION

Botulinum toxin is a neurotoxic protein produced by the bacterium Clostridium botulinum. Although botulinum toxin is the cause of the disease botulism and can be used in a terrorist attack, there are also many other uses for botulinum toxin. Botox, a derivative of botulinum toxin, is used for cosmetic purposes. Botulinum toxin is also used in medicines to control certain conditions marked by involuntary muscle contractions. The objective of this paper is to present a strong review of botulinum toxin so that one can see all the good and bad that is botulinum.

Clostridium botulinum is an anaerobic, Gran positive spore forming, rod shaped organism. The spores are heat-resistant and are widely distributed in nature. They occur in both cultivated and forest viscera of crabs and other shellfish (FDA, 1992). *Clostridium botulinum* was named, in the late 1700s, after a sausage (botulus being the Latin word for sausage), when 13 people ate from the same sausage and got the disease. In 1949 botulinum toxin was discovered to be a blocker of neuromuscular transmissions (Caya et al, 2004).

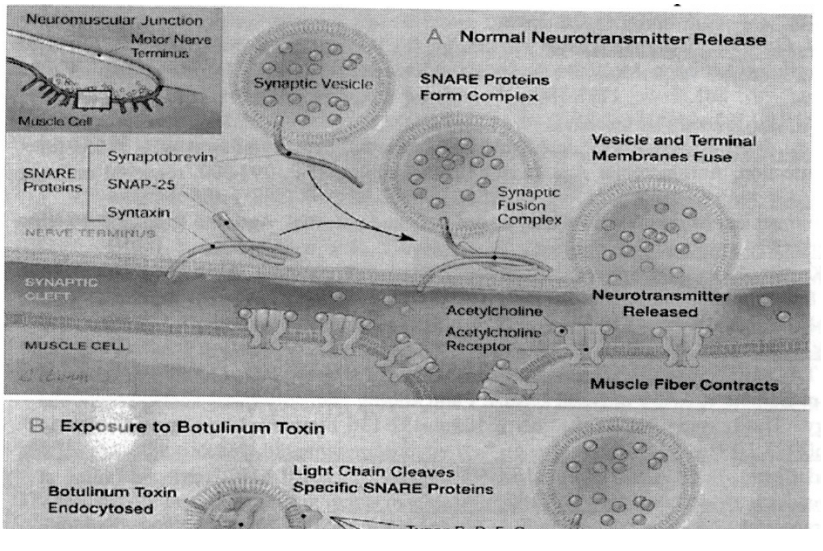

Figure 1. (Arnon S.)

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Botulinum toxin is "the most poisonous substance known," says Stephen S. Arnon, M.D., head of the Infant Botulism Prevention Program as the California Department of Health Services (Vanhelova, 1995), with a lethal dose of about 200-300 pg/kg, meaning that one hundred grams could kill every human on earth.

There are seven antigenically distinct serotypes associated with C. botulinum; A, B, C, D, E, F, and G. Most cases however are associated with types A, B, E, and F. The toxin works by entering the release of the neurotransmitter substance, acetylcholine, (see illustration) which initiates the signal for muscle contraction. In short it results in a progressive flaccid paralysis.

The classic symptoms of botulism include blurred vision, double vision, drooping eyelids, slurred speech, difficulty swallowing, dry mouth, and muscle weakness. Infants with botulism appear lethargic, feed poorly, are constipated, and have a weak cry and poor muscle tone. These are all symptoms of the muscle paralysis caused by the bacterial toxin. If untreated, these symptoms may progress to cause paralysis of the arm, legs, trunk and respiratory muscles (Josko, 2004). In food-borne botulism, symptoms generally begin 18 to 36 hours after eating a contaminated food, but they can occur as early as 6 hours or as late as 10 days. There are three clinical forms of botulism poisoning, distinguished by the manner in which they are obtained: food-borne botulism, wound botulism and infant botulism. Less than 200 cases of botulism are reported each year in America. Of these cases approximately 25% are food-borne, 72% are infant botulism, and the rest are wound botulism.

Food botulism is the classic case of this disease. It occurs after the ingestion of preformed neurotoxin in inadequately processed food. This form of botulism is still the most widespread form worldwide. The most common culprits in food-botulism are improperly prepared home-canned foods, especially low acid vegetables such as corn, carrots, asparagus, and beans. The reason home-canned foods are susceptible to botulinum is because the spores are very durable and can withstand temperatures as high as 120 °C. This is especially problematic in high altitudes where the boiling temperatures is <100 °C (Talaro, 2002).

In infant botulism the toxin is produced when C. botulinum spores germinate in the intestines. Although we ingest these spores all the time, because they are nearly everywhere, infants are more susceptible due to the lack of a protective gastrointestinal bacterial flora and in part due to the relatively reduced levels of clostridial-inhibiting bile acid as compared to adults (Cox and Hinkle, 2002).

Wound botulism is simply the entry of the bacterium through a break in the skin. A significant number of new cases involve intravenous drug use; needle puncture sites may become infected with organisms, including C. botulinum. Under conditions of tissue necrosis and anaerobiosis, such as those seen in a subcutaneous abscess, C. botulinum spores can germinate and produce the neurotoxin, which will make its way to a neuromuscular junction of the skeletal muscles (Caya et al, 2004).

The respiratory failure and paralysis that occur with severe botulism may require a patient to be on a breathing machine (ventilator) for weeks, plus intensive medical and nursing care. After several weeks, the paralysis slowly improves. If diagnosed early, foodborne and wound botulism can be treated with an antitoxin which blocks the toxin from circulating in the blood. This can prevent patients from worsening, but recovery still takes many weeks. Physicians may try to remove contaminated food still in the gut by inducing vomiting or by using enemas. Wounds should be treated, usually surgically, to remove the source of the toxin-producing bacteria. Good supportive care in a hospital is the mainstay of therapy for all forms of botulism. Currently, antitoxin is not routinely given for treatment of infant botulism (Sobel, 2005).

Therapy for botulism consists of supportive care and passive immunization with equine antitoxin. Optimal use of botulism antitoxin requires early suspicion of botulism. Timely administration of passive neutralizing antibody will minimize subsequent nerve damage and severity of disease but will not reverse existent paralysis (Arnon, et al 2001).

BIOTERRORISM

The potency of botulinum has caused it to become a very important subject I bioterrorism. The United States has been investigating the possibility of the weaponization of botulinum toxin during the early years of World War II. Botulinum toxin has been developed as an aerosol weapon by several countries. It is estimated that one gram of aerosolized toxin can kill 1.5 billion people. Later on, after the 1991 Persian Gulf War, Iraq admitted to the United Nations inspection team to having produced 19,000 L of concentrated botulinum toxin, of which approximately 10,000 L were loaded into military weapons. These 19,000 L of concentrated toxin are not fully accounted for and constitute approximately 3 times the amount needed to kill the entire current human population by inhalation. It is noteworthy that Iraq chose to weaponize more botulinum toxin than any other of its known biological agents (Shulka and Sharma, 2005).

In the event of an attack, early recognition of outbreaks of botulinum depends on heightened clinical suspicion. Aerosol dissemination may not be difficult to recognize because a large number of cases share a common temporal and geographical exposure and will lack a common dietary exposure. However, identification of the common exposure site initially may be difficult because of the mobility of persons exposed during the incubation period. Though botulism and botulinum toxin are not contagious a microbe intentionally modified to produce botulinum toxin might be contagious.

Extremes of temperature and humidity will degrade the toxin, while fine aerosols will eventually dissipate into the atmosphere. Depending on the weather, aerosolized toxin has been estimated to decay from between less than 1% to 4% per minute. At a decay rate of 1% per minute substantial inactivation of toxin occurs by 2 days after aerosolization.

The potency of botulinum toxin has led to speculation that it might be used to contaminate a municipal water supply. This scenario is unlikely for two reasons. First, botulinum toxin is rapidly inactivated by standard potable water treatments (e.g., chlorination, aeration). Second, because of the slow turnover time of large-capacity reservoirs, a comparably large inoculum of botulinum toxin would be needed. In contrast with treated water, botulinum toxin may be stable for several days in untreated water or beverages. Any outbreak of botulism, should bring to mind the possibility of bioterrorism, but certain features would be particularly suggestive.

The following are features of an outbreak that would suggest a deliberate release of botulinum toxin:

- Outbreak of a large number of cases of acute flaccid paralysis with prominent botulinum palsies
- Outbreak with an unusual botulinum toxin type (i.e., type C, D, F, or G, or type E toxin not acquired from an aquatic food)
- Outbreak with a common geographical factor among cases (e.g., airport, work location) but without a common dietary exposure (i.e., features suggestive of an aerosol attack)
- Multiple simultaneous outbreaks with no common source

Therapeutic botulinum toxin represents an impractical bioterrorist weapon because a vial of the type-A preparation currently licensed in the United States contains only about 0.3% of the estimated human lethal inhalational dose and 0.005% of the estimated lethal oral dose (Arnon et al, 2001).

Recognition of a covert release of a finely aerosolized botulinum toxin would probably occur too late to prevent additional exposures. When exposure is anticipated, some protection may be conferred by covering the mouth and nose with clothing such as an undershirt, shirt, scarf, or handkerchief. In contrast with mucosal surfaces, intact skin is impermeable to botulinum toxin.

In the United States, an investigational pentavalent (ABCDE) botulinum toxoid is distributed by the CDC for laboratory workers at high risk of exposure to botulinum toxin and by the military for protection of troops against attack. A recombinant vaccine is also in development. The pentavalent toxoid has been used for more than 30 years to immunize more than 3000 laboratory workers in many countries. Immunization of the population with botulinum toxoid could in theory eliminate the hazard posed by botulinum toxins A through E. However, mass immunization is neither feasible nor desirable for reasons that include scarcity of the toxoid, rarity of natural disease, and elimination of the potential therapeutic benefits of medicinal botulinum toxin. Accordingly, pre-exposure immunization currently is neither recommended for nor available to the general population. Botulinum toxoid induces immunity over several months and, so, is ineffective as post exposure prophylaxis (Dressler and Hallet, 2006).

Due to the fact that current antitoxins are ineffective once the botulinum toxin entered the neuronal cells, it is essential that the development of a practical post-exposure prophylaxis takes place. One possibility could be the use of photo-chemically irradiated botulinum toxin in the presence of micronutrient riboflavin (vitamin B2) to result in oxidation of the toxin. Encouragingly, one study has shown significant protection using this method. At this stage more research is being done and the findings will be reported in due time (Eubanks, et al, 2005).

Another possibility for vaccinating against botulinum toxin is the inoculation with a plasmid that produces a fragment C of botulinum toxin inside the body. Once the harmless fragment is transcribed and translated, the body will elicit an immune response against it, just like a standard vaccine. This has only been tried in mine but still has great potential. Because the production of a DNA vaccination does not require botulinum toxin, only the plasmid that produces a response, it would be a great solution for the lab workers that would otherwise prepare the vaccine in unsafe conditions (Shyu, et al 2000).

MEDICAL/COSMETIC USES

Most things with destructive powers, if harnessed, can be used for great advancements in human civilization. Botulinum toxin (BTX) is no exception, with uses that range from treating excessive eye contractions to cosmetically taking away frown lines. The treatments gained FDA approval in 1989 and the use of cosmetic Botox was approved in April 2002.

Strabismus (squint), blepharospam, hemifacial spasm, and glabellar (forehead) lines are all problems that occur from over active muscles. The effect of BTX is to weaken the muscle to the point where the muscle will only contract when the individual wants it to. Although the FDA has approved for these conditions, and the National Institute of Health consensus conference of 1990 also included BTX as safe and effective therapy for the treatment of adducter spasmodic dysphonia, oromandibular dystonia and cervical dystonia, there are many off label uses. Off label uses include the treatments of: facial wrinkles other than glabellar lines, migraine headaches, tremor disorders, sphincter dysfunction and other spasticity disorders (Klein, 2004).

Adductor spasmodic dysphonia (ADSD) is defined as a movement disorder primarily affecting voice production. Voice characteristics of ADSD include a strained-strangled, harsh, low mono-pitch voice quality with stoppages and pitch breaks resulting from hyperadduction of the vocal folds. Botox is the treatment choice for ADSD and the overall efficacy has well been established. However, Botox injections do not result in speech intelligibility similar to that of normal, non-ADSD speakers (Bender et al, 2004).

Most of the time when referring to the uses of botulinum toxin we mean type-A (Botox), but type-B (Myobloc) has also gained approval by the FDA (for cervical dystonia not cosmetic use) and a comparison must be made so that we understand the benefits of both, and use the better of the two for our advantage. There were several studies done to try to see the effects of type-B injections for facial rhytides. Botox was also found to have a longer duration of effect and Myobloc was found to have a quicker onset of action but the duration of effect is dependent on dose. Another important feature that myobloc has is, if a person develops neutralizing antibodies that prevent clinical effects from one type of botulinum they could still have the other type of work. Resistance has been reported in 3% to 5% of patients with cervical dystonia who were treated with large doses of botulinum type-A therefore type-B will be a good option for them. Whatever the differences are, type-B is found to be safe and effective and with further trial and

clinical experience it is conceivable that each type will have its own set of indications (Sadick and Matarasso, 2004).

There are some uses for BTX that are under experiment. One study is proposing promising results for obesity treatment. The pilot study has assessed eight subjects with severe obesity. The subjects were injected with 500 UI of BTX-A in the gastric antral region at ten different points. The treatment works by reducing gastric mobility therefore leading to early satiety. Overall, a single dose of BTX-A was well tolerated by all eight patients and a reduction in weight was shown at one month. By the third month a subgroup of patients showed further weight loss (Albani et al, 2005).

Another investigated use of BTX-A is the treatment for non-neurogenic detrusor over activity (overactive bladder). Twenty patients with this bladder problem were given suburothelial injections of 200 UI of BTX-A. The injection was performed at 40 sites of the posterior and lateral walls of the urinary bladder. Although the bladder capacity was increased, the bladder sensation was greatly impaired and the postvoid residual urine volume increased significantly. These unfavorable results might prevent the wide spread use of this therapy, but is still a good option for people who cannot tolerate the usual treatments of antimuscarinic drugs or intravesical vaniloid therapy. The next step would be to adjust the doses in order to help perfect the therapy (Kuo, 2005).

Botulinum toxin was initially intended to treat disorders characterized by excessive muscle contractions. It was noted to also alleviate pain. After studying the effects on pain, botulinum toxin was found to not only block acetylcholine but also block one or more pain neurotransmitters. This new found use is being researched for therapy of migraine and tension-type headaches. The studies done did not have compelling results and more research needs to be done (Schulte-Mattler and Martinez-Castrillo, 2006).

In order to be effective botulinum toxin injections must be administered into the muscle rather than interstitial tissue. Electromyogrophy (EMG) and ultrasonography are two guidance techniques that can be used to ensure proper chemodenervation. Chemodenervation refers to use of a chemical to prevent a nerve from stimulating its target muscle. The advantages of EMG guidance are precision, safety and less chance of side-effects. EMG guidance is used for cervical muscles and deep or small limb muscles. Ultrasonography is used for injections in the urinary system and salivary glands (Pathank, et al, 2006).

As of 2006, Botox injection is the most common cosmetic operation in the United States. It is used for facial wrinkles by weakening or paralyzing the muscles that cause facial rhytides. Though the effect is temporary, it is extremely popular because it has a low risk of side effects and is an easy technique to acquire.

CONCLUSION

There are some difficulties that prevent or slow advancement in the fields that are part of botulinum toxin. First of all, the danger involved with handling the toxin does not allow rapid changes in medicine. Second, the distribution of mass vaccination is almost impossible because the small market size combined with the high price of development (Casadevall, 2002). Another reason that might be stopping mass vaccination is the inevitability of attack, meaning that even if money is spent on defense against botulinum toxin type-A the terrorists will just attack with

another one of the seven types. Plus they can always use a different agent such as anthrax or any other biological weapon.

The ability of botulinum toxin to be used both in a bioterrorist attack and in numerous medical fields is what makes it so amazing. The fact that the bacterium Clostridium botulinum is so durable and can be found all over the world shows that society cannot just ignore it and hope for the best. Research must continue in the fields of medicine and in the fields of prevention.

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