BOTULISM: A FREQUENTLY FORGOTTEN OLD MALADY

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SUMMARY

A frequently forgotten old malady called botulism has been recognized for more than a century. This ailment occurs worldwide, afflicts human of all age groups from infants to elderly and affects Oriental people more often in several regions of China. Occurrence in Taiwan is uncommon, and therefore, it is often overlooked. The outbreaks of human botulism in various regions of the world, the clinical types, the molecular mechanisms, and the electrophysiologic findings will be highlighted. [International Journal of Gerontology 2007; 1(3): 118–124]

Key Words: Botox, botulinum neurotoxin, botulism, Clostridium botulinum, paralysis

Introduction

Botulism, a Latin name for sausage, was first reported by van Ermengem in 18971. He eloquently observed that in a group of musicians who developed a unique clinical syndrome of autonomic dysfunction with ocular and bulbar weakness, the rapid and subsequent descending paralysis led to death within 16 to 60 hours after ingestion of ham. He also noted that unlike myasthenia gravis, the pupillary constriction of eyes was impaired in this syndrome. A century later, botulism was redefined as a neuroparalytic illness caused by the action of an exotoxin, the botulinum neurotoxins (Botox) that inhibit the release of acetylcholine at the peripheral nerve endings, subsequently blocking the presynaptic neuromuscular transmission2–5. Thus, the paralysis of muscles, which involves the ocular, bulbar and skeletal muscles and eventually the respiratory muscles, may be widespread, leading to death or moribund outcome. Until three decades ago, the chemical and molecular structures of botulinum neurotoxin and the mechanisms of action were identified6–8.

Botox is a mixture of several protoplasmic proteins6,7,9–17 that are released from cells after autolysis18. The toxins are resistant to low pH and are readily destroyed by alkali, such as 0.1 N sodium hydroxide. The neurotoxin is a unique protein produced by an obligate anaerobic, spore-forming, Gram-positive, rod-shaped bacterium called Clostridium botulinum (C. botulinum). C. botulinum has a widespread distribution in the terrestrial and marine environments throughout the world. To date, there are more than eight types of botulinum neurotoxins recognized, based on their distinct antigenicity. They are types A, B, C1, C2, D, E, F, and G10–21. Most colonies of C. botulinum in culture produce only one single type of neurotoxin. The exceptions are types C and D, and types A and F, that can be produced by the same colony. Different cultural characteristics of C. botulinum has been identified. The germination of the spores, the outgrowth, and toxin production by C. botulinum do not occur in food with pH 4.5 or less.

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Accepted: March 15, 2007
Molecular Properties of Botox

The chemical structures and molecular properties of Botox have been described in detail in a number of articles. By using polyacrylamide gel electrophoresis with sodium dodecyl sulfate, all types of Botox, except type E, showed a similar disulfide-linked di-chain molecular structure. The heavy chain (H-chain) is the larger subunit with molecular weight of about twice as heavy as that of the complementary light chain subunit. H-chain can be cleaved by trypsin or chymotrypsin into H-1 and H-2 fragments. All of these subunits are antigenically distinctive. The maximal toxicity of all Botox depends upon the integrity of the disulfide-linked di-chain configuration.

Six other physical and molecular properties of Botox are listed as follows:
1. The neurotoxic crystals following protein purification from type A culture yields a homogeneous entity of 90,000 daltons (90 kDa) protein molecule.
2. The molecular moiety of the crystal has a hemagglutination activity that is being absorbed by red blood cells without affecting the toxic titer. This specific hemagglutination effect was first reported by Lamanna et al in 1971.
3. Two protein peaks have been recognized from the ion-exchange chromatography by using diethylaminoethyl Sephadex column. The first peak is the alpha fraction (20% of the applied protein) that shows high toxicity, approximately three- to fivefold more toxic than the crystals per se, and it reveals little hemagglutinating activity. The second peak is the beta fraction that has the reverse properties.
4. Botox molecules show a core strand within a coil of a hemagglutinin helix on electron microscopic examination.
5. On ultracentrifugation of type A culture, Botox shows toxic peaks at 7.2S, M-complex, L-complex, and 19S components.
6. On gel filtration, the neurotoxic peak reveals a homogeneous molecule of 150,000 daltons (150 kDa).

Relative Toxicities of Botulinum Neurotoxin Complexes

The toxicity of botulinum neurotoxin complexes depends upon several factors, some of which are intrinsic factors and others extrinsic. The relative toxicity potencies of the components of type A culture, 7S, M-complex, L-complex, and 19S, are 1, 12, 20, and 360, respectively. For type B culture, the potency of 7S/ M-complex/L-complex is 1:20:16,000. By oral route of intoxication, the larger complexes of Botox have the greater potency of toxicity, probably due to the associated protein on the surface of the molecules which protects the neurotoxins per se from inactivation in the gut and gives them a greater opportunity to be absorbed before being inactivated. Further, pure components of Botox are more easily detoxified than the complexes by pepsin, pancreatin, and gastric and intestinal juices.

The estimated LD₅₀ of Botox for humans is 5 to 50 ng/kg of body weight. Once the toxin and cell binding occurs, the axon terminals are permanently destroyed, and partial recovery is only possible when the sprouting of axonal terminals makes new neuronal connections.

How does Botox affect the nervous system? Three steps of action on the axon terminals and the neuromuscular transmission have been recognized.
1. Botox irreversibly binds to the axon terminal. It is calcium independent and minimally temperature dependent. At the earliest stage, the toxin does not interfere with the neuromuscular transmission. Use of antitoxin in early phase of botulism is still effective, because the antitoxin reacts with the toxin and neutralizes its neurotoxicity.
2. Translocation step. Botox is internalized into the cell and exerts its effect in the axoplasm, and thus, use of antitoxin becomes ineffective.
3. Lytic step. The toxins interfere with the release of acetylcholine from the axon terminals to the neuromuscular junctions. The lytic step is calcium milieu dependent and temperature sensitive.

Clinical Forms of Human Botulism

Botulinum toxins affect humans by several routes, and thus cause various clinical forms.

Food-borne botulism is caused by ingestion of preformed neurotoxin in the contaminated food. It is intriguing that clinical botulism will not develop in all people who ingest the toxin. The diversity of vulnerability of every individual to this neurotoxin may dictate different genetic backgrounds of the susceptibility. It has been reported that 31% of 36 patients died of food poisoning caused by C. botulinum toxin type A in 14
different areas of Japan. Most of these victims had eaten a special local food product used for gift in Kumamoto. The commercially available food product was made of fried lotus-rhizome solid mustard.

In vivo Botox production in human body has two common routes. In infant botulism, Botox are produced in vivo after multiplication of *C. botulinum* in the infant intestinal tract. Type A and B Botox are the most common cause of infant botulism. It has been reported that as low as 10 out of 280 cases of sudden infant death syndrome (SIDS) were caused by infantile botulism. In an elegant pathologic study of 70 necropsies of infants died of SIDS, as high as 15% of all cases of SIDS were shown to bear Botox and *C. botulinum* of different types (A, B, C, F, and G) in the contents of the ileojejunum or colon. The second route of human in vivo botulism is well recognized as “wound botulism”. The Botox are produced in the infected wound and circulated to the body by hematogenous spread.

In some cases of human botulism, neither vehicle nor toxin can be identified. We call this “unclassified human botulism”. Some other form of unidentified new strain may play a role. In an autopsied series of 95 patients with unexplained death, four adults and an 18-month-old infant had *C. botulinum* type G isolated from their intestines.

### Diagnosis of Human Botulism

The diagnosis of botulism can only be reached if the clinician keeps the possibility of botulism in mind. The clues to rapid diagnosis are as follows.

Clinical signs of acute descending neuropahtic syndromes start from ocular and bulbar weakness, followed by more generalized limb and respiratory paralysis. Consciousness remains clear prior to the respiratory failure and the subsequent complication of brain hypoxia. It is important to exclude many other diseases that resemble botulism, such as Guillain–Barré syndrome and myasthenia gravis.

Demonstration of the source of Botox intoxication is utmost important. Necessary survey and work-up has to be done for confirming the food-borne botulism or the wound botulism, the endemic occurrence, or the sporadic unclassified human botulism. Different cultural backgrounds and common habits for processing food-stuff may explain the high incidence of human botulism in several geographic regions of the world. The higher incidence of human botulism in certain parts of China is shown in Figure 1. Taiwan is an area of low incidence. Shih and Chao have reported 986 outbreaks of botulism during the period from 1958 to 1983. The outbreaks affected 4,377 individuals and resulted in 548 deaths in China. The incidence was highest in Xinjiang province. Type A botulinum toxin was the most common in the northwest region, whereas type B in the north, and type E in the northeast of China. The most frequent offending food was homemade strong smelling preserved bean curd (74%). We speculate that although this type of bean curd is very popular in Taiwan, botulism is uncommon in Taiwan probably because of the habit of Taiwanese using high-temperature cooking process before eating bean curd and good public sanitation. An eloquent ecologic study on *C. botulinum* in the soils of Taiwan has been reported.

Identification of the strains of the invading microorganisms, *C. botulinum*, and the types of Botox they produce may be rewarding. In the 12-year period of a survey among 336 infants with suspected infantile botulism, 113 out of 336 patients had positive stool culture for group I (proteolytic) *C. botulinum*. Among them, 69 showed type B botulinum neurotoxin, and 28 patients revealed type A toxin. Type E, type F, and two or more different strains of organism were also found in a few cases. The neurotoxins could be identified in the feces of 98 out of 111 culture-positive victims, whereas it could only be seen in the serum of 9 out of 67 stool culture-positive infants. Therefore, early detection of the neurotoxins is more sensitive in the stool specimens than in the serum. In affected Chinese adults, types A and B are the most common toxins encountered. In other parts of the world, such as the northern Quebec of Canada, type E neurotoxin is more common (52 of 61 outbreaks). The outbreaks were attributed to eating raw, parboiled or fermented meats (59%) from marine mammals, fermented salmon eggs, or fish (23%). Similarly, an outbreak of type E botulism has been reported in the Middle East country Iran. In contrast, the largest outbreak of food-borne botulism in the United Kingdom in June 1989 was attributed to type B botulinum intoxication that came from improperly processed hazelnut yoghurt contaminated with *C. botulinum* spores. Different strains of food-borne botulism were reported in the United States.

Electrodiagnosis of human botulism include objective evidence showing a failure of neuromuscular transmission, such as a decrementing response following
repetitive stimulation of nerves (Figure 2). These decrementing responses simulate myasthenia gravis. However, botulism differs from myasthenia gravis in that the former causes decrease in acetylcholine quantal release from the autonomic and motor nerve endings, whereas the latter reveals an immune-mediated defective function of the postsynaptic acetylcholine receptors. Further, nerve conduction studies reveal a pattern of axonal change with reduced amplitude of the compound muscle action potentials and nerve action potential in case of botulism, but not in the patients with myasthenia gravis. The nerve conduction velocities are relatively preserved in botulism (Figure 3). The characteristic features on electrophysiologic tests may also distinguish botulism from another common acute motor paralytic syndrome, called the classic form of Guillain–Barré syndrome. Rarely, a patient with botulism can be associated with Guillain–Barré syndrome.

A pathologic study of biopsied muscles taken from the biceps brachii of three patients with chronic type A botulism revealed not only the ultrastructural alterations at the presynaptic region, but also the postsynaptic structures such as denuded nerve terminals at the motor end-plate and hypertrophy of the junctional folds. These findings were not seen in patients with Eaton–Lambert myasthenic syndrome. Finally and most importantly, it should be emphasized that the autonomic dysfunction is an early sign of botulism. The control of heart rate and blood pressure responsiveness is remarkably impaired in the early stage of botulism. Loss of heart rate variability and blood pressure
variability can be seen in the first 48 hours of onset of botulism (Figure 4). At recovery, the sympathetic functions normalized earlier than the parasympathetic functions, and the neuromuscular transmission recovers earlier than the autonomic function42. In contrast, the autonomic dysfunctions are not early signs of myasthenia gravis.

Regarding the bioassay, the intraperitoneal injection of Botox, prepared from the patients' specimen, into a group of 18–20 g female Swiss-Webster mice yields toxic effect that causes a certain number of mouse lethality/mortality. The dose that produces 50% of mouse lethality (LD₅₀, lethal dose 50) is defined as one mouse unit. Commercially available Botox for therapeutic usage contains 50 ng of freezed and dried toxin hemagglutinin in one-vial preparation, which is equal to 2,000 mouse units. Therefore, one mouse unit is approximately equal to 0.4 ng of the protein toxin.

Treatment of Human Botulism

The keystone to the success of treatment of human botulism depends on the early detection of the disorder. Use of botulism antitoxin is effective only in the early stage of the disease. There is lack of evidence-based data to support use of plasma exchange in botulism, although it has been tried on some occasions. Supportive treatment, especially respiratory care, is utmost important. Recovery of the upper airway muscles may take as short as 12 weeks to as long as 12 months following appropriate ventilation support and exercise performance43. Nutritional support and extensive rehabilitation may be helpful. Guanidine hydrochloride (50 mg/kg) may be useful to reverse in part the motor paralysis5. However, the neurologic recovery from the axonal nerve injury is often slow and incomplete.

Acknowledgments

This work was supported in part by grant number MMH-9565.

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