Botulinum Toxin: A Friend or an Enemy?

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Authors' contributions

This work was carried out in collaboration with each and every authors. Each author contributed equally to wrote the draft of the manuscript and managed literature searches, analyses of the study and literature searches.

Review Article

ABSTRACT

Botulinum toxin is utilized in many drugs for the treatment purposes in healthcare. Besides healthcare, it is also utilized in the cosmetic industry. Botulism toxin is a very good friend as far as we use it with precaution and follow all guidelines for controlled doses for medication. Botulinum toxin is also known as Botulinum neurotoxin, which has been classified as seven serotypes, structurally similar but different in their antigenic and serological properties. Toxins are specific proteases, which act by degrading the protein component essential for exocytosis. BoNT/A is widely applied in neurological treatment as remodelling of neuromuscular junctions. Despite causing neuromuscular disease that could prove fatal, BoNTs are of great interest and may unveil their true potential in medical applications. They could become very useful and valuable research tools, which may lead to novel applications from cosmetic interventions to development of potent anti-cancer drugs.

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1. INTRODUCTION

Botulism refers to a serious paralytic illness that is caused by toxin-producing strains of *Clostridium spp*. The toxins produced by the bacteria are nerve toxins which are very potent in blocking neuromuscular motor nerves; thus causing paralysis in affected muscle tissues. The casual agents of botulism are *Clostridium botulinum* and sometimes those of the *Clostridium butyricum and Clostridium baratii* strains [1,2,3,4].

Botulinum toxins are neurotoxic enzymes produced by *Clostridium botulinum.* They are perhaps one of the most potent toxins known to mankind [1,2]. Botulinum toxins inhibit release of acetylcholine at the neuromuscular junction and in cholinergic sympathetic and parasympathetic neurones. They have become the first biological toxins approved for clinical use. Moreover, they are increasingly being produced in preparations for treatment of various neuromuscular disorders [1-3]. Justinus Kerner (1786-1862) was the first person to provide a complete description of botulism. He described botulinum toxin as "fatty poison" because of its association with outbreaks in Germany when people consumed smoked sausage [1,2]. Therefore, the toxin was commonly known as "sausage poison". The bacteria produce these toxins by growing in improperly-cooked meat products. Kerner was the first physician to envision botulinum toxins for therapeutic uses and hence, coined the name botulism [1,2].

1.1 Chemical Structure

Botulinum toxins, which are also known as Botulinum neurotoxins (BoNTs) are potent toxins that leads to botulism. These neurotoxins are classified into seven different serotypes; types A,B,C,D,E,F and G, which are structurally similar but differ by their antigenic and serological properties [5,6]. All BoNTs are synthesized as single chain polypeptides with a molecular weight of 150kDa [7]. These toxins are activated when the polypeptide chains are proteolytically cleaved between amino acids 448 and 449, resulting in the formation of one light chain and one heavy chain as mentioned in Fig. 1 [7]. A disulphide bond covalently links the light chain and heavy chain together, and the final product is a potent substance [8]. The toxins are further activated when the disulphide bond is reduced, converting the light chain into a proteolytic enzyme. This process occurs after internalization of the neurotoxin by the target cell.
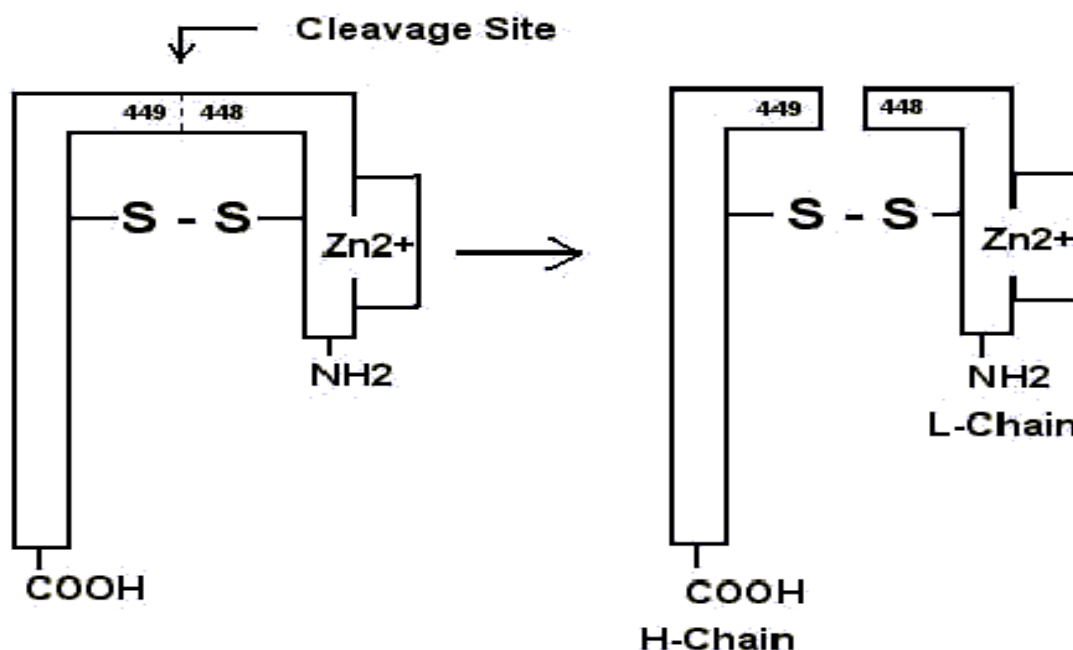

Fig. 1. Schematic structure of botulinum toxin

All different subtypes of botulinum toxins have a similar three-dimensional structure of the N-terminal as the amino acid sequences are highly conserved. However, the amino acid sequences of the C-terminal are variable [8]. The role of the C-terminal domain is more crucial compared to the N-terminal, as the removal of the N-terminal from the heavy chain will not affect the membrane binding ability. In contrast, the removal of about ten residues from the C-terminal will hamper the ability of the toxin to bind to neurons [8]. The light chain on the other hand is a protease associated with a zinc atom, which plays a vital role in the activity of the toxins. It is important in preventing the fusion of the acetylcholine-containing vesicles with the terminal membrane of the motor neuron [9].

1.2 The Mechanisms of Action

BoNTs primarily target the peripheral cholinergic nerve endings and blocks the release of acetylcholine, causing paralysis [7]. BoNTs cause paralysis by blocking the neurotransmitter release at the peripheral. This complex mechanism involves the binding of the toxins to receptors on the presynaptic membrane, uptake of the toxin into nerve terminals, translocation of the toxin across endosomal membranes and the inhibition of transmitter release from the presynaptic terminal [7,10]. When the toxin is present in the systemic circulation, it diffuses to the presynaptic membrane [8], where it is bound to the specific glycoprotein structure present on the cholinergic nerve terminal by the C-terminal domain of the heavy chain [11]. The bound toxin is then internalized into nerve terminals by endocytosis. At the same time, the N terminal of the heavy chain forms a specific channel that allows the translocation of the light chain across the presynaptic membrane into the cytoplasm. As the light chain is part of the toxin that blocks neuroexocytosis in the cytosol, it is of utmost importance that this part reaches the cytosol. The movement of the toxins across the endosome membrane into cytosol is dependent on the pH. At low pH, the structure of the toxin undergoes conformational changes exposing its hydrophobic domain, which enables it to pass through the hydrocarbon core of the lipid bilayer. Once it reaches the endosomal membrane, both the heavy and light chains separate from where the light chain is neutralized in the cytosol [7,11]. The toxins are specific proteases, which act by degrading the protein component essential for exocytosis. The soluble N-ethylmaleimide-sensitive factor attachment protein receptor

(SNARE) complex is a transport protein chain, which is responsible for the release of acetylcholine. Different types of BoNTs target various parts of the SNARE complex [11]. Botulinum toxin types A (BoNT-A) and E (BoNT-E) acts on the synaptosomal-associated protein (SNAP-25) while types B, D, F and G targets vesicle associated membrane protein (VAMP) [5,11]. Toxin type C1 acts by cleaving syntaxin [5]. The cleaving of the proteins blocks the release of neurotransmitters and hence, paralysis ensues.

2. CLINICAL APPLICATIONS

2.1 Cosmetic Industry

BoNT-A is most widely used for cosmetic procedures, that include brow lifting, reduction in lateral canthal lines, glabellar furrows, and transverse forehead lines, etc. Recently, several studies have been conducted and researchers suggested that BoNT-B can also be used in treatment of wrinkles alongside with BoNT-A [12]. However, BoNT-B seemed to have faster rate of onset compared to BoNT-A.

2.2 Treatment of Migraines

Migraines are throbbing headaches that occur more frequently in women than in men. As the cause of migraine is unknown, treatments are merely to alleviate the pains during an incidence of migraine. Treatment of migraine with by BoNT-A injection seemed to inhibit contraction of cranial muscle nerves that acts on myofascial components and vascular components that eventually inhibits release of acetylcholine (Ach). Thus, BoNT-A alleviates symptoms of migraine. It has also been proposed that BoNT-A may also block other neurotransmitters than Ach. Thus with acetylcholine and potentially other types of neurotransmitters being inhibited, the cascade of reactions occurs which eventually lead to prevention of migraine. Effects of this treatment have also been found to be longer compared to traditional methods. Patients generally tolerate it well and it causes fewer side effects [13].

2.3 Suppression of Symptoms of Allergic Rhinitis

Allergic rhinitis is an inflammatory condition where it affects the mucous membranes of the nose, eyes, eustachian tubes, middle ear, sinuses, and pharynx, though the mostly affected area is in the nose. BoNT-A was shown to be able to reduce the symptoms for 4 weeks. It was

determined that vasoactive intestinal peptide immunoreactive fibers in the nasal mucosa were markedly decreased after application of BoNT-A. Thus, local application of BoNT-A can be used to reduce sensitivity of sensory neurons around the mucosa, decreasing sensitization of nasal mucosa [13].

2.4 Reduction in Excessive Sweating

Hyperhidrosis is a condition where a person experiences excessive sweating even during conditions where thermal regulation is not needed, for instance in cold environ-ments. BoNT-A has been demonstra-ted to decrease perspiration in certain areas of the body in patients with hyperhidrosis when administered topically. Administration by intradermal injection is only used if patients fail to respond to local therapy. However, more studies are needed to determine if BoNT-A is able to act directly on sweat glands [13].

2.5 Treatment of Sialorrhea

Amytrophic lateral sclerosis (ALS) is a disorder [14] that affects voluntary movements of the body. Excessive salivation is one of the symptoms of this disease. A study was conducted to determine if Botox® was able to treat sialorrhea in patients with ALS. Results showed that application of 20U in parotid glands and 30U in submandibular glands were sufficient in reducing saliva in patients. It was found that there were no adverse reactions in tested patients. Therefore, it might be a good alternative in the treatment of ALS [15].

2.6 Treatment of Blepharospasm

Blepharospasm is a condition where the eyelids are forcibly closed. It has been reported that BoNT-A has been found to be useful in treatment of various types of muscular disorders [16]. It has also been reported that application of BoNT-A may reduce symptoms of focal dystonia, which is also known as "writer's cramp". Aside from BoNT-A, BoNT-B is also commercially available as Myobloc which is administered via injection in treatment of complications due to cervical dystonia [17].

2.7 Other Applications

BoTNs have also been utilised for many other clinical indications such as spasticity management, allodynia treatment, etc [18].

3. DEVELOPMENT OF ANTITOXIDANT THERAPEUTICS

Despite all of these advances and progress being made over the years, identification and characterization of the protein receptors targeted by BoNTs remain a challenge. Furthermore, the mechanism of specificity of binding domains and light chain translocations of the neurotoxins are yet to be fully understood. In addition, the molecular structures and studies of BoNTs should be explored to examine the effects of physiological changes such pH on the tertiary structure of BoNTs. To understand the mechanism and its effect would be crucial in development and rational design of therapeutics against BoNTs. Serotype-specific inhibitors could be developed through further structural studies of endopeptidase domains of the BoNTs [19,20].

3.1 Therapeutic use of BoNTs

By displacing the pathogenic light chain domain of BoNTs, the highly neurospecific BoNT heavy chains could be utilized in delivery of engineered molecules to neurological tissues. Thus, the therapeutic use of fragments of BoNTs holds unexplored potential and promises in treating various neurological disorders such as Alzheimer's disease and Parkinson's disease in the future [19].

3.2 Potential Use of BoNTs as an Anticancer Drugs

Clostridium spores are also used as anticancer drugs. The technology that first pioneered this concept was the 'Clostridium-directed enzyme pro-drug therpy (CDEPT). *Clostridium* spores are aimed at targeting hypoxic regions of solid tumours. These spores will be localized and exclusively germinated in solid tumours as spores are not able to germinate in healthy tissues. Anti-cancer compounds or pro-drugs converting enzymes as in CDEPT could be expressed by genetically modified *Clostridium* hosts. It would potentially lead to localized destruction of solid tumour tissue without affecting neighbouring healthy tissues [19].

4. ADVANTAGES AND DISADVANTAGES

The duration of effectiveness of botulinum toxins is basically dependent on the rate of clearance from the circulatory system and the elimination rate from nerve terminals [21]. Among seven

neurotoxins, comparative studies have demonstrated that BoNT/A has the longest paralyzing effect compared to others [22]. For BoNT/A the duration of therapeutic efficacy is weeks to months, and some may last for more than a year. Compared to BoNT/A types B, E, and F toxins show shorter duration of efficacy [23]. Variations were seen related to the intensity of disease, the muscles affected, and the dose administered. BoNT/A is widely applied in neurological treatment as remodelling of neuromuscular junctions. Numerous clinical studies have proved the effectiveness in treatment of long-term pathological disorders in humans due to extended synaptic blockage [22]. A study has been conducted to demonstrate the effect of BOTOX-treated surgical wounds in a rat model, and showed that the wound size became larger, reduced infiltration of inflammatory cells, and decreased fibrosis [24]. A large amount of collagen was produced, so it might be used to decrease fibrosis of surgical wounds without causing damage to epithelial growth. BoNT/B is an alternative method which can be used for patients who do not respond to BoNT/A. Interestingly, there are few studies, which indicated that BoNT/B (MYOBLOC) has a faster onset than BoNT/A [21]. However, the therapeutic effect of BoNT/A in patients with cervical dystonia was significantly longer than for BoNT/B [21]. Botulinum neurotoxin D (BoNT/D) is harmless to humans due to lack of specific receptors on human neurons, but is responsible for botulism outbreaks in animals including cattle [22]. BoNT/E might be a useful tool for synaptic inhibition purposes, for example, in pre-operative and post-operative cases to prevent immobilization of damaged joints [22]. Notably, BoNT/E disables synapses for the shortest period and without changing synaptic structure, thus it is an ideal clinical tool for reversible silencing of neurons [22]. Botox (a generic name for onabotulinum) was introduced in the early 19th century [21]. Botox was used to treat many clinical symptoms and drastically becoming popular when Botox was introduced in cosmetics. Despite its broad application, it also has many side effects and disadvantages.

The disadvantage of BoNT/A is that multiple injections are needed to maintain the desired effects, as the action is reversible. Additional injections lead to increased costs and the effects last only for 3 to 6 months. A well trained and experienced doctor is required for Botulinum administration because the amount of Botulinum toxin to be injected needs to be carefully calculated based on muscle size, the specific muscles and the desired effects. It often takes a series of injections before the optimal dose of a particular patient can be obtained [25]. BoNT/A is also useful in treating muscle disorders for example, blepharospasm and dystonias. However, Botulinum toxin does not work for all patients. One third of the patients may experience lack of response. There have been cases reported where Botox is not effective after repeated use in which the patient had conferred resistance to BoNT/A. The reason for development of resistance is due to the production of antibodies against the toxin, especially in patients treated with large and small doses of Botox. Some individuals need multiple injections to achieve optimum and long-term response, whereas other patients might experience low responses even after several injections [26,27]. Botox applied for cosmetic purposes has been considered safe and effective. Nevertheless, it does cause some side effects such as nausea, fatigue, malaise, appearance of flulike symptoms and skin rashes. There might be some localised reaction such as bruising, swelling, pain, erythema and minor headache. Moreover, poor technique, lack of experience and lack of knowledge of the facial muscles may lead to complications. The complications are diplopia, ectropion, cocked eyebrows, asymmetric smile, difficulties in eye closing, eye brow ptosis, and eyelid ptosis due to muscle weakness of neighboring muscles [27].

5. BOTULINUM INTOXICATION IN HUMANS

5.1 Types of Botulism

There are several types of botulism which include both natural and man-made types. Natural botulism includes food borne botulism which is caused by consumption of contaminated food/water with these neurotoxins. Infectious botulism is also known as infant botulism and adult intestinal toxemia botulism which causes intoxication through the ingestion of *Clostridium* spores of a toxin-producing strain. It results in colonizing the gastrointestinal tract and leads to spore germination. Consequently, the neurotoxins will be produced and absorbed directly in the gastrointestinal tract of the host [1,2,3,4,28].

In children and adults, the normal gastrointestinal tract is resistant to *Clostridium* colonization, however, infants of 1 week to 11 months possess

poorly developed microflora of the gut. Hence, *Clostridium* spores may germinate and produce toxin-forming cultures in the gut of infants. Factors influencing the colonization of the infant gut are poorly understood. Infections have been reported in cases whereby infants were bottle-feed after changing from breast-feed. Thus, it was speculated the infantile botulism could be foodborne due to contaminated milk products. Adults may also suffer from similar colonization of the gut by *Clostridium* bacteria when their gut flora or the physiologic condition of the gut had been altered by drugs or medical interventions like surgery [1,2,3,4,28].

Similarly, both infant botulism and adult intestinal toxemia are caused by consumption of *Clostridium* spores, which leads to colonization of the gastrointestinal tract and release of toxins. Wound botulism occurs when open wounds are contaminated or exposed to *Clostridium* spores [1,2,3,4]. Wound botulism is a rare circumstance whereby an individual will acquire botulism through wound infections. This form of botulism occurs when Clostridium botulinum or its spores contaminate open wounds. The *Clostridium* spores germinate in abscess or deep wounds such as in muscle, nerves, and soft tissues that have an anaerobic environment to support neurotoxin production [1,2,3,19,4]. The locally produced toxin becomes systemically absorbed and circulated to other parts of the body; causing wide-spread systemic botulism in affected individual [1,2,3,4,28]. At present, wound botulism is diagnosed among drug users who subcutaneously inject drugs using contaminated needles or impure heroin. The incubation period from infected wounds varies from 4 to 17 days. Furthermore, mixed infections are often seen in wound botulinum cases and this may occur due to the presence of two or more strains of botulinum toxin-producing *Clostridium spp* [32]. 'Unnatural' or man-made botulisms are the results of these neurotoxins being used by humans for a particular purpose. Iatrogenic botulism occurs when an over-dose of neurotoxins are accidentally being administered into humans for cosmetic or therapeutic purposes. Inhalation botulism is caused by either accidental inhalation of these aerosolized neurotoxins or being exposed to through bioterrorism [1,2,3,4,29].

5.2 Diagnosis of Botulism Toxicity

For the various clinical indications, different specimens have to be collected for laboratory diagnosis. It is of upmost importance to collect and transport the suitable specimens accordingly and correctly to ensure safety and to determine an accurate diagnosis [29]. Botulinum toxins are perhaps regarded as one of the most potent and toxic substances found in nature. The lethal dose to 50% of an exposed population for botulinum toxin is estimated to be 1 ng/kg parenterally and 3 ng/kg by inhalation. As discussed earlier, only a very minute amount of these toxins was required to cause neurological disorders in humans. Hence, it is of upmost importance to detect and diagnose botulism as quickly as possible to avoid dire and fatal consequences [1,19,29,30]. Diagnosis of botulism is mainly symptomatic and presumptively based on direct examination of the patient. History and travel records will be helpful to identify possible exposure to *Clostridium spp.* or botulinum toxins [1,2]. Patients may present a wide range of symptoms associated with the epidemiology and toxin involved. However, the key clinical syndromes of *Clostridium* spp. toxin exposure include an afebrile, descending, symmetrical, flaccid paralysis with prominent bulbar palsies, absence of fever, and clear sensorium [1,2]. Other signs and symptoms include dysphagia, weakness of muscles and tendons, bladder distention, nausea, vomiting, diarrhea, and so on. The onset of botulism symptoms is between 2 hours to 8 days but there will be a peak at approximately 48 hours. It also depends on which type and dose of toxin the patient has been exposed to [1,2].

6. LABORATORY INVESTIGATION METHODS

6.1 Bioassay Method

The most definitive laboratory test for botulism is the demonstration of the toxigenicity by the presence of toxins in clinical specimens of affected patients. The standard test is the mouse lethality test [1,2,28,31]. Biological assays involve the intraperitoneal injection of culture supernatant or a serum specimen into a mouse. A second mouse will be challenged with culture supernatant or serum specimen after being heated to 100°C or treated with antitoxin against botulinum toxin. Diagnosis will be based on the observation and detection of botulism-specific symptom development in mice. The presence of toxins will be confirmed if the first mouse develops flaccid paralysis and/or dies while the second remains protected [2,28,31].

6.2 Microbiological Analysis

Microbiological studies can be performed as well in the case of botulism. The causal agent, *Clostridium botulinum* can be presumptively identified after culturing on agars such as anaerobic blood agar, Brucella agar with 5% sheep blood, egg yolk agar, and phenyl ethyl alcohol blood agar, at 35-37°C under anaerobic atmosphere with less than 2% of oxygen [1-3,19,28,31,32]. *C. botulinum* colonies will appear gray-white, circular, with irregular edges, and usually beta haemolytic on blood agar [1,2,3,19, 28,31,32]. Bacteria will be stained deeply purplish-blue (Gram-positive) by Gram stain and appear to be rod-shaped with oval subterminal spores. These spores may be demonstrated by using phase-contrast microscopy of direct wet mounts. Other presumptive identification of *C. botulinum* includes aerotolerance testing, carbohydrate fermentation tests, and demonstration of specific volatile metabolic products from chopped meat medium via gas-liquid chromatography [1,2,3,19,28,31,32]. The aerotolerance test was performed via subculturing on clinical specimen suspected of *C. botulinum* onto culture media such as Brucella (BRU) agar, Blood agar (BA), or Chocolate agar. These culture plates are incubated under both anaerobic conditions and 70% CO_2. Theoretically, obligate anaerobes will only grow on the BRU/BA media under anaerobic conditions while some aerotolerance microbes such as *Clostridium spp*. will grow on Chocolate agar under 70% CO_2 conditions [32]. In the carbohydrate fermentation tests, the various species of *Clostridium spp.* could be differentiated through a series of biochemical profiles based on their ability to ferment carbohydrates such as glucose, mannose, lactose, and mannitol [33]. Volatile and non-volatile acid, namely acetic, butyric, isobutyric, and isovaleric acids, produced by *Clostridium spp.* on chopped-meat medium are species specific and also based on the toxins they produce [34]. However, microbiological studies are only presumptive diagnosis of botulism and not useful because the bacteria itself is not causing the symptoms. Thus, isolation of *Clostridium spp.* only provides evidence for the presence of the organism but not its toxin [1,2]

6.3 Toxin Neutralization Assays

The principle of this assay is to detect the presence of antibodies against BoNT in patient sera. This could be assessed by subjecting cell cultures of macrophages (J7747 cell line, or other B cell lines) to patient sera. The ability and presence of the antibodies present in the sera are determined by the viability of these cells after being incubated and exposed to the Botulinum toxins added to the culture. Viable cells can be visualized by adding reagents such as diphenyltetrasolium bromide (MTT) as they are able to metabolize these reagents and generate a vivid colour. Hence, dead cells affected by the toxin will induce colour formation [34]. In the presence of antibodies against Botulinum toxins, the toxins will be neutralized by the binding action of the antibody and therefore, subdue the toxic effect on the cells. Thus, this would prevent the cells from being killed and remain viable. Culture plates are then analyzed by using a plate reader to determine the absorbance level of each well. The amount of toxins can be quantitated and the concentration of toxin that is required to kill half of the cell population determined [34].

6.4 Enzyme-linked Immunosorbent Assay (ELISA)

Enzyme-linked immunosorbent assay (ELISA) has been used to detect botulinum neurotoxin [35]. A solid test matrix is initially coated with polyclonal or monoclonal capture antibodies against one or more botulinum toxins prior to addition of samples that contain the neurotoxin of interest. Another toxin antibody is then added to bind to the primary antibody-bound neurotoxin complex. Ultimately, a signal is generated following the addition of an anti-toxin molecule that carries enzymes such as alkaline phosphatase or horseradish peroxidase, as a chromogenic substrate is cleaved enzymatically [31]. The recent advancements in signal amplification have improved and enabled increased assay sensitivity as compared to previous results. However, immunological methods such as this require antibodies of high quality, which are not commonly available. False positive results can rise from inactivated toxins, which have undergone heat treatment as well. Also, false negative results due to reduced affinity to monoclonal antibodies can occur as a result of genetic differences between neurotoxins of various serotypes [31]. Despite of the many drawbacks and interferences, ELISA performance may be enhanced by extending incubation times and using fetal bovine serum to block interfering substances in the case of infant botulism, which has been shown to be even more reliable than other assays. Furthermore, the ELISA procedure can be executed in one

day, which makes it most suitable for the preliminary screening of individuals with suspected botulism [31].

6.5 Endopeptidase Assay

The endopeptidase assay can be used to detect botulinum toxin through its particular zinc-endopeptidase activity [2,19,28]. Various types of botulinum neurotoxins cleave different parts of the SNARE complex proteins or the synaptic proteins specifically. The cleaved peptide is detected by either immunological techniques or the production of fluorescence from the quenched chromophore-labeled peptide [2,19,28]. The endopeptidase assay detects only biologically active neurotoxins, is very specific, and is more sensitive than other assays in general [2,19,28]. Its sensitivity can be enhanced further by using mass spectrometry to detect the cleaved peptide [31]. Absence of cross-reactivity between the various botulinum neurotoxins or with tetanus toxin has also been reported. But as for commercial solutions, only type A toxin can be detected using this endopeptidase assay [31]. Furthermore, this assay is costly as it requires both expensive equipment and expertise. No concrete evidence of its compatibility with complex matrices such as serum and foods has been reported too. Therefore, further research is necessary for the validation of this assay for all types of botulinum toxins, and with complex matrices [31].

6.6 Other Laboratory Investigations

Electrophysiologic studies have also proven useful in aiding diagnosis of botulism. In the case of botulism, increase in the M-wave amplitude with repetitive stimulation often occurs and this may provide early clues to muscle weakness. Besides that, electromyogram may demonstrate low-amplitude and short-duration motor unit action potentials, in which are less specific changes caused by botulism. A single-fiber electromyography may demonstrate increased blocking and jitter as well. Lumbar puncture may be indicated in view of Botulism of suspected central nervous system involvement [2,3,19,28,31].

7. DISCUSSION

Botulinum toxin preparations are increasingly being recognized for the potential and benefits in treatment of various neuromuscular diseases. However, Botulinum toxins can be misused for other purposes [1,19,29]. Vaccination of the general population is not warrant because of cost and risks of adverse reactions against the vaccines. In addition, botulism of all forms is rarely reported cases since the implementation of improved hygiene and sanitation controls in the meat-production industry. Moreover, therapeutic and cosmetic applications of these purified botulinum toxins will be restricted to vaccinated subjects. Therefore, the current therapy and postexposure are only available in the form of passive immunization through administration of botulinum antitoxins for the general populations. Hence, there is wide spread fear of massive casualties when botulinum toxins are released intentionally [1,19,29].

8. CONCLUSION

In addition to causing neuromuscular disease that could prove fatal, botulinum neurotoxins are of great interest and may yet unveil their true potential in medical applications. They could become very useful and valuable research tools which may lead to novel applications from cosmetic interventions to development of potent anti-cancer drugs. As the structure of toxins and mechanism of action are slowly explored and understood, vaccines, serotype-specific inhibitors, and even novel therapeutics will pave the way to prevention, or even eradication of botulism.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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