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Review Article

Botulinum Toxin and Its Biological Significance: A Review

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Abstract

Botulism is a severe neuroparalytic bacterial disease caused by gram positive, anaerobic, spore-forming microorganisms, Clostridium botulinum, referred to as Botulinum Neurotoxin (BoNT) producing bacteria. The objective of this paper is to review botulinum toxin and its biological significance. This bacteria can produce seven types of toxins (A - G) known as BoNT. BoNT is highly potent preformed toxin that affects humans, all warm-blooded animals, and fishes due to consumption of contaminated silage, carcass, water, industrial by product, and canned foods like meat, milk, fruits and vegetables. Botulism is an important disease in the world, particularly where stock graze under range conditions and are subject to periods of protein and phosphorous deficiency. There is no geographical limitation for botulism because sporadic outbreaks occur in the most countries. The main route of transmission of botulism is by oral ingestion and wound infection with spore. The clinical signs occur within 24 hour up to 17 days. BoNT contain zinc endopeptidase that blocks vesicle of acetylcholine binding with the terminal membrane of the motor neuron and causes flaccid muscle paralysis with lateral recumbency, generalized muscle weakness and dysphagia. Finally results in death due to respiratory arrest, paralysis of pharyngeal and diaphragmatic muscles. There is no specific lesion during postmortem examination but may be seen in chronic case. Diagnosis is based on clinical sign and laboratory examination like, ELISA, MPT and culture for isolation of bacteria. Although this toxin in animals represents a serious environmental and economic concern because of the high mortality during the outbreak, it provides some biological significance after extraction by genetic recombination like in cosmetics, chemotherapy, and biological weapons. There is no effective treatment but ampicillin used as antibiotics. Prevention by polyvalent vaccine and proper feeding management is better than treatment. Therefore it recommended that the farmers should store animals feed with proper ventilation to avoid multiplication of bacterial spore.

Keywords: Botulism; Bacteria; Neurotoxin; Zinc endopeptidase; Paralysis

Introduction

Botulism is a severe neuroparalytic bacterial disease affects humans, all warm-blooded animals, and fishes [1]. It is caused by exposure to botulinum neurotoxins (BoNTs), which are produced by gram positive, anaerobic, spore-forming microorganism's genus Clostridium, referred to as BoNT-producing clostridia [2]. BoNTs act on nerve endings to block acetylcholine release. Their potency depends on two factors: their enzymatic activity and their selective affinity for binding neurons. It is also an exotoxin-induced flaccid paralysis in animals and human. Because of its bio-warfare agent and potent toxin BoNT causes disorder in living beings [3].

The word 'botulism' is derived from the Latin word 'botulus' meaning 'sausage'. Hence, the name 'botulism' refers to the poisoning caused due to consumption of sausages. In 1869, Muller report that eating fish cause food poisoning. He was the first person to call the term 'Botulism' in his report. The bacteria are found in the intestinal tracts of some healthy fish, birds and mammals, and the gills and viscera of shellfish (crabs) [4].

The causative agent, Clostridium botulinum is appear as soil-borne pathogen, prefers to grow in decaying organic matter [5]. This toxin is a protein in nature and heat labile neurotoxin which affects acetylcholine release at the neuromuscular junction and causes botulism [6]. It is fatal bacterial disease because of neuronal paralysis which cannot be reversed by therapeutic options. However administration of antitoxin was recommended as the first line of management. Human botulism treated effectively with antitoxin, mechanical ventilation, and other symptomatic therapeutic measures [7]. But, the availability of antitoxin in developing countries is limited. However, antitoxin therapy would be effective if it is injected before the toxin reaches motor-end plate [1].

An important source of intoxication is contaminated silage, canned foods, either from consumption of food that has not been heated properly before canning or from food that was not properly cooked from the can before consuming. Three important types of botulism are identified, namely food botulism, infant botulism and wound botulism [8].

In the recent year, sporadic incidences and high amount of poultry outbreak with bovine botulism have been reported from different parts of the world [9,10]. This disease in animals represents a serious

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environmental and economic concern because of the high mortality during the outbreak and used as biological weapons. More than a million deaths have been reported during outbreaks in particular area in a year with losses of 50,000 birds [1]. Therefore the objective of this paper is to review botulinum toxin and its biological significance.

Literature Review

Etiology

Botulism is disease caused by a toxin produced by the bacterium *Clostridium botulinum* which is a spore forming, gram positive, an anaerobic bacteria. This bacteria multiplies in an oxygen deficient (oxygen-free environment). In this condition with warmth and moisture, *C. botulinum* multiplies fast and produce highly lethal toxin. All warm-blooded animals can be paralyzed because the toxin blokes nerve function. As result this toxin is known as neurotoxin [11].

Based on their genotypic, phenotypic, and biochemical characteristics, these strains of microorganism can be divided into six groups: *C. botulinum* (groups' I-IV), *C. butyricum*, and *C. baratii*. Groups I and II, *C. butyricum*, and C. baratii are associated with human botulism, and group III causes animal botulism [3]. Group IV organisms (*C. argentinense*), are associated with wound botulism [12] .This bacteria can produce seven types of toxins (A, B, C, D, E, F and G). Most clostridial strains produce only one toxin type. All of the botulinum toxins cause the same clinical signs but different in severity of the disease. Knowing the type of toxin is important in selecting an antiserum for treatment because antiserum produced against one type is not effective for others. In people, botulism is caused by types A, B and E. Types C and D are the most common causes of disease in other mammals and birds. Type C is especially common in birds, mink and horse. Cattle that fed poultry litter and dogs that eat contaminated bird carcasses are also affected by type C. Types A and B affect horses in the U.S. Type E toxin is found in aquatic environments, and can cause botulism in fish and fish-eating birds. In addition *C. botulinum* type C also produce C2 toxin, which causes an enterotoxin with gastrointestinal signs. In humans, botulism is caused by group I or group II organisms [13].

Group I and II organisms can producing type A, B, E, and F toxins while group III organisms produce type C, D, and their mosaic C/D and D/C toxins [14]. Group I contains proteolytic strains and group IV, *C. butyricum*, and C. baratii can produce type G, E, and F toxins, respectively [15]. And group II consists of nonproteolytic strains that form B, E or F toxins. Group I and II *C. botulinum* strains differ in heat resistance. Spores from group I organisms are more resistant to heat, growth temperatures, and other characteristics that inhibit the types of foods where they tend to grow. Group III strains produce toxins C or D that cause botulism in animals. Group IV produces the type G toxin which reclassified as *C. argentinense*. This species cause outbreak of human botulism in Switzerland [13].

Epidemiology

Mammals are susceptible to botulinum neurotoxin and develop botulism with the same clinical sign to humans. Most of the cases are caused by *C. botulinum* group III, even if groups I and II are also reported in animal botulism. Horses are very sensitive to BoNTs and equine botulism occurs sporadically worldwide, both as feed poisoning and as toxico-infectious forms. Avian botulism is usually caused by BoNT type C1, to which most birds seem to be susceptible. It is also very serious problem in fish farming. Contaminated silage becomes major cause for outbreak of botulism in cattle [2]. There is no geographical limitation for botulism because sporadic outbreaks occur in the most countries. The source of the toxin and risk for the disease varies from regions to region due to food storage, feeding and management practices. Outbreaks of disease occur with ingestion of toxin in feeds that is common in northern USA and Europe. Additionally outbreaks in animals on pasture are reported from South Africa, Australia and Gulf coast of USA [16].

Geographic distribution: The geographical distribution of bacterial strain that conducted in the USA indicates, type A was found in the neutral and alkaline soil in the west whereas type B and C in damp or wet soil all over, except that B was not found in south. Type C was found in soils in Gulf coast and type D in alkaline soil in west. The prevalence of the disease is high in area where, canning fruits and vegetables is more common like tropical countries [17].

Botulism is an important disease in the world, particularly where stock graze under range conditions and are subject to periods of protein and phosphorous deficiency. It has been reported in feedlots and in dairy cattle under intensive feeding systems. There are seven recognized types of botulism organisms but only two, types C and D, are important in cattle [11]. The distribution of the organism is not the same and more common in certain geographical areas because the environmental factors can influence the occurrence of botulism For instance, it is common in cattle from areas with phosphorus-poor soils, like in southern Africa [13]. The toxin does not affect Fly larvae and other invertebrates. However, feeding on toxigenic carcasses make this organism victim of the toxin. Ingestion of a single toxigenic maggot could be lethal. This is described as the carcass-maggot cycle of botulism [18].

Animal botulism: The primary contamination route for either animal botulism or human botulism is the ingestion of preformed toxins in foods or feeds. Raw material, such as grass, hay, rotting vegetation, and slaughterhouse waste, decay of vertebrate carcasses, invertebrates, and sewage may support BoNT-producing clostridia growth and toxin production. Animals may directly ingest decaying organic matter containing toxin, or from the consumption of zooplankton or invertebrates, such as larva that carry toxin. A second form of animal botulism is due to absorption of BoNTs produced *in vivo* in the intestinal tract. This form of botulism, seen in chickens and horses is known as toxicoinfection. A third form of animal botulism is caused by the germination and production of toxin by *C. botulinum* spores in infected wounds. The last 2 forms are often referred to as toxicoinfectious form of botulism [19].

Susceptibility: The susceptibility of cattle to botulinum poisoning is depends on presence of the following factors: phosphorous and protein deficiency, carcass and bone chewing, bacterial distribution, toxin, unvaccination and improper vaccination [11]. The exposure to poultry litter as feed or bedding may be risk factor in the occurrence of cattle botulism [9]. Phosphorus deficiency in cattle may result in pica that tend to chew on cadavers and bones to balance their mineral deficiency which means a high risk of BoNT ingestion [20- 22].

Transmission: The main route of transmission of botulism is by oral ingestion and wound infection with spore. Because all species of Clostridium can produce spores that make them dormant and highly resistant to disinfectants, heat and environmental conditions that destroy vegetative cells. These spores can survive for many years until favorable conditions allow them to germinate and grow. *C. botulinum* spores are common in the soils, in sediments in lakes, streams and coastal waters. Also found in the intestinal tracts of some healthy fish, birds and mammals, and the gills and viscera of shellfish (crabs). Also the toxin has been detected in snails, earthworms, maggots feeding on contaminated carcasses, and nematodes. Because invertebrates are not affected by the toxin, they are involved in transmitting it to species such as birds [13].

Pathogenesis

Botulinum toxin is a dichain polypeptide: a heavy chain of 100 KDa is attached by a single disulfide bond with 50 KD of light chain, which contain zinc endopeptidase that blocks vesicle of acetylcholine binding with the terminal membrane of the motor neuron and causes flaccid muscle paralysis. This toxin is the most lethal toxin and all seven types act in similar ways. Thus results in death due to respiratory arrest, paralysis of pharyngeal and diaphragmatic muscles [23]. Mental functioning is not impaired by BoNTs, so the patient remains alert and conscious throughout the disease [2].

It produced during bacterial vegetative growth as inactive single-chain polypeptides then activated by bacterial or tissue protease. Naturally this toxin found as progenitor toxins containing the neurotoxin and nontoxic associated proteins which protect neurotoxin from environmental factors [1]. The genes for encoding BoNTs found in the chromosome or on extrachromosomal elements, such as plasmids or bacteriophages [24]. But toxin genes for group III organisms are carried by bacteriophages that exert rapid change on lysogenic cycle. Molecular and genomic analysis of the bacteriophage genome describes that this phage exists as a circular plasmid prophage in the lysogenic state but does not accepted by host chromosome [25].

The mechanism of action of Botox follows steps in the system of the body. Frist, active toxin is absorbed in the small intestine by binding to the receptors on the apical surface of gut epithelial cells. Second, released into the systemic circulation, reaching all peripheral cholinergic nerve endings. Third, in these sites, the toxin binds to specific receptors. Then internalized into the cytosol of the nerve terminus, where it blocks the release of acetylcholine, finally results in characteristic paralysis [26].

Post-mortem examinations

Postmortem examination is not usually evident but may be seen in chronic case [27]. Diffuse intestinal hemorrhage may be observed as some strains of *C. botulinum* type C and D because of an enterotoxin they produce. But these changes are not sufficient to specifiy confirmatory diagnosis. However examination of stomach contents like decayed carcass material, bones, maggots may be consistent [28,29].

Clinical signs

There are three forms of botulism, food-borne, wound and intestinal form. Those cause disease by absorption of botulinum toxin into the circulation. The incubation period on the mucosal surface of the wound depends on the rate and amount of toxin absorbed [30].

Food-borne botulism (classical botulism) was the first form of the disease reported in literature. Food poisoning due to this toxin was emerged as a problem when food preservation became a common practice in the world. Infant botulism, recognized as a clinical disease over three decades ago in USA. The initial neurological symptoms of infant botulism are largely the same as in other forms of botulism, but these are usually missed by parents and doctors because the infant cannot verbalize them. The most common sources of infection for infants appear to be honey and environmental exposure. Another form of botulism is analogous to tetanus, in that BoNT is determined from *C. botulinum* growing *in vivo* in abscessed wounds called wound botulism. Most cases occur in physically active young males who are presumable at higher risk of traumatic injuries. Wound botulism has emerged following subcutaneous injection of spore [2].

All forms of botulism results in progressive, symmetrical, flaccid paralysis that starting from the hindquarters with weakness, muscle tremors, stumbling, ataxia, eyes appear closed, pupils dilated, papillary reflexes sluggish and recumbency, finally results in death. It may be in peracute, acute, and chronic forms. In general, the clinical signs occur within 24 hours up to 17 days. The incubation period of food borne botulism in monogastric animal is shorter than in ruminants which show clinical signs after a week. The incubation period for toxicoinfectious botulism is longer (4 to 14 days) [31].

Diagnosis

Clinical diagnosis: Clinical signs of animal botulism are not specific but indicative. Confident diagnosis is based on signs observed in sick animals, the duration of the outbreak, the postmortem findings and by ruling out other differential diagnoses. In cattle, flaccid paralysis, the epidemiology of the outbreak, the clinical chemistry like hyperglycemia and neutrophilia support the diagnosis [1].

Laboratory diagnosis: Laboratory confirmation can be done by following methods; first, by examination of BoNTs in serum, gastrointestinal content, liver, and wound; second by BoNT-producing clostridia in gastrointestinal content, liver, and wound; third by BoNTs or BoNT-producing clostridia in feed or the close environment of the sick animal and by antibody response in an animal with symptoms of botulism [27,32]. Detection of toxin by protecting with monovalent toxin allows diagnosis of botulism with testing of toxin in plasma or tissue before death of animals. In addition to this the toxin can be demonstrated by ELISA technique, injecting intra peritoneal the extract of food or culture into mice or guinea pig and isolation of bacteria by culturing [33].

Enzyme Linked Immuno Sorbant Assey (ELISA): This test is used to show that an animal has antibodies to against toxin in its blood serum. Antibodies arise from either natural exposure to a toxin or from vaccination. The test can identify the type of toxin involved (type C or D) with natural infection, and the level of antibodies in the animal. Because of cross reactions following vaccination, it is not possible to differentiate between type C and D vaccination titres. This test is useful for assessing the success of a vaccination program. In unvaccinated herds the ELISA test is very useful as a positive result shows natural exposure. However, it is an expensive test. It can be used together with the fecal culture test to confirm that animals have

been exposed to botulism. A repeat sample taken from survivors two weeks following the outbreak should indicate rising levels of antibodies if botulism infection has occurred [11]. However toxin detection by ELISA test appears less sensitive than mouse bioassay [33].

Mouse protection test (MPT) (Toxin neutralization test): Detection of toxin using bioassay in mice coupled with toxin neutralization with polyvalent antitoxins used but the sensitivity is low in both ruminants and horse because they are more sensitive than mice to botulism toxin. The test results in paralysing mice with an injection of a toxic bacterial or toxic serum from an affected animals and then protecting them with specific type C or D botulism antiserum. It is good for identifying the presence of toxic botulism bacteria and is used with the ELISA test. However, it is not so useful in proving that a paralyzed beast has botulism. This is because only very low doses of toxin are present for short periods in the bovine serum and the mouse is relatively resistant to the toxin compared to cattle [11]. After demonstration of BoNTs in serum, feed material, or intestinal content inject into the mouse and taking bioassay is the gold standard for laboratory confirmation of botulism. But negative mouse bioassay does not always mean no botulism, because the toxin may be present at a level below the limit of detection or may have been biodegraded by microbes in the intestinal tract of the animals [33].

Culture for isolation of bacteria: Examination of the toxin in feed stuff, fresh stomach content or vomitus assists diagnosis of botulism. The spoilage of food or swelling of cans or presence of bubbles inside the can indicate clostridial growth. Food is homogenized in broth and incubated in Robertson cooked meat medium and blood agar or egg yolk agar, which are incubated anaerobically for 3-5 days at 37°C [16]. The botulism organism can be grown from any gut contents or even carcass material. Once the organism is grown in the laboratory, tests are carried out to show that it is *C. botulinum*, and to identify the type. This test will show that a toxic bacterium may be present but it does not prove that it was the cause of death. It may have been present without ill effect [11].

Biological significance of botulinum toxin

Preparations of botulinum toxin: Serotype A (BTX-A) is the most commercially available toxin for clinical use .Also the efforts have been made for the commercial production of serotypes B, C, and F. The two available market preparations of BTX-A are by the trade names Dysport and Botox BTX-A is prepared by laboratory fermentation of Clostridium botulinum cultures. Crude botulinum toxin is a protein with a molecular weight of about 190, KDa. After purification, the toxin is diluted with human serum albumin, bottled in vials, lyophilized (freeze-dried), and sealed. Each freeze-dried vial contains 100 units (U) of BTX-A which is reconstituted with preservative-free normal saline (1-5 ml) just before use. The toxin should be used within 4 hours of reconstitution. Within these four hours; reconstituted botulinum should be clear, colourless and free of particulate matter. The shelf life of the packaged product is 36 months when stored at 2°C to 8°C. The potency of BTX-A is measured in mouse units (MU). One MU of BTX-A is equal to the amount of toxin that kills 50% of a group of 20g Swiss Webster mice within 3 days of intraperitoneal injection(LD50) [39].

Importance of botulinum toxin in cosmetics: Today, BoNT is the most commonly performed cosmetic procedure in the world. The main significance of botulinum toxin in cosmetic use is on the muscles of facial expression gives beautiful apperance. Because this toxin reduces hyperfunctional muscles and eliminating the overlying skin line or ridge [35]. It is also used in treatment of glabellar lines, horizontal forehead lines, wrinkles correction, brow lift, nasal scrunch, rejuvenation of mouth and mandibular contouring [36]. Now days, glabella is the only FDA-approved site for cosmetic injection of BOTOX -A in the USA. Because it is the most common site for patients and physicians to begin treatment with BoNT-A. Injections of the small muscles in this area are technically simple to perform and they result in a high degree of patient satisfaction. Close attention should be paid to the eyelid and eyebrow for possible ptosis and redundant eyelid skin that made patient dissatisfayed following treatment Stretching the skin in this area will form creases and repeated treatment should be given within 3-4 month intervals to reduce wrinkles in the area where treated [38]. One recent study has publicized that glabellar treatment may help convey positive and relaxed emotions correctly and that BoNT-A injections of the glabella can be beneficial for patients, who believe their faces are not communicating their emotions properly [36,38]. Botulinum neurotoxin type A injection is a simple, safe, and very effective treatment of the aging face, reducing wrinkles through the temporary and reversible paralysis of treated muscles [35,36].

Importance of botulinum toxin as therapeutic agent: The first batch of botulinum toxin type A manufactured by Scott and Schantz was named Oculinum. Later by 1991, the manufacturing facility and license were turned over to Allergan and got a new name Botox. The clinical use of botulinum toxin is to change extra ocular muscle to different position during surgical treatment of strabismus (heterotropia). In animal botulinum toxin produced long lasting, localized, dose dependant muscle weakness with no systemic toxicity and necrotizing side effects. This toxin is used in humans according to Investigational New Drug (IND) license for the treatment of strabismus, blepharospasm, hemifacial spasm, cervical dystonia (torticollis), thigh adductor spasm and, hyperhidrosis. At now a day number of label used botulinum toxin. Such as in tremor, spasticity, over active bladder, anal fissure, achalasia, various pain disorders including headache. The most recent indication of botulinum toxin (botox) is used for wrinkles and various cosmetic activities [4].

Currently available pharmaceutical preparations of botulinum toxins for the treatment of human diseases in ophthalmology, neurology and dermatology are marketed under the trade names *Botox®(USA)*, *Dysport® (United kingdom)*and *Xeomin®* (*South America and Asia*) (based on botulinum neurotoxin A), Neuronox (South korea) and *Myoblock® /Neuroblock®* (based on botulinum neurotoxin B). With the exception of Xeomin, this is practically devoid of complexing protein [2] (Table 1).

Clinical use of botulism: Botulism toxin has become the first biological toxin which is licensed as drug for treatment of human diseases. As of January 2008, two BoNT serotypes (A and B) are approved for clinical use in the United States by Food and Drug Administration (FDA) [2]. Cervical dystonia (spasmodic torticollis) is abnormal head and neck posture due to tonic involuntary

Table 1: Comparison of different botulinum toxin drugs.

NO	Properties	Preparations			
		Botox	**Dysport**	**XEOMIN**	**NEUROBLOCK**
1	Formulation	Powder	Powder	Powder	Ready to use solution
2	Storage condition	< 8°C	< 8°C	< 25°C	< 8°C
3	Self life	36 months	24 months	36 months	24 months
4	Botulinum toxin type	A	A	A	B
5	SNARE target	SNAP25	SNAP25	SNAP25	VAMP
6	Purification process	Precipitation and chromatography	Precipitation and chromatography	Precipitation and chromatography	Precipitation and chromatography
7	pH value	7.4	7.4	7.4	5.6
8	Stabilization	Vacuum drying	Freeze drying	Vacuum drying	pH reduction
9	Excipients	Human serum albumin, NaCl	Human serum albumin, lactose	Human serum albumin, sucrose	Human serum albumin, disodium succinate, NaCl, H_2O, hydrochloric acid
10	Biological activity	100 MU-A/vial	500 MU-I/ vial	100 MU-M/vial	1.0-2.5/10 k MU-E/ vial

Source: Amarnath et al., [4].
MU-A: Mouse unit in the Allergan mouse lethality assay; MU-I: Mouse unit in the Ipsen mouse lethality assay; MU-M: Mouse unit in the Merz mouse lethality assay; MU-E: Mouse unit in the Solstice mouse lethality assay [4].

Table 2: Clinical use of botulinum neurotoxin.

NO	Indication	Example
1	Dystonias	Cervical dystonia, Oromandibular dystonia, Pharyngolaryngial dystonias, Jaw closure/opening dystonias, Occupational cramps, Limb and axial dystonias
2	Spasticity	Cerebral palsy, Brain injury, Spinal cord injury
3	Eyelid spasm	Blepharospasm, Hemifacial spasm, Eyelid twitch
4	Exocrine gland hyperactivity	Focal hyperhidrosis, Relative sialorrohoea, Crocodile tears syndrome
5	Movement disorders	Tremors, Bruxism, Tic
6	Pain syndromes	Migraine, Back spasm
7	Urinary bladder dysfunction	Sphincter- detrusor dyssenergia, detrusor hyperreflexia
8	Opthalmology	Strabismus, Entropion, Protective ptosis
9	Cosmetology	Hyperactive facial lines-brow lines, Frown lines,
10	Gastroenterology	Achalasia, Anal fissures, Anismus
11	Gynecology	Vaginismus
12	Urology	Sterile prostatitis
13	Dentistry	Muscle spasm associated with temporomandibular joint pathology
14	Veterinary	Barking dogs

Source: Dhaked et al., [2].

contraction in cervical muscles. The most effective treatment for cervical dystonia is botulinum toxin [39]. Hemifacial spasm is an involuntary, irregular, clonic or tonic movement of the facial muscles which innervated by seventh cranial nerve on one side of the face. That is caused by vascular compression of the facial nerve which is effectively managed by BOTOX [40]. Blepharospasm is involuntary tight contraction of the eyelid as form of dystonia. Patients face strong eye closure in which opening and closing the eyes is difficult due to contraction of periocular muscles. It is treated by botulinum toxin injected through orbicularis oculi muscle [41].

Botulinum toxin is used in Grave's disease to treat double vision by reducing oscillation and improve vision in rapid involuntary movement of the eye from side to side or up to down called nystagmus [42]. Spasticity is resistance to the passive movement of a limb that is maximal at the beginning of the movement and gives way as more pressure is applied. It is increased muscle tone mainly on upper motor neuron lesion due to stroke. Botulinum toxin therapy is used to reduce muscle tone in limbs and improve muscle functions [43]. Hyperhidrosis is excessive sweating than normal physiological activity. It can be generalized, regional and localized. Local hyperhydrosis is treated by botulinum toxin injected intradermally to block the release of acetylcholine from sympathetic nerve fiber that stimulates sweat glands. Injection of BoTN at localizes tissue can stop sweating but it is reversible [44] (Table 2).

The lethal dose of the Botox preparation for a person of 70 kg is to be 2,500-3,000 units. The dose for large muscles (e.g. gastrocnemius) is 100-400 units and the recommended dose for cosmetic purposes is less than 30 units are injected directly into the targeted muscle. whereas, for smaller muscles or deeper muscles, detected through electrostimulation, (e.g. orbicularis oculi) 1-2 sites of injection

and a quantity of 3–4 units are effective, and a large muscle (e.g. gastrocnemius) requires 4-5 injections and 300-400 units [2].

Botulinum toxin type A for prophylactic treatment of chronic migraine: Migraine is a neurological condition resulting from spasm and subsequent over dilation of certain arteries in the brain which causes visual disturbance. It is disorder characterized by recurrent episodes of headache with related symptoms such as nausea, vomiting, photophobia and phonophobia. The pain is usually unilateral (one side of the head) and aggravated by physical activity. It lasts for 4-72 hours and may force to bed rest. Migraine prophylaxis with Botulinum Toxin (BTX) type A has the ability to reduce the disease burden and it poses a potential benefit for the afflicted patients [45].

Botulinum toxin against tetanus-induced rigidity and spasms: Botulinum toxins contain zinc metalloproteinases that enter into nerve terminals of lower motor neurons and attacks synaptic vesicle proteins. Botulinum toxin A cleaves synaptosomal-associated protein (SNAP-25), botulinum toxins B, D, F, and G cleave synaptobrevin (which is also attacked by tetanus toxin); botulinum toxin C cleaves SNAP-25 and syntaxin. Compared to tetanus toxin, the botulinum toxins undergo less axonal and trans-synaptic transport. Therefore, the effects of botulinum toxins remain fairly confined to the nerve terminals of lower motor neurons, inhibiting release of acetylcholine and activation of voluntary muscles. For this reason they may have a role in reducing the muscular hyperactivity in tetanus patients. Botulinum toxin A was used successfully to control muscle rigidity and spasms. Theoretically, the action of botulinum toxin could be more rapid in tetanus, in which the activity of the lower motor neurons is much increased [46].

As cancer treatment: The therapeutic potential of clostridial toxins is not only for neurotoxin for the inhibition of neurotransmitter release, but also used as anticancer drug. The technology termed 'Clostridia Directed Enzyme Pro-Drug Therapy' (CDEPT) in which intravenously injected clostridial spores are used to target hypoxic regions of solid tumours? Spores get localized to solid tumours exclusively for germination, as they cannot grow in healthy tissues. Genetic modification of the clostridial host to express anti cancer compounds or pro-drug converting enzymes (as in CEDPT), has the potential to lead the localized destruction of solid tumours tissue [2]. It is well known that radiotherapy and chemotherapy can induce complications such as spastic contractures and painful muscle spasms. In such cases, treatment with BoNT-A injections can be helpful [47]. BoNT-A was also used to reduce ocular motility disturbances such as diplopia induced by plaque brachy therapy in the treatment of uveal melanoma [48] and to compensate diplo-pia-associated tumour (nasopharyngeal carcinoma) resulting from sixth nerve palsy [49].

Basic pharmacology of botulinum toxin

Botulinum toxin is composed of botulinum neurotoxin and non toxic protein. Structurally this toxin is made up of two basic pillars from botulinum toxin component and added excipients which use for stabilization and pH calibration. Lactose, sucrose and serum albumin are used for stabilization whereas buffer system is used for pH calibration. Botulinum neurotoxin has a heavy amino acid chain and a light amino acid chain. Heavy chain has a molecular weight of 100 KD and light chain has a molecular weight of 50 KDa which connected by a disulfide bond. The total complex weight may be a factor determining diffusion of the toxin from the site where it is injected [4].

Mechanism of action of botulinum toxin: After botulinum toxin gets to the body, light chain cleaves transport cascade proteins which transports acetylcholine vesicle to synaptic cleft from intracellular space [50]. When BT is injected into a target tissue, the heavy chain of the botulinum neurotoxin binds to glycoprotein structures specifically found on cholinergic nerve terminals because BT's have high selectivity for cholinergic synapses. After interance, the light chain of the botulinum neurotoxin binds with high specificity to the SNARE protein complex. Then the light chain's proteolytic cleavage of the SNARE protein complex prevents the docking of the acetylcholine vesicle on the inner surface of the cellular membrane and results in blockade of vesicle fusion. The inhibition of acetylcholine exocytosis by BT is terminated by restoration of the SNARE protein complex turnover [51]. Botulinum toxin can be used to treat hyperactive smooth muscles, such as the distal oesophageal sphincter in achalasia, the internal anal sphincter in anal fissures and anismus, and the pylorus in gastroparesis [52]. Botox is used to treat painful muscle hyperactivity disorders with substantial pain relief. This pain relief was qualified to reduction of the muscle hyperactivity. However, formalin-induced pain in animals can be reduced by BT direct analgesic effect. Probably such effect of BT is based on an action on neurotransmitters other than acetylcholine [53].

Generally, the botulinum toxin derived drugs have good adverse effect profiles. The adverse effects can be divided into three major categories such as obligate, local and systemic. Obligate effect is in born effects caused by therapeutic principle itself. Similarly local effect is caused by diffusion of botulinum toxin from the target tissue into adjacent tissue and systemic effect is adverse effects in tissues distant from the injection site and based upon botulinum toxin transport with in the blood circulation [54]. But systemic adverse effects of BT-B causes smooth muscle affection when heart burn, accommodation difficulties and obstipation occurs. When BT is used to treat hyperhidrosis, hypersalivation, hyperlacrimation, minmal adverse effects has been observed; such as dryness of eye or mucosa, exocrine glandular tissue [52].

Importance of botulinum toxin as bioweapon

In 1920, Hermann with his colleagues in the University of California studied the basis of use of botulinum toxin and isolate pure botulinum toxin type A as a stable acid precipitation for the first time which used in World War I as a biological weapon. Later US government made further research on this toxin as biological war weapons during World War II. Began from this era botulinum toxin used as drug [4].

Treatment

The first critical therapeutic step that given for botulism intoxicated animal is polyvalent antitoxin which is effective against circulating toxin before reaching the neuromuscular junction. Antibiotic administration is indicated for inhalation pneumonia or wound infection. Aminoglycosides may potentiate neuromuscular weakness and a non-depolarizing type of neuromuscular block. Beta-lactams successfully used to treat poultry affected by the toxicoinfection form of botulism [20,55].

In cattle with botulism, administration of Vitamin AD3E and activated charcoal aid the clinical recovery. Besides, strictly avoiding anti-clostridial antibiotics, fluid therapy, and calcium therapy may facilitate the clinical recovery. Upon fluid administration, the pulmonary congestion existed in the poor health cattle might have worsened the anoxia. Administration of antibiotics like penicillin, aminoglycosides, and tetracyclines further worsen the neuronal paralysis by increasing the availability of botulinum neurotoxin. Cattle in early botulism have fair chances of recovery with the modified therapy [56]. Ampicillin was used for antibiotic treatment in a dosage of 10 mg/kg body weight [57]. The animals with botulism can be treated with administration of intravenous isotonic saline and water to manage dehydration with activated charcoal and B-complex vitamin injections [58].

Prevention and control

The measures that taken to prevent feed borne botulism are based on; vaccination, providing safe and high-quality feed to farm animals, properly storing animal feed, inspecting water sources for dying or dead small animals and birds, avoiding spreading poultry litter that contains birds or dead animals on pastures and avoiding using poultry litter as bedding material [1].

Equine antitoxin used as passive immunization therapy. Botulism can be prevented by administration of a pentavalent (ABCDE) botulinum toxoid, which is a recombinant vaccine in development [30]. Immunization has been successfully adopted for broilers grown on farms with recurrent cases of the disease. Usually, 2 doses of vaccine administered about 14 days apart are used. The degree of protection by toxoid vaccination is influenced more by the time and number of inoculations than by the amount of toxoid injected. Foals can be vaccinated as early as 2 weeks of age and their immunization is also achieved by vaccinating pregnant mares, considering antibotulism antibodies found in the colostrum. Vaccinated foals or adult horses have to receive an annual booster [1].

Conclusion and Recommendation

Botulism is a severe neuroparalytic disease caused by highly potent toxin called BoNT. This toxin affects all animals including human. It is an important disease in the world, particularly in the farm subject to periods of protein and phosphorous deficiency. It is common in poultry farm, feedlots and in dairy cattle under intensive feeding systems. The source of infection is contaminated silage, water and packed food. After the toxin enter into the body, shows clinical sign with in 24hr-17days. Diagnosis is based on clinical sign and laboratory examination. It causes high mortality due to neurological disorder. Treatment by antibiotics is effective, before the toxin reaches synaptic junction. Rather than this, prevention with polyvalent vaccine and proper feed management is fair. Botulinum toxin is hazard to life; however it used as drug by using molecular technique. This toxin is a group of highly potent drugs with specific mechanism of action. They are not at the end of their development cycle, but rather at the starting phase. Careful use of botulinum toxin and imparting knowledge about its various clinical applications to the physicians will ensure that it will be an important treatment option for improving quality of life of patients. Based on above conclusion, the following future line should be forwarded:

- The farmers should store animals feed with proper ventilation to avoid multiplication of bacterial spore.
- The farmers should provide supplement feed in the ration to protect protein and mineral deficiency like calcium and phosphorous. This is risk factor for transmission of disease.
- The farm workers should properly dispose carcass of dead animals either by burring or incineration.
- Botulinum toxin to be used as drug, the recombinant DNA technology should be developed well concerning on wide range production of toxin.

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