REVIEW ARTICLE

Botulinum toxin and its clinical aspects: An overview

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Botulinum toxin (BTX), a potent neurotoxin which is produced by the bacterium *Clostridium botulinum*, consists of eight distinct neurotoxin serotypes referred to as (BTX type-A [BTX-A], B, C, D, E, F, G, H) all of which inhibit acetylcholine release at the neuromuscular junction. BTX-A, by blocking acetylcholine release at neuromuscular junctions, accounts for its therapeutic action to relieve dystonia, spasticity, and related disorders. A wide variety of medical conditions such as bruxism, hyperhidrosis, achalasia, focal dystonia, upper motor neuron syndrome, blepharospasm, and chronic migraine are now treated with BTX. The cosmetological applications include correction of lines, creases, and wrinkling all over the face, chin, neck, and chest. Side effects are generally rare and minimal. Injections with BTX-A are well-tolerated. Discovery of further newer indications of this neurotoxin can enlighten the path of research in the field of neuroscience.

Key words: Botulinum toxin, cosmetic uses, neurotoxin

INTRODUCTION

Neurotoxins, an extensive class of exogenous chemical compound, adversely affect the functioning of both developing and mature nervous tissue through the inhibition of neuron cellular processes. These inhibitory processes usually range from membrane depolarization mechanisms to interneuron communication. By inhibiting the neuronal ability to perform their expected intracellular functions, or pass a signal to a neighboring cell, neurotoxins can induce systemic nervous system arrest as in the case of botulinum toxin (BTX), or even nervous tissue death. The time required for the onset of symptoms upon neurotoxin exposure can vary between different toxins, being on the order of hours for BTX and years for lead. Though neurotoxins are often neurologically destructive, their ability to specifically target neural components is important in the study of nervous systems.[1]

Botulinum toxin, a protein and a neurotoxin, consists of eight serologically and antigenically distinct, but structurally similar toxin types referred to as BTX type-A, B, C, D, E, F, G, H, which are produced by the bacterium *Clostridium botulinum*. The toxin is a two-chain polypeptide with a 100-kDa heavy chain joined by a disulfide bond to a 50-kDa light chain.[2] This light chain is an enzyme (a protease) that attacks one of the fusion proteins (SNAP-25, syntaxin or synaptobrevin) at a neuromuscular junction, preventing vesicles from anchoring to the membrane to release acetylcholine. By inhibiting acetylcholine release at the neuromuscular junction, the toxin interferes with nerve impulses causing flaccid paralysis of muscles in botulism, as opposed to the spastic paralysis seen in tetanus.

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MECHANISM OF ACTION

Normally, acetylcholine diffuses across the synaptic cleft at the neuromuscular junction to bind acetylcholine receptors on the motor end plate of the muscle cell. The binding of acetylcholine to its receptors triggers an increase in the opening of sodium and potassium ion channels which initiates depolarization of the motor end plate and ultimately causes a muscle contraction. This toxin binds to cholinergic nerve terminals where it is internalized and released into the cytoplasm of the neuron. Forming a complex with neuronal proteins, it causes the proteolysis of SNAP-25 – a synaptosomal-associated protein utilized in synaptic vesicle fusion with the nerve terminal membrane. This is followed by a decrease in the frequency of acetylcholine released at the synaptic cleft, leading to the inhibition of its exocytosis. There is a loss of acetylcholine receptors at the motor end-plate thus resulting in loss of neuronal activity in the target organ, and eventually muscular denervation. This neurotoxin, therefore, interrupts a vital step in the contraction process of a skeletal muscle

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causing temporary muscle paralysis.[2] Eventually, however, the muscle initiates the formation of new acetylcholine receptors. As the axon terminal begins to sprout with the growth of branches to form new synaptic contacts, there is a gradual return to full muscle function, usually with minimal side effects [Figure 1].

Recent data, however, suggests that the neurotoxin also plays a role in reducing the release of inflammatory mediators like calcitonin gene-related peptide, substance P, glutamate etc., which causes pain.[3]

Botulinum toxin is denatured at temperatures >80°C (176°F). It is the most acutely toxic substance known, with an estimated human median lethal dose of 1.3–2.1 ng/kg intravenously or intramuscularly and 10–13 ng/kg when inhaled. Toxins are differentiated according to their antigenic differences: Types A to G. For type-A toxin, the toxic dose is estimated at 0.001 mcg/kg; the lethal dose for a 70-kg person by the oral route is estimated at 70 mcg, by the inhalational route 0.80 mcg to 0.90 mcg and the intravenous route 0.09 mcg to 0.15 mcg. The toxins are identified by neutralization with type-specific antitoxin; minor cross-neutralization between types C and D and types E and F has been observed. The toxins are produced by vegetative cells and released by cell lysis. Some toxins are fully activated by the bacteria that produce them as in case of proteolytic strains of type A, B, and F, while some require exogenous proteolytic activation as in case of types E and nonproteolytic types B and F. BTX can be absorbed from eyes, mucous membranes, respiratory tract or nonintact skin. Although BTX is a lethal, naturally occurring substance, it can also be used as an effective and powerful medication. Researchers discovered in the 1950s that injecting overactive muscles with minute quantities of BTX-A would result in decreased muscle activity by blocking the release of acetylcholine from the neuron by preventing the vesicle where the acetylcholine is stored from binding to the membrane where the neurotransmitter can be released. This will effectively weaken the muscle for a period of 3–4 months. Thus, notably unique feature of BTX is its relatively common therapeutic use in treating dystonia and spasticity disorders, as well as in inducing muscular atrophy.

Three forms of BTX-A (Botox®, Dysport® and Xeomin®) and one form of BTX-B (MyoBloc®) is available commercially for various medical and cosmetic procedures [Table 1].

THERAPEUTIC USES OF BOTULINUM TOXIN

Upper Motor Neuron Syndrome

For muscles affected by the upper motor neuron syndrome having weakness, decreased motor control, altered muscle tone, and impaired ability to effectively lengthen, BTX-A is now used as a common treatment. Severe muscle imbalance

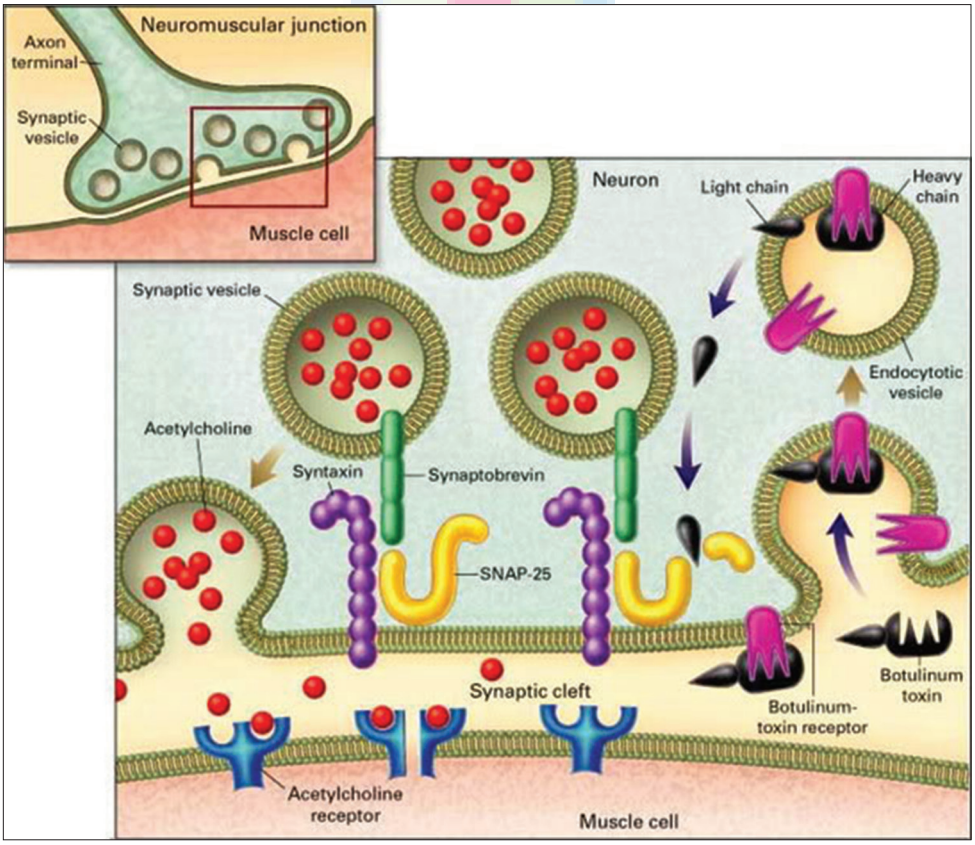

Figure 1: Mechanism of action of botulinum toxin

Table 1: Clinical uses of BTX

Indications	Method of delivery	Dosage	Duration of treatment effects	Effects	Side effects
Upper motor neuron syndrome	Administering into the muscle belly, thus helping to dampen the signals between nerve and muscle	Dosage ranging from 100 to 2000 units	Effect lasts for 3–4 months	Decrease its level of contraction can allow improved reciprocal motion, improved ability to move and exercise	Nausea, fatigue, bronchitis, pain in extremities and muscular weakness
Cervical dystonia	BTX B injected IM	Injection: Initial, 2500–5000 units IM	Clinical improvement generally begins within the first 2 weeks after injection with maximum clinical benefit at approximately 6 weeks postinjection	Relieving pain and lessening dystonic posturing by working on altering sensory input in the central nervous system in addition to its effects on the neuromuscular junction	Dysphagia, upper respiratory infection, neck pain, headache, increased cough, flu syndrome, back pain, rhinitis, dizziness, hypertonia, soreness at injection site, asthenia, oral dryness, speech disorder, fever, nausea and drowsiness
Blepharospasm	Injected on eyelid, the brow and the muscles under the lower lid	1.25–2.5 units into each of 3 sites per affected eye	Benefits begin in 1–14 days after the treatment and last for an average of 3–4 months	Inducing localized and partial paralysis	Ptosis, blurred vision, diplopia, tearing
Severe primary axillary hyperhidrosis	Injected into the axilla	50 units per axilla	Mean treatment effect lasts for 6 months	Localized, long-lasting but reversible decrease in cholinergic transmission	Nonunderarm sweating, respiratory infections such as cold or flu, headache, fever, neck or back pain and anxiety
Esophageal achalasia	Injected into the LES	80–100 units	Effect is mere transient lasting for about 6 months	Temporarily paralyze the nerves that signal the LES to contract, thereby helping to relieve the obstruction	Chest pain, heartburn. Damage to the esophageal wall and lining are rare
Focal dystonia	Local intradermal injections of BTX	Dosages may range from 100 to 450 units	Effect lasts for 3–4 months	Temporarily weaken the muscle reducing the spasm	Dysphagia, upper respiratory infection, neck pain, headache, increased cough, flu syndrome, back pain, rhinitis, dizziness, hypertonia, soreness at injection site, asthenia, oral dryness, speech disorder, fever, nausea and drowsiness
Migraine disability	Injected IM into the head and neck	Starting dose 155 units IM; maximum, 360 units IM	Effect lasts for 3–4 months	Inhibit the release of peripheral nociceptive neurotransmitters, which may then have a knock-on effect on the central pain processing systems that generate migraine headaches	Neck pain, headache, worsening migraine, muscular weakness, and eyelid ptosis
Bruxism	Five or six injections into the masseter and termporalis muscles and less often into the lateral pterygoids	25–40 IU per muscle	Effect lasts for about 3 months	Dilute solution of the toxin partially paralyzes the muscles and lessen their ability to forcefully clench and grind the jaw thereby aiming to retain enough muscular function to enable normal activities	Dizziness, mild difficulty swallowing, respiratory infections such as cold or flu, pain, nausea, headache and muscle weakness may occur

BTX – Botulinum toxin; IM – Intramuscularly; LES – Lower esophageal sphincter

with some muscles being hypertonic and lacking active lengthening may restrict joint motion. Thus, injecting an overactive muscle to decrease its level of contraction can allow improved reciprocal motion, so improved ability to move and exercise. Administering the injection into the muscle belly, can help to dampen the signals between nerve and muscle.[4,5]

Cervical Dystonia

Cervical dystonia, also known as spasmodic torticollis, is a painful chronic neurological movement disorder causing the head to involuntarily turn to the left, right, upward and/or downward. BTX-A is commonly used to treat this condition though its efficacy is lost after a time period of 12–16 weeks. Patients developing immunoresistance to this medication must use BTX–B. Treatment using BTX-B is comparable to BTX-A with an increased frequency of side effects such as dry mouth.[6]

Blepharospasm

Blepharospasm, a condition of abnormal contraction or twitch of the eyelid, is generally of two types: Essential and reflex blepharospasm. Essential blepharospasm is characterized by repeated forceful spasmodic contractions of the orbicularis oculi muscle frequently resulting in prolonged eyelid closure and severe visual disability while reflex blepharospasm can be attributed to any pain in and around the eye. BTX injections, inducing localized and partial paralysis, are a preferred method of treatment in this case.[7] They are usually given on the eyelid, the brow, and the muscles under the lower lid. The injections are carried out with a very fine needle. Benefits begin in 1–14 days after the treatment and last for an average of 3–4 months. Long-term follow-up studies have shown it to be a very safe and effective treatment, with up to 90% of patients obtaining almost complete relief of their condition. Side effects include drooping of the eyelid (ptosis), blurred vision, and double vision (diplopia). Tearing may occur, though all are transient and recover spontaneously. Providing the dose is kept small, and the injections carried out at a minimum of 3-month intervals, repeated treatments remain effective over a long period.[8]

Severe Primary Axillary Hyperhidrosis

The nonmuscular use of BTX-A laid its inception with its capacity to inhibit sweating. The efficacy of this toxin was thus utilized in the treatment of hyperhidrosis (excessive sweating). BTX-A was later approved for the treatment of severe primary axillary hyperhidrosis-excessive underarm sweating with an unknown cause which remained unmanageable by other topical agents.[9]

Esophageal Achalasia

Esophageal achalasia is characterized by incomplete lower esophageal sphincter (LES) relaxation, increased LES tone, and lack of peristalsis of the esophagus. LES pressure and relaxation are regulated by excitatory (e.g. acetylcholine, substance P) and inhibitory (e.g. nitric oxide, vasoactive intestinal peptide) neurotransmitters. Persons with achalasia lack noradrenergic, noncholinergic, inhibitory ganglion cells, thereby causing an imbalance in excitatory and inhibitory neurotransmission. The result is a hypertensive nonrelaxed esophageal sphincter. Patients with this condition often demonstrate dysphagia, regurgitation, and sometimes chest pain. BTX may be injected into the LES to paralyze the muscles holding it shut. As in the case of cosmetic Botox, the effect is mere transient lasting for about 6 months. Botox injections cause scarring in the sphincter that may increase the difficulty of later Heller myotomy or surgical cleaving of the muscle. This therapy is recommended only for patients who cannot risk surgery, such as elderly persons in poor health.[10]

Focal Dystonia

Focal dystonia is referred to as a neurological condition affecting a muscle or group of muscles in a specific part of the body causing involuntary muscular contractions and abnormal postures. Misfiring of neurons in the sensorimotor cortex, a thin layer of neural tissue covering the brain is thought to cause contractions. This condition is often treated with local intradermal injections of BTX, which compels the body to create new programs by blocking the nerve impulses to the contracting muscles. The injections temporarily weaken the muscle reducing the spasm. A different wrist position is necessary to compensate for the relaxed muscle. In this case, the injection acts as a tool to facilitate the patient developing a modified motor program. Botox only helps to reduce the symptoms of the disorder though not acting as a cure for it.[11]

Migraine and other Headache Disorders

BTX-A has been shown to decrease migraine disability in patients with mild to severe migraine randomized to BTX-A or placebo in a double-blind, placebo-controlled trial.[12] Onabotulinum toxin A received Food and Drug Administration (FDA) approval for the treatment of chronic migraine on October 15, 2010. The toxin is usually injected into the head and neck to treat these chronic headaches. This toxin has been reported to improve headache symptoms when used prophylactically for patients with chronic migraine who exhibit headache characteristics consistent with: Pressure perceived from outside source, shorter total duration of chronic migraines (<30 years), "detoxification" of patients with coexisting chronic daily headache due to medication overuse, and no current history of other preventive headache medications.[13]

Bruxism

Bruxism, an oral parafunctional activity, is characterized by excessive grinding of the teeth and/or excessive clenching

of the jaw. Bruxism is a widespread condition with its global prevalence ranging from 8% to 31% in the general population affecting children, adults, the elderly, and may in fact be more frequent in patients with developmental disabilities. It is most often a result of psychological stress and manifests both nocturnally and diurnally. Subsequent signs of bruxism may include myofascial pain and limited range of motion of the mandible. Chronic bruxism may lead to tooth wear, periodontal disease, headaches, and other temporomandibular joint disorders. BTX has been shown to provide treatment in a range of bruxism-related conditions, such as in patients with developmental disabilities, nocturnal bruxism and myofascial pain. A randomized controlled trial based on thirty people with bruxism reported that Botox reduces the myofascial pain symptoms. In 2013, a further randomized control trial investigating Botox in bruxism started. The use of Botox is a longer-term solution to the problem of bruxism. Current treatment with Botox involves a bilateral injection into the masseter and temporalis muscles. However, the injection of Botox into the temporalis muscle has not conclusively been found to eliminate bruxism. Rather, the bilateral action of Botox on the masseter muscles, just superior to the angle of the mandible, has been found to be effective in numerous clinical trials. The neurotoxin functions by causing muscular paralysis by inhibition of acetylcholine release at neuromuscular junctions. Botox injections are used in bruxism on the theory that a dilute solution of the toxin will partially paralyze the muscles and lessen their ability to forcefully clench and grind the jaw thereby aiming to retain enough muscular function to enable normal activities such as talking and eating. This method of treatment typically provides relief for 4–6 months. At the conclusion of the cycle of relief, Botox may be re-administered for continued management of the condition. Similarly, because the neurotoxin provides a treatment that is reversible, it gives patients the option to stop the therapy at any time. The neurotoxin may also work to inhibit periodontal mechanoreceptors providing a solution to problems with jaw closure related to bruxism. Bruxism may also result in masseteric hypertrophy. Botox may provide a much less invasive option for this condition compared to surgery. This treatment typically involves five or six injections into the masseter and termporalis muscles, and less often into the lateral pterygoids. It takes a few minutes per side, and the patient may start feeling the effects by the next day and may last for about 3-month. Occasionally, adverse effects may occur, such as bruising can occur, but this is quite rare.[14]

COSMETIC USES OF BOTULINUM TOXIN

In cosmetic applications, a Botox injection, consisted of a small dose of BTX, can be used to prevent the development of wrinkles by paralyzing facial muscles. After many trials, on April 12, 2002, the FDA announced regulatory approval of BTX-A (Botox cosmetic) to temporarily improve the appearance of moderate-to-severe frown lines between the eyebrows or glabellar lines. Since then, cosmetic use of BTX-A has become widespread. As of 2007, it has been the most common cosmetic operation, with 4.6 million procedures in the United States.[15]

Dermatologically Botox/Botox Cosmetic is usually used at the labeled dose of 20 Units (for glabellar lines) or 100 Units (for severe primary axillary hyperhidrosis). Each vial of Botox contains either 100 Units of *C. botulinum* type A neurotoxin complex, 0.5 mg of Albumin Human, and 0.9 mg of sodium chloride or 200 Units of *C. botulinum* type A neurotoxin complex, 1 mg of albumin human, and 1.8 mg of sodium chloride in a sterile, vacuum-dried form without a preservative. Common side effects associated with the use of Botox cosmetic are problem with swallowing, breathing or speaking. Botox and Botox cosmetic may even cause loss of strength or general muscle weakness or vision problems within hours to weeks of taking Botox or Botox cosmetic.[16]

Neuromodulator injections of BTX-A can improve frown lines, deep wrinkles in the sky between the eyebrows and on the bridge of the nose, across the forehead and at the corners of the eyes. BTX injected directly into the target muscle, treats vertical lines between the eyebrows and on the bridge of the nose, the squint lines or crow's feet at the corners of the eyes, the forehead horizontal lines and the platysmal muscle bands often visible on the neck, commonly known as turkey neck. It may also be used for eyebrow positioning. Once the muscle is weakened and relaxed, it cannot contract. Since there is no way to make the undesirable facial expression, the lines gradually smooth out from disuse, and new creases are prevented from forming. Other muscles like those needed to raise the eyebrows are not affected, so a natural expression is maintained. BTX may not be as effective on the smile lines around the mouth because the muscle action in this area is needed for expression and important functions like eating.[17] The wrinkle-preventing effect of Botox normally lasts about 3–4 months, but can last up to 6 months.

The global botox market is forecast to reach $2.9 billion by 2018. The entire global market for facial esthetics is forecast to reach $4.7 billion in 2018, of which the US is expected to contribute over $2 billion.[18]

Other uses of BTX A that are not specifically approved by the FDA but widely known include treatment of pediatric incontinence, anal fissure, vaginismus, painful bladder syndrome, benign prostatic hyperplasia, allergic rhinitis.[19-23]

In addition, Botox A has been found to aid in weight loss by increasing the gastric emptying time-an experimental study conducted in an obese rat model system.[24]

SIDE EFFECTS

Botulinum toxin type-A has effects other than peripheral action; indirect effects may also occur on the spinal cord and brain, which are caused by changes in the normal balance of efferent and afferent signals. Side effects associated with administration of BTX-A fall into three broad categories: (1) Diffusion of the toxin can lead to unwanted inhibition of transmission at neighboring nerve endings, (2) continued blockade of transmission can cause some effects similar to anatomic denervation, such as muscle atrophy, (3) immunoresistance to BTX-A is another undesirable side effect.

Side effects are minimal and however predictable from the mode of action and chemical structure of the molecule. The two major areas of side effects are paralysis of the wrong muscle group and allergic reaction. However, bruising at the site of injection is not considered a side effect of the concerned toxin. It is caused due the mode of administration and is prevented by the clinician applying pressure to the injection site, but may still occur, and lasts around 7–11 days.

In cosmetic use, makeup may be worn after treatment, but care should be taken to avoid pressing or massaging the area for several hours. In rare instances, patients may develop temporary weakness of the neighboring muscles, headache, a temporary droopy eyebrow or eyelid, double vision, uneven smile or loss of the ability to close eyes. This wears off in around 6 weeks. If severe lower lid weakness occurs, an exposure keratitis may result and if the lateral rectus is weakened, diplopia results. Treatment is symptomatic.

When injecting the masseter muscle of the jaw, loss of muscle function will result in a loss or reduction of power to chew solid foods. Patients receiving injections into the neck muscles for torticollis may develop dysphagia because of diffusion of the toxin into the oropharynx. When this occurs, it usually lasts only a few days or weeks. Some patients may require soft foods. Although a swallowing weakness does not herald systemic toxicity, if it is severe, patients may be at risk of aspiration. Some patients experience neck weakness, which is especially noticeable when attempting to raise the head from a supine position.[25]

All cosmetic treatments are of limited duration and can be as short as 6 weeks, but usually the effective period lasts from 2 to 3 months. At the extremely low doses used medicinally, BTX has a very low degree of human and animal toxicity. Individuals, who are pregnant, have egg allergies or neuromuscular disorders are advised to avoid Botox.

Botox takes away or dampens the emotional expressions in a particular situation. That may be due to less interaction between facial muscle movement and brain.

Other systemic side effects include an influenza-like illness and rarely, brachial plexopathy, which may be immune-mediated.[26] Gallbladder dysfunction can be attributed to autonomic side effects of the toxin and a case of necrotizing fasciitis in an immunosuppressed woman with blepharospasm have been noted.[27]

BOTULINUM TOXIN TYPE-A IN BOTULISM

Botulinum toxin can cause botulism, a rare, serious, life-threatening, and sometimes fatal paralytic illness in humans. The toxin enters the human body in one of the three ways: By colonization of the digestive tract by the bacterium in children (infant botulism) or adults (adult intestinal toxemia), by ingestion of toxin from foods (foodborne botulism) or by contamination of a wound by the bacterium (wound bacterium). Infant botulism is caused by consuming the spores of the botulinum bacteria, which then grow in the intestines and release toxin. Adult intestinal toxemia or adult intestinal colonization botulism is a very rare kind of botulism that occurs among adults by the same route as infant botulism. Finally, iatrogenic botulism can occur from an accidental overdose of BTX. In the United States, an average of 145 cases is reported each year. Of these, approximately 15% are foodborne, 65% are infant botulism, and 20% are wound. Adult intestinal colonization and the iatrogenic botulism also occur, but rarely. Symptoms of botulism can be characterized as weakness of muscles supplied by cranial nerves, controlling eye movements, chewing, swallowing. Double vision, drooping of both eyelids, loss of facial expression, and swallowing problems may occur. Most infant botulism cases cannot be prevented because the bacteria that causes this disease is in soil and dust. The bacteria can be found inside homes on floors, carpet, and countertops even after cleaning. Even, honey can contain the bacteria that causes infant botulism so, children < 12 months old should not be fed honey. Honey is thus considered safe for persons' 1-year of age and older. Wound botulism can be prevented by promptly seeking medical care for infected wounds.[28] Severe botulism can lead to reduced movement of respiratory muscle, thereby causing the problem with gaseous exchange. This can be sometimes experienced as dyspnea and at severity, can lead to respiratory failure.

As the toxin is highly biologically active, an estimated dose of 1 μg/kg body weight is sufficient to induce an insufficient tidal volume and resultant death by asphyxiation. Due to its high toxicity, BTX antitoxins have been an active area of research. It has been shown that capsaicin which is an active compound responsible for heat in chili peppers, can bind

the transient receptor potential cation channel subfamily V member 1 receptor expressed on cholinergic neurons and inhibited the toxic effects of BTX. Two primary botulinum antitoxins are available for treatment of botulism.

- Trivalent (A, B, E) botulinum antitoxin is derived from equine sources using whole antibodies (Fab and Fc portions)
- The second antitoxin is heptavalent (A, B, C, D, E, F, G) botulinum antitoxin, which is derived from "despeciated" equine IgG antibodies, which have had the Fc portion cleaved off, leaving the F (ab') 2 portions. This less immunogenic antitoxin is effective against all known strains of botulism.[29]

CONCLUSION

Botulinum toxins are a promising treatment for patients with dystonia, spasticity disorders, and muscular atrophy. The widespread use of this compound has revolutionized the field of cosmetology. Precise knowledge of the functional anatomy of muscles and experience with the procedure is an absolute necessity to correctly use BTXs in clinical practice. The discovery of new indications for the use of BTX should promote continued research and development of this remarkable compound.

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