



A Review on Neurotoxins from *Clostridium botulinum* against Neuro-muscular Disorders

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Authors' contributions

This work was carried out in collaboration between both authors. Both authors read and approved the final manuscript.

Article Information

DOI: 10.9734/JABB/2021/v24i730223

Editor(s):

(1) Dr. Joana Chiang, China Medical University, Taiwan.

Reviewers:

(1) P.Brindha Devi, Vels University, India.

(2) Rakesh Sharda, Nanaji Deshmukh Veterinary University, India.

(3) Ionica Mihaela Iancu, Agriculture Science University, Romania.

Complete Peer review History: <https://www.sdiarticle4.com/review-history/72279>

Review article

Received 06 June 2021

Accepted 11 August 2021

Published 17 August 2021

ABSTRACT

Neuro-muscular disorders cause a series of serious complications in the human body, where some lead to considerable morbidity and mortality occasionally. Neurological diseases result in dystrophy, inhibited growth, etc. This present review aimed to emphasize the employment of neurotoxins against neuro degenerative disorders. The source of neurotoxins includes botulinum (*Clostridium botulinum*), snakes like *Vespa orientalis* and some medically important arthropods like hornets and spiders. The review not only describes the potential of the neurotoxins in the treatment but also elucidates the mechanism of action of lethal toxins like botulinum. Safety and dosage regimens of various toxins with the help of proven study data would aid in endorsing researchers for further research on toxins making them more superior targeted drugs.

Keywords: *Botulinum; Toxins; Neuro-muscular Disorders; memory; palsy.*

1. INTRODUCTION

Diseases like cerebral palsy, which have some serious complications such as excited movement

of the lower and upper extremities is ideally observed in the early phases of life lacks a proper treatment regimen. Cerebral palsy generally affects the whole life of the child

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interfering in growth [1]. Musculoskeletal complications are also found to develop in cerebral palsy. Loss of strength, muscle tone and balance are some of the few major complications of the muscular system [2]. Spasticity of cerebral palsy generally effects the longitudinal muscle growth and worsens the condition by decreasing the muscle volume and shortens the spastic muscle, thereby making the child prone to difficulty in walking [3]. Treatment targeting spasticity is therefore regarded ideal in the treatment of cerebral palsy. On the more negative side, palsy not only causes hindering in growth but also adds to psychological stress and memory loss such as dementia. Dementia is an acquired syndrome of decline in memory and at least one other cognitive domain, such as language, visio-spatial, or executive function, that is sufficient to interfere with social or occupational function in an alert person [4].

Toxins, especially neurotoxins have lethal effects on the human body. They are a mixture of two or more compounds like peptides, amines etc [5]. They are most likely to display deleterious effect on the human body. For instance, botulinum is responsible for causing neurophylactic disease. The botulinum toxins are considered as one of the most potent neurotoxins known to mankind [6]. Similarly, neurotoxins from venomous snakes also cause severe neurological damage and finally leading to death by nerve damage or respiratory failure. Surprisingly, toxins are currently employed to combat neurological disorders like Alzheimer's disease, stroke, etc [7]. Botulinum is employed for treating facial nerve dysfunction, palsy, etc. Thus, the present review focuses on the employment of the neurotoxins for managing neuro-degenerative disorders, muscular skeletal disorders and other conditions like depression and memory malfunctions.

2. NEUROTOXINS FROM VENOMOUS ANIMALS AS POTENTIAL DRUGS

Reptiles and arthropods, such as snakes and spiders, which are considered as a bane for humans are now turning out into a big boon. The venom of these creatures, which is considered to be neurotoxic in nature, possesses pleiotropic and synergistic effects over the neuromuscular system. Snake venom has been incorporated into the medicine since ages [8-9]. *Echis carinatus*, which is a venomous viper, has been potentially proved for its neuro-protective effect. It was affirmed that the venom of this snake was

found to possess stimulating effect on neurons [8]. The venom of *Echis carinatus* in a study was successful in treating chronic degenerative illness and depression. This may be the first report on cognition by this particular venom [10]. Peptides are complex chemical structures obtained from the snake venom of few snakes, which play an active role in combating severe conditions like neuropathic pain. A similar effect was observed in the venom of Amazonian viper *Bothrops atrox* [10]. This venom's low molecular mass peptide fraction Ba-V obtained was found to carry cyclosporine-A (CsA) like effect to inhibit the calcium/phosphate-induced swelling, which indicates it's potential to prevent mitochondrial permeability transition (MPT) is one of the prime aspects in neuro protection [10]. Hornets on the other hand are found to have deadly neurotoxins. Some studies conducted on cats, dogs and mice, proved that the fractions of the hornet's venom possess anticholinesterase activity [11]. The venom of hornet enumerated positive results against neurodegeneration and amnesia [5]. There was a significant memory enhancement observed and was proved that there was an improvement in the step down passive avoidance test, which indicates a clear improvement in memory. Latrotoxins are the category of neuro toxins that are found in the genus *Latrodectus*. One of the prime compounds is α - latrotoxin. They were originally found to exhibit effects on the presynaptic membrane. α -Latrotoxin binds to both CL (isoforms) and neurexins close to the active zone on the presynaptic membrane. However, these receptors do not function in any intracellular transduction mechanism [12]. Latrotoxins are also found to conduct intracellular activation of exocytosis. There was an arguably important mechanism of depletion of acetyl choline vesicles, which stays as an important mechanism in treating many diseases related to muscle spasticity.

3. SIGNIFICANCE OF BOTULINUM

A proper neurotoxic mechanism was postulated after the discovery of botulinum in the early 19th century [13]. Botulinum toxin serotype-A (BoNT-A), a form of botulinum toxin affects the neuromuscular junction by revealing a reversible action on muscle tone and other prime functions [14]. Employing the use of this toxin for treating various disease conditions like facial movement disorders, limb and neck dystonias etc. did prove its therapeutic potential to a greater extent. Acetylcholine inhibition at the sites of neuromuscular junctions is one of the

fundamental mechanisms of botulinum [15], which expresses a key role in reducing muscle plasticity. Table 1 illustrates the various activities of botulinum assessed by various researchers. This elucidation gives a key importance and possible scope regarding the employment of botulinum in various treatment regimens and symptomatic approaches against musculoskeletal disorders. The praxis of the botulinum in the management of spasticity in ambulant children was thus recommended [16]. Though many researchers have raised an issue over the safety concerns and efficacy of botulinum [17], some have postulated that the botulinum toxin-A (BTX-A) prevent the deformity in children suffering with cerebral palsy and thus reducing the need for surgery [18-19].

4. BOTULINUM AGAINST MUSCULO SKELETAL DISORDERS

Botulinum neurotoxins are used as effective agents against hypersecretory syndromes as they possess a potential in blocking the acetylcholine release [15]. Blepharospasm is a facial movement disorder related to the eyelid closure. Botulinum toxins are employed for treating such conditions. It was proved that BoNT-A was effective by 90% in the treatment against blepharospasm [20]. Disease conditions like oromandibular dystonia, which showed poor response toward systemic medications like clonazepam and antispasmodic, could be successfully inhibited by the employment of botulinum. Tan and Jankovic [21] observed a great improvement in patients with jaw-closure dystonias. Among the potential applications, cervical dystonia was best studied and yielded impressive results. According to a meta-analysis data from relevant drug trials [22], mean two to three point improvement was observed on the Tsui scale at the peak effect. Among the studies on pediatric spasticity, one of the trials displayed statistically significant improvement at two weeks [23]. Hypersecretory syndromes are some of the socially inelegant or awkward conditions occurring in children. Hypersecretory syndromes include sialorrhea/ptyalism, hyperhidrosis, etc. Poor swallowing control is a common symptom in such neurological conditions [24]. With the prime mechanism of the botulinum to act on the SNARE (soluble N-ethyl maleimide- sensitive factor attachment protein receptor) complex, the symptoms could be reduced. Studies (controlled) on Parkinsonism patients revealed that the drooling, which is regarded as one of the major complications of these disease conditions was

far significantly reduced [25]. Surprisingly, there were no adverse events recorded, unlike the previous anticholinergic medications. In hyperhidrosis, it was reported in a pilot study that absolute sweat was reduced by 40 % relative to placebo at 4 weeks by the usage of BOTOX® [26]. Fig. 2 depicts the mechanism of botulinum in aiding in the elongation of muscle fibers. In the condition of palsy, the muscle fibers are found in the contracted state. The botulinum injections to the muscle tissues would aid in the process of stretching, which in turn would reduce the difficulty in walking.

5. MECHANISM OF THE BOTULINUM IN COMBATING NEUROMUSCULAR DISORDERS

The botulinum is secreted by *Clostridium botulinum*. There are seven serotypes of the botulinum. The toxins contain 150 KD single chain polypeptides after which they form heavy and light chains [14]. The heavy chains bind to the SV2 receptor on the pre-synaptic membrane. This process mediates the toxins (botulinum) to the axon terminal. These toxins intervene with SNARE complex (soluble N-ethyl maleimide-sensitive factor attachment protein receptor). This leads to the docking of the neurotransmitter containing intra-axonal vesicles with the pre-synaptic membrane with the help of SNARE complex which results in the eviction of the acetylcholine into the pre synaptic cleft. The toxin (Botulinum A) is primarily responsible for the cleavage of the SNARE component SNAP- 25. Fig. 3 describes the mechanism of botulinum in reducing muscle spasticity. The schematism describes right from the action of the botulinum in moving into the axon terminals to the extrusion process of the acetylcholine into the presynaptic cleft. Reduction of the acetylcholine outflow at the neuromuscular junction is the major objective behind the employment of botulinum. Achievement of the Miniature EndPlate Potentials (MEPP'S) is the pivotal step, which occurs by depolarization of post synaptic membrane by each packet of acetylcholine. The botulinum here would prevent acetylcholine secretion but not MEPP frequency. The motor EPP (end plate potential) is reduced below the muscle membrane threshold and the ability to generate muscle fiber action potentials and subsequent contraction is declined. The muscle fibers supplied by the nerve terminal affected significantly by botulinum toxins [27], although the gross histologic appearance of the neuromuscular junction remains normal [28].

After the effective impairment of the acetylcholine from the axon terminal, the regeneration process of the nerve initiates. The regeneration process starts from the nodes of Ranvier. These may begin after botulinum toxin administration. The sprouts form a new neuromuscular junction and endplate with adjacent muscle after which, the muscle fiber atrophy may occur before the full maturation of the motor end plates. As the SNARE complex is neutralized initially, it will be regenerated and the acetylcholine resumes from the original nerve terminal. The physiological inactivity lasts for 3-4 months [29].

6. SAFETY OF TOXIN'S

The safety of toxins in various treatments is always a concern as toxins, especially from sources like venom and secondary metabolites as they might carry concealed effects and deserve to be screened before usage on living tissue as most of the toxins or related derivatives have necrotic potential. Snake venom for example which is employed for treating various neuromuscular disorders is highly toxic for the living tissue, provided it is purified or used in de venomized form. Devenomization is a process where some photochemical reactions occur [30]. Photo-oxidation is the process resorted where the venom is exposed under gamma, visible and ultraviolet radiations using methylene blue as a

sensitizer to generate antigenically-active or detoxified species, which can also be used in the production of anti-venom [31]. When the toxophoric regions are altered, snake venom proteins are said to possess significant pharmacological effects. Radiation methods are employed to shorten the immunization process and for a better detoxification process [9]. However, usage and dosage of botulinum is a huge controversy. There has always been a concern raised by science whether botulinum is safe for long-term usage? The botulinum was effectively employed in the medication in late 60's [14]. After initial success over the treatment of blepharospasm, facial nerve dysfunction and strabismus, BoNT-A has passed through the US FDA (United States Food and drug administration) after further purification. Reputed pharmaceutical companies like Allergan, Merz, Lanzhou etc. marketed the BoNT-A with numerous brand names. Dosing is the principal aspect during screening of any drug especially when agents like toxins are employed in the treatment. After a study on non-human primates by Scott and Suzuki, BoNT-A was affirmed to be non-lethal when given intramuscularly to eight rhesus monkeys [32]. The approximate safe dose in a 75 kg/b.w human was supposed to be 1800 U. Though death was recorded in some instances, a direct casual link was not established.

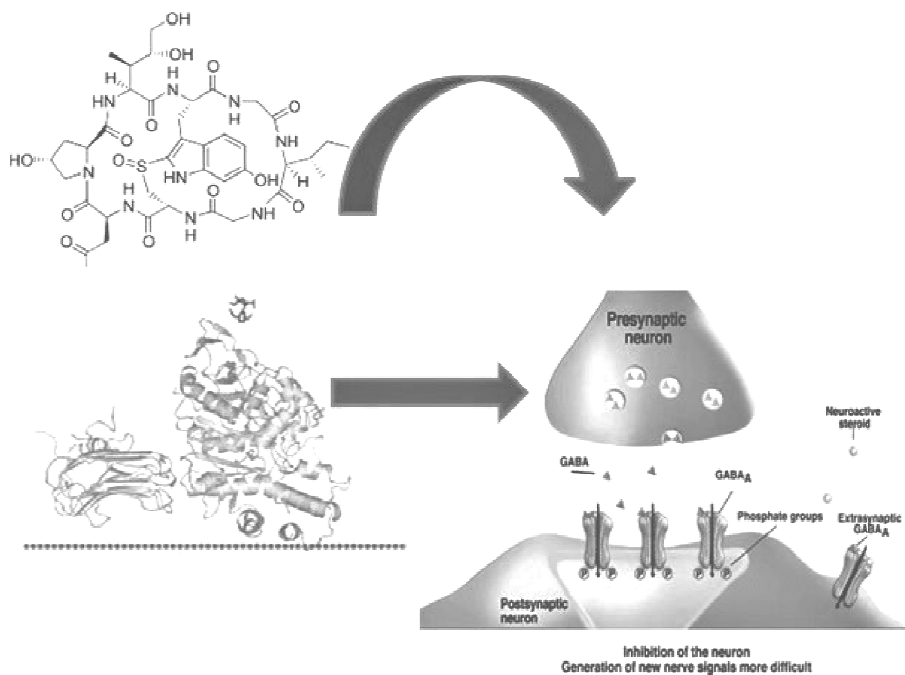


Fig. 1. Toxins from various classes of insects and animals against neuro disorders

Table 1. Reports on the botulinum

Study	Observation	Reference
Assessed the employment of botulinum toxin treatment on upper limb function in school-age children with bilateral spastic cerebral palsy.	As daily activities are one of the prime aspects in school children's life, these activities were screened and they have found that BoNT-A could aid in escalating the performance of school children in their daily activities.	Jee et al. [1]
Usage of BTX-A Injection	The muscle fibers in spastic cerebral palsy are found short and the BTX aids in longitudinal growth of the muscle fibers.	John & Kevin [14]
Botulinum for treatment of cerebral palsy in children (spasticity)	The safety of the BTX-A was evaluated which was found to be safe. There was also a recording of functional improvement.	Kristie et al. [33]
BTX-A with and without rehabilitation.	They have affirmed that the BTX-A did improve the muscle tension and motor function.	Jianjun et al. [34]
The safety of botulinum in upper limb spasticity after stroke was screened.	The usage of distort (BTX-A) in patients with post stroke upper limb spasticity was found to be significant.	Bakheit et al. [35]
Assessed Three-dimensional ultrasound to evaluate the medical gastrocnemius muscle belly length in children with spastic diplegia	A significant difference between the gastrocnemius muscle lengths and diplegia was found.	Fry et al. [36]

BTX-A, Botulinum toxin A

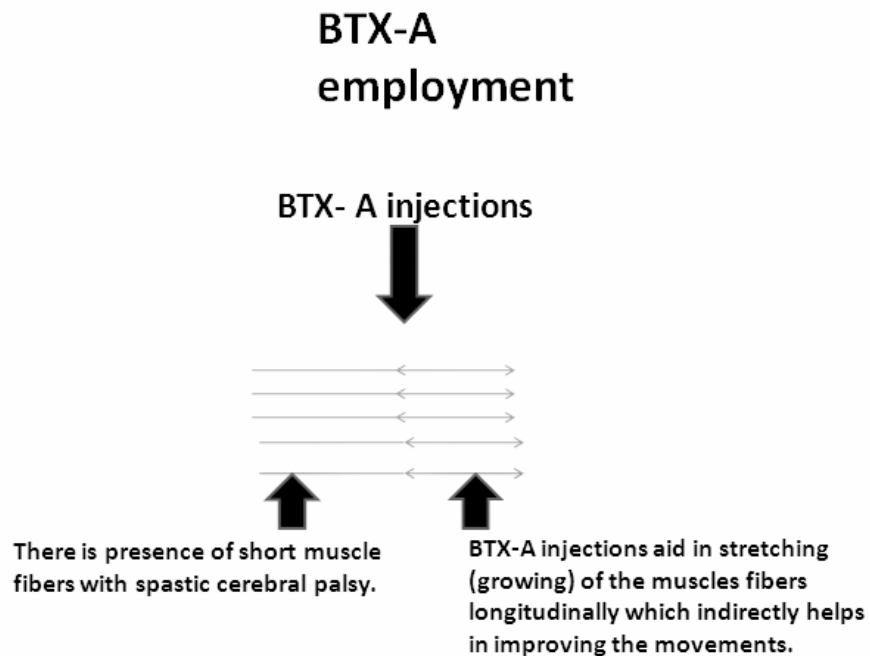


Fig. 2. Role of BTX-A in stretching of the muscle fibers, thereby aiding in treatment for palsy (BTX-A, Botulinum toxin A)

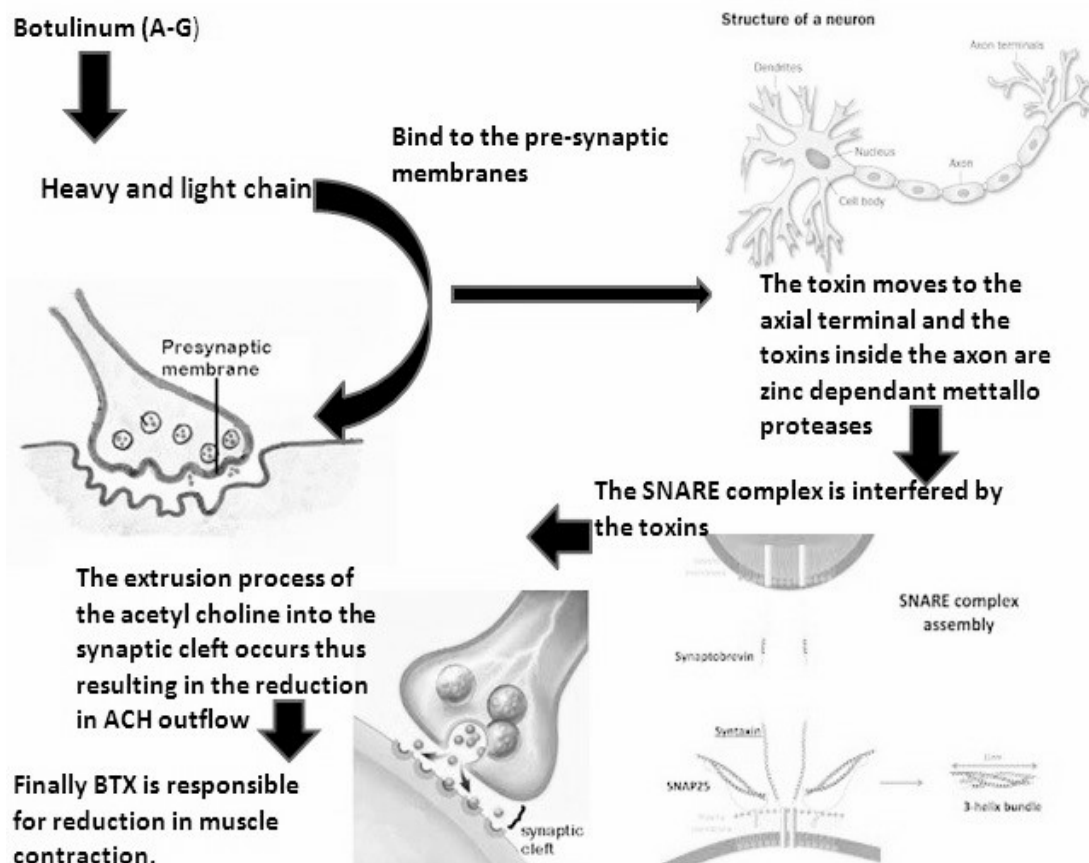


Fig. 3. Mechanism of botulinum in devitalizing the acetylcholine and reducing muscle spasticity

The field of medicine has been dependent on toxins, especially venom of insects and animals since decades. The proper pretreatment of toxins by methods such as de venomization [9], which would devitalize the lethal effects of toxins would aid in further safe usage on living tissues. Neuromuscular disorders like memory loss or amnesia, palsy etc. generally result from the non-progressive brain damage. Potential toxins such as botulinum have inhibitory effects on acetylcholine [28]. Active impairment of acetylcholine would aid in reducing the muscle spasticity. Snake venoms as emphasized in the current review, act on some of the receptors, which help in escalating the memory and other prime functions. This present review article covers all the aspects of the toxins and their active role in acting against the neurodegenerative and other neuromuscular diseases. The employment of BTX-A, latrotoxins like α -Latrotoxin etc from sources like arthropods and neurotoxin venom from sources like *Vespa orientalis* etc are some of the sources of

neurotoxins, which might provide greater therapeutic avenues.

8. CONCLUSION

Summing up, we can conclude that many neurotoxins from sources like snake venom, botulinum (*Clostridium botulinum*), hornets etc can be a rich source of neuroprotective agents which can be employed in treating stubborn neuromuscular disease conditions. Handling conditions like spastic movements and other similar conditions require interdisciplinary approaches. By the emerging botulinum and other neurotoxins, there is a hope rising in the field of medicine and biotechnology and thus leading to a path of novel treatment.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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