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Article Review: Botulinum Toxin; Structure and Mechanism of Action

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ABSTRACT

The anaerobic, Gram-positive bacteria *Clostridium botulinum* produces a neurotoxin known as botulinum toxin (BoNTs). In this review, the botulinum toxin's chemical makeup, mode of action, and various uses—particularly medical ones—are discussed. The induction of flaccid paralysis by botulinum neurotoxins (BoNT) requires internalization of neural cells, translocation of the catalytic domain into the cytosol, and cleavage of one of the three soluble N-ethylmaleimide sensitive factor attachment protein receptors (SNARE). By blocking nerve signals from reaching muscles, botulinum toxin temporarily weakens muscles. An acute paralytic illness known as botulism can result from accidentally consuming high amounts of botulinum toxin, such as from inadequately canned foods. Botulinum toxins are now used to treat a wide range of medical problems, including strabismus, focal dystonias, hemifacial spasm, other spastic movement disorders, migraines, hypersalivation, and hyperhidrosis, as well as numerous chronic ailments that only partially respond to medical treatment. The FDA authorized the use of Botox® (Botulinum toxin-A) in 2002 for the temporary reduction of glabellar forehead frown lines for cosmetic purposes.

Keywords- Botulinum toxin, Neurotoxin, Gram positive, exotoxins, BOTOX.

I. INTRODUCTION

One of the most poisonous biological substances ever found is the "wonder poison" known as botulinum toxin (BoNTs) [1]. The gram-positive, anaerobic rod-shaped bacterium *Clostridium botulinum*, which is widely found on plants, in soil, water, and animal intestinal tracts, is responsible for producing this neurotoxin [2]. The exotoxin-producing bacteria Clostridium botulinum produces eight distinct exotoxins, known as A, B, C 1, C 2, D, E, F, and G. Type A is the most potent toxin, followed by types B and F. Types A, B, and E are frequently linked to systemic botulism in humans [3]. After being broken down by internal or external proteases, the majority of BoNTs are released as dichains. Generally speaking, dichain BoNTs are more potent than single-chain BoNTs. The most frequent causes of infection in humans are BoNTA, B, E, and maybe F. BoNTs C and D are the culprits for infections in mammals and birds [4, 5]. Botulinum toxin is a complicated mixture of proteins that includes both various non-toxic proteins and botulinum neurotoxin (Bovine Toxin). People are susceptible to foodborne, wound-related, neonatal, and adult colonization kinds of botulism. Reports of these incidents are forwarded to the Centers for Disease Control and Prevention (CDC) in the United States. The botulinum toxin is created by the neurotoxin-producing Clostridium spp (6). Human botulism is a rare but potentially lethal form of food poisoning. In the United States, the case fatality rate was 60% until the 1950s. Yet, today 10% of cases result in death because of advancements in intensive care and the accessibility of therapeutic antitoxin. The majority of these fatalities are either the result of long-term ventilator care difficulties or a delay in diagnosis (6). The first commercially produced toxin

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was type A, which is now sold by two companies: Ipsen (Dysport) and Allergan (Botox). The development of type C and type F toxins is ongoing, but the other serotypes are not currently commercially available. Naturally, Clostridium botulinum has a negative reputation and periodically, outbreaks of botulism still occur (7). The aims from this review are the study the chemical structure, mode of action and medical application of Botulinum toxin.

II. BOTULINUM TOXIN STRUCTURE

It is most likely that bacterial lysis is what releases clostridial neurotoxins from the cell because they are constantly generated as a single, 150 kDa, inactive polypeptide chain without a leader sequence. A heavy chain (H, 100 kDa), a light chain (L, 50 kDa), connected by disulphide bonds, and one atom of zinc make up the active di-chain neurotoxins that are released when these toxins are broken down by bacterial or tissue proteases inside of an exposed, particularly protease-sensitive loop. The breaking of this interchain S-S bond by reduction eliminates toxicity and is essential for cell penetration (8). A functional division of the heavy chain into an amino terminal domain (Hn) and a carboxyl terminal domain (Hc) is possible (9), see figure (1).

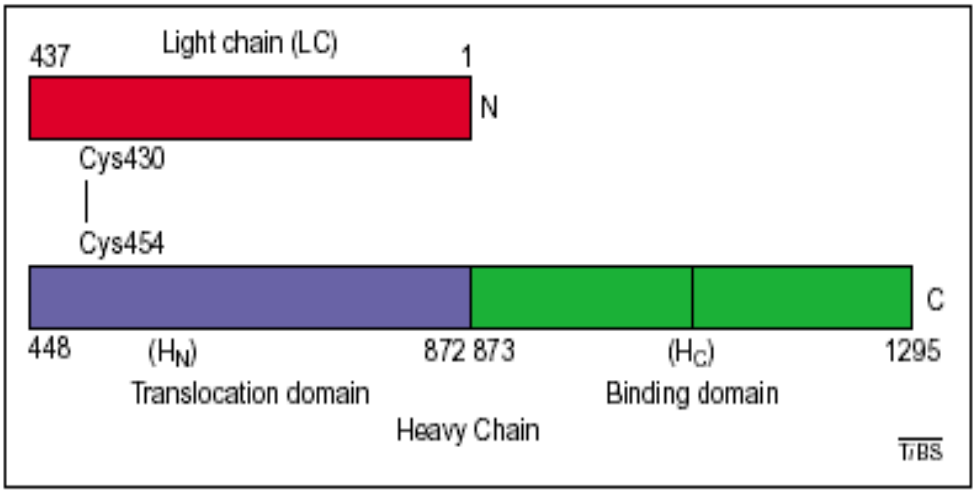
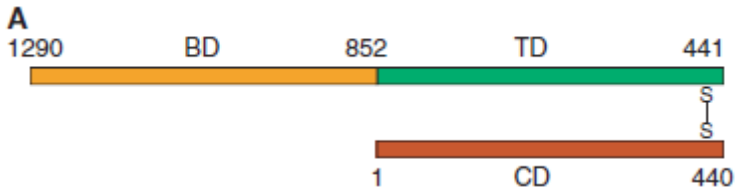


Figure (1): Botulinum toxin structure

III. CRYSTAL STRUCTURES OF BONT⁄A AND B

BoNTA and B's crystal structures have been identified (10, 11). BoNTA and B exhibit structural similarities as a result of their high sequence homology (39% identity and 56% similarity) (12). Because BoNT-B has a higher resolution (1.8 A) and folds that are comparable to A and A, we will talk about it. The three distinct structural domains that make up BoNTB are the catalytic, translocation, and binding domains (Fig. 2). Contrary to the catalytic domain (LC), which has an a-b fold, the translocation domain (NH) is predominately helical, with two long (100 A) helices generating a coiled-coil. The belt region, which is made up of residues 481 through 532, forms a wide loop around the catalytic domain. Despite being closely connected to the catalytic domain in three dimensions, this region is a peculiar BoNT-specific property that connects to the translocation domain in the primary sequence. The binding domain is composed of two subdomains, HCC and HCN. HCN is composed of a 14-stranded b-barrel with a jellyroll form that is typically observed in lectin-binding proteins [13]. Loops and b strands with a b-trefoil fold make form the majority of the HCC domain [14]. The three domains are arranged in a linear fashion, with the translocation domain in the middle. Because it is tipped away from the translocation domain, the binding domain only occasionally contacts with it. It has a close relationship with the catalytic domain, which is on the other side of the translocation domain. Rarely do BoNTA or B's binding domain and catalytic domain interact with one another. The huge cavity containing the catalytic zinc is partially encased by the belt region. BoNTB has a larger cavity than BoNTA.



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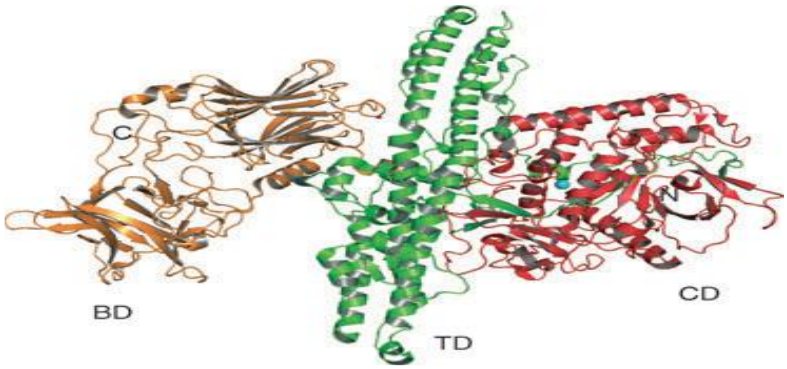

Fig. 2. Type B of the clostridial neurotoxin. BoNT B is shown in (A) as a linear representation, with the individual domains colored as in (B). Additionally indicated is the interchain disulfide bond. Ribbon illustration of BoNT equals B. In terms of fold and domain organization, BoNT A and BoNT B are comparable. Receptor binding (BD), translocation (TD), and catalytic (CD) domains are each represented by an orange, green, or red color. Zinc appears as a cyan ball.CD is encircled by the belt zone (also shown in green). There are marks on the N- and C-terminals.

IV. RECEPTOR-BINDING DOMAIN

The receptor-binding domain is divided into two subdomains (Fig. 3). The N-terminal (HCN) and C-terminal (HCC) domains, which contain a jelly-roll motif and b-trefoil fold, respectively, are connected by a brief helix (13, 14). Despite the low sequence identity of the C-terminal region, all botulinum and tetanus toxins share comparable binding domains. The variety in the sequence is reflected in the size of the connecting loops. Currently, it is unclear what functions the N-terminal domain (HCN) performs. There is no evidence that the fold can bind any ganglioside sugar group, despite the fact that it can bind carbs. However, subsequent studies have shown that there is only a little interaction between phosphatidylinositol phosphates and BoNT-A HCN (15).

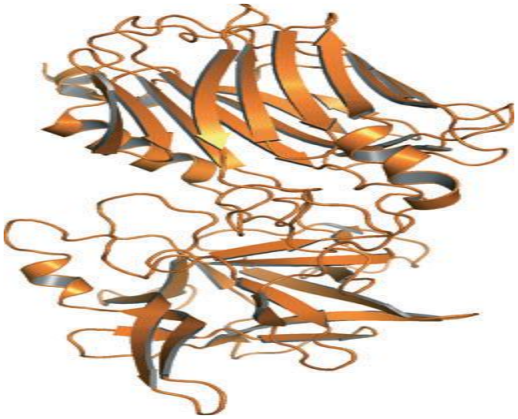

Fig. 3. The portion of BoNT B that binds receptors. The HCC domain contains a b-trefoil fold and the HCN domain comprises a lectin binding motif, both of which provide binding spaces for the receptors.

V. TRANSLOCATION DOMAIN

Toxins are internally processed once they bind to membranes in a process that depends on temperature and energy. The hydrophobic vesicle barrier must be breached in order for the neurotoxins to move from the vesicles into the cytosol. This is accomplished by bringing the pH down to acidic levels, which enables the translocation domain to alter conformation and then penetrate the membrane to build a channel that will allow the catalytic domain to escape the endosome. The transmembrane region of BoNTs has been predicted. This region, 653-673 in BoNTB and 650-672 in BoNTA, is near the translocation domain's tip and next to one of the long helices (Fig. 4), but it does not take on a helical shape in the crystal structures. When the pH drops, it is hypothesized that the area will adopt a helical structure. Nevertheless, structural analysis of BoNT-B at different pH levels (down to 4) did not reveal any alteration in this area (16). Nonetheless, crystal packing can be to blame for this. The N-terminal region of the translocation domain (449-545 in BoNT A), particularly the loop that wraps around the catalytic domain and is referred to as the belt area because it does so, is the most fascinating section of the structure. The translocation domain's structure has not yet been determined,

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despite the fact that the catalytic and binding domains' individual crystal structures have been determined. This is partially because the translocation domain is hydrophobic and forms aggregates, which makes crystallography difficult. The belt region may also lose its conformation and fold back up or down if the catalytic domain is not present to sustain it.

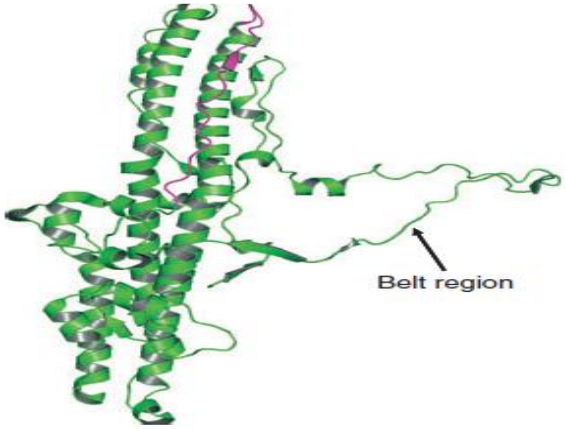

Figure (4): The BoNT translocation domain B. When the catalytic domain separates, the belt region that surrounds it loses its hydrophobic interaction. Magenta represents the anticipated transmembrane region.

VI. CATALYTIC DOMAIN

Both structurally and biochemically, this area of BoNTs has received the most attention. BoNT has a zinc endopeptidase in its catalytic domain that resembles thermolysin (10,11,17). All BoNT serotypes have crystal structures of their catalytic domains (LCs), and they all share a similar fold (18, 19). A blend of a helices and b sheets make up the compact globule that is the fold. The main sequence of LC contains the distinctive zinc-binding motif, HExxH+H. A sizable open cavity with a strong negative electrostatic potential contains the active site zinc, which is bonded there (Fig. 5). By two histidines and one glutamate, the zinc ion is coordinated. A water molecule that performs the nucleophilic function of providing the fourth coordination. The nucleophilic water molecule and the first Glu in the zinc-binding motif form a strong hydrogen bond that forms the basis of the catalytic process. Surprisingly, the architecture and considerable sequence conservation of the active sites of all BoNTs are strikingly similar. In 10 A, the conserved residues interact with zinc in the same exact ways.

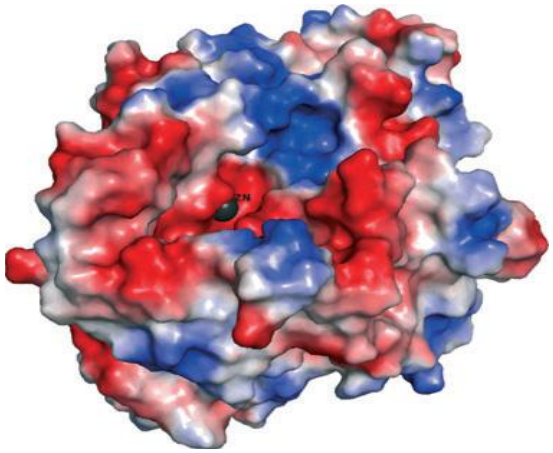

Figure (5): Catalytic domain electrostatic potential surface of BoNT B. A deep hollow that is extremely electronegative contains zinc. As a greyish spherical, zinc is depicted.

VII. ROLE OF ZINC IN BONTS

Proteins may use zinc for structural, functional, or a combination of these purposes. A structural zinc is often coordinated by four amino acids, whereas a catalytic zinc is typically coordinated by three amino acids and one water [20]. In BoNT, a water molecule, three amino acids, and the zinc are coordinated. Nonetheless, tertiary structural investigations led some to believe that its function might be structural [21]. However, structural research has unequivocally demonstrated

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that removing zinc does not alter the conformation and that its function is functional because the catalytic activity is lost when zinc is removed [22].

VIII. BOTULINUM TOXIN MOLECULAR MODE OF ACTION

Botulinum neurotoxin disrupts excitatory synaptic transmission by binding to the neuronal membrane when it enters the cytoplasm of the axon terminal at the neuromuscular junction (Halpern & Neale, 1995). This results in flaccid paralysis. Three stages of toxin-mediated paralysis take place:

1) Internalization
2) Disulfide translocation and decrease
3) Restricting the release of neurotransmitters (24).

The action potential depolarizes the axon terminal of the motoneuron, which causes acetylcholine to be released from the cytosol into the synaptic cleft. This release of acetylcholine is mediated by the soluble N-ethylmaleimide-sensitive factor attachment protein receptor (SNARE) complex, a transport protein chain. When BT is injected into a specific region, the heavy chain of the botulinum neurotoxin binds to certain glycoprotein structures found only on cholinergic nerve terminals. The great selectivity of BT for cholinergic synapses is a result of this particular docking. After internalization, the light chain of the botulinum neurotoxin specifically binds to the SNARE protein complex (25). Different target proteins are used by various BT serotypes. BT-A cleaves the 25 kDa synaptosomal-associated protein (SNAP-25) (25). BT-B degrades synaptobrevin II, sometimes referred to as vesicle-associated membrane protein (VAMP). The SNARE protein complex is proteolytically cleaved by the light chain, preventing vesicle fusion and cholinergic vesicle docking on the inner surface of the cellular membrane. When a muscle is the intended target tissue, paresis through chemical denervation takes place. The glandular production stops when an exocrine gland is the target tissue (see Fig. 6). The BT-induced inhibition of cholinergic exocytosis is lifted after the SNARE protein complex turnover is restored. Despite the fact that endplate lengthening and axonal sprouting are seen, it is believed that these modifications are just transient and are not the reason the BT effect vanishes (26).

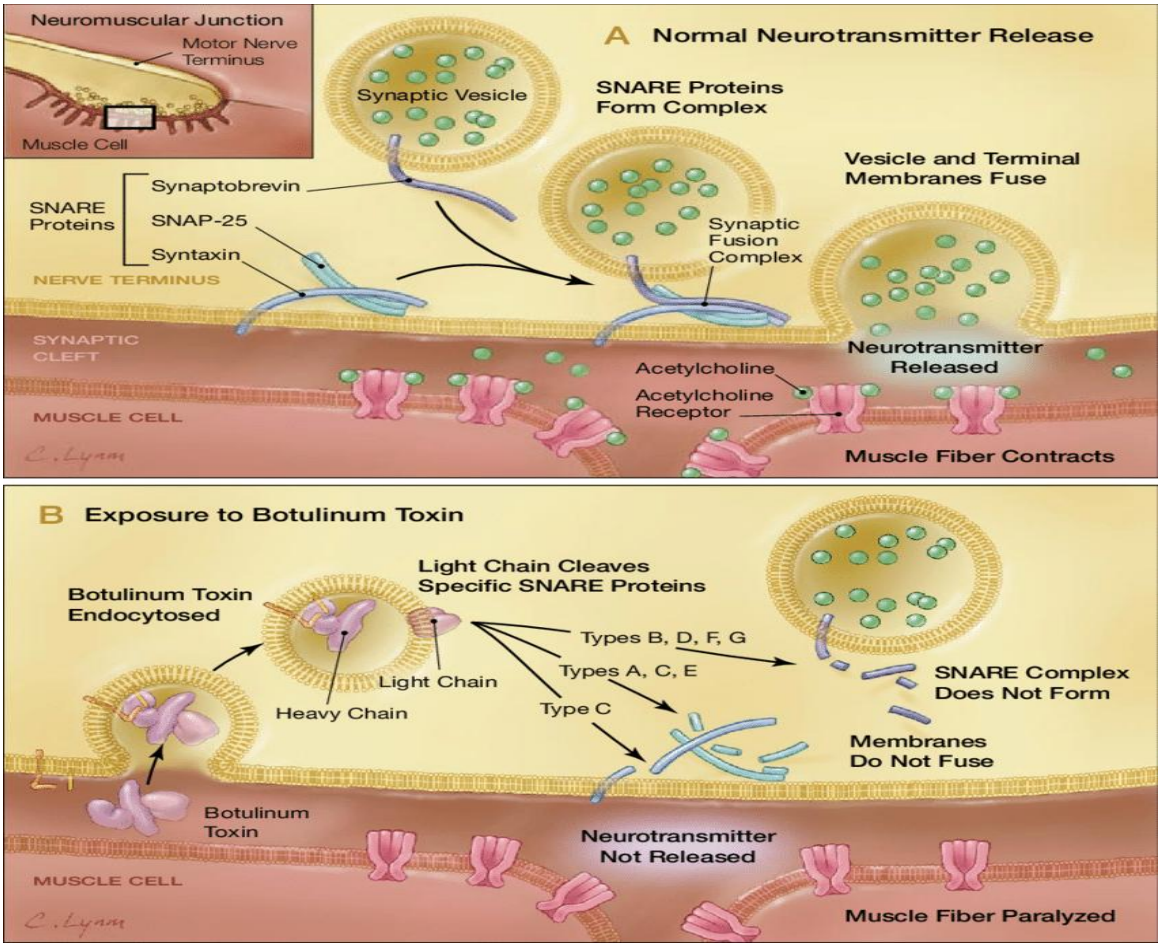

Figure (6): A. Normal neurotransmitter release, B. Exposure to botulinum toxin.

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IX. STRUCTURE-BASED DRUG DISCOVERY FOR BOTULISM

Each of the three crucial steps—binding, translocation, and catalytic activity—in the toxicity pathway of botulinum neurotoxin can be targeted to block the substance's action. This can be achieved by creating small molecules to clog the catalytic site, binding site, or translocation domain channel (27).

Chimeric BoNTs for therapeutics

The development of vaccines is also focused on the binding domain of BoNT. LC-HN is being developed as a potential vaccination by Foster and colleagues. For medicinal purposes, chimeric molecules of specific BoNT domains are being created (28). Barbieri altered the catalytic domain through mutation to use it therapeutically to target cells other than neural cells (29). The chimeric molecule will allow BoNTs to be used as a treatment for additional diseases.

Clinical applications

Nowadays, many medical conditions are treated with botulinum toxins, particularly hemifacial spasm, focal dystonias, and different spastic movement disorders [30]. Along with these, exciting clinical data have been published for a variety of other purposes, including headaches, hypersalivation, hyperhidrosis, and a few chronic conditions that only partially respond to conventional medical treatment. It can occasionally be used as an alternative to surgery. It looks to be a promising alternative to sphincterotomy in patients with persistent anal fissures since it is successful in treating achalasia (31). Botulinum toxin is an excellent treatment for several autonomic issues that cause excessive gland secretion, such as ptyalism or gustatory sweating, which typically happen following parotid gland surgery. Surprisingly, the response appears to continue a lot longer than it would under circumstances brought on by extremely active smooth or striated muscles [32].

Cosmetic uses of Botulinum toxin

Since BTX-A was allowed to treat glabellar lines, botulinum toxin (BTX) is currently used more frequently for cosmetic purposes. Traditional Botox® treatments primarily targeted the appropriate facial expression muscles in the upper face. Examples of its current applications include the correction of lines, creases, and wrinkles on the face, chin, neck, and chest, depressor anguli oris, nasolabial folds, mentalis, medial and lateral brow lifts, and the reduction of facial shadows and maintenance of a smooth outline of the jaw and cheeks from all angles. Axillary or palmar hyperhidrosis is a localized condition that is resistant to topical or systemic treatment (33). The clinical safety and efficacy of BTX-A led to its approval for cosmetic usage among the several BTX variants. BTX-A has been shown in the literature to be effective in getting rid of gummy smiles due to its simple and secure application, low doses, quick initial impact, low risk, and reversible effects (34).

X. FORMULATION OF BOTOX

Botulinum toxin preparations of serotypes B, C, and F are being developed, but only serotype A is now available for purchase on the market for clinical usage. Botulinum toxin A is sold under the names Dysport® and Botox®. The doses and units of effectiveness of the two formulations, unfortunately, have generated a lot of uncertainty (35). Botox® appears to be more potent even though doses are stated in mouse units (i.e., the quantity of toxin required to kill 50% of a group of 18–20 g female Swiss–Webster mice). One Botox® unit is three times more potent than one Dysport® unit, according to a recent study. Botox® is the brand name for a lyophilized, sterile form of botulinum toxin type A. It is made with the help of the Hall strain of C. botulinum, which is then purified via a series of acid precipitations to generate a crystalline complex comprising the toxin and other proteins (35). About 20 units of specific activity are contained in each nanogram of the neurotoxic protein complex in Botox®. Each vial of Botox® (U) contains the Clostridium botulinum types a neurotoxin complex, which is sterile, vacuum-dried, and free of preservatives (34) along with 0.5 milligrams of human albumin and 0.9 milligrams of sodium chloride. It was replaced with a fresh batch of neurotoxic complex with the batch code BCB 2024. In terms of clinical efficacy and safety, a unit dose of the new Botox® results in a similar reaction to a unit dose of the prior Botox®. The botulinum toxin known as Dysport® is produced using purification techniques based on columns and is available in 500-unit vials that can be stored at room temperature. Along with Europe, a few other countries also sell it. Variations in these toxins are likely related to differences in the bacterial strain, preparation, diffusion, and potency testing (33).

XI. ADVERSE EFFECTS OF BOTOX

- After injection, a few uncommon systemic effects are possible, including a widespread rash and flu-like symptoms in a tiny percentage of persons.
- The toxin is thought to have spread from the immediate injection site, which is the only serious clinical issue. Muscle deterioration in nearby locations may result from such distribution.
- Following a botulinum toxin injection in the neck muscles for cervical dystonia, for instance, swallowing difficulties happen in roughly 5% of cases.

- Antibody resistance may emerge over time. This is probably present in 5% of people and is typically brought on by the production of antibodies against both toxic and non-toxic elements of the botulinum complex (36).

XII. CONCLUSIONS

- Understanding the mechanisms of action in each area has been aided by the structural, biochemical, and biophysical data on botulinum neurotoxins.
- Botulinum toxin is made up of a single polypeptide chain that is activated by a proteolytic cleavage process to generate a dichain molecule, which is made up of a heavy chain and a light chain and is connected by a disulfide bond.
- Botulinum toxin A can be utilized as a medication at small therapeutic doses (BOTOX).

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