BOTOX and Its Effect on Wrinkles

Rochelle Eckstein

ABSTRACT

Clostridia Botulinum (C. Botulinum) is a bacterium that produces a unique exotoxin, botulinum toxin (BTX). BTX induces flaccid muscle paralysis by inhibiting the release of acetylcholine at the cholinergic nerve endings. Recently, Botox, developed from BTX, has become a popular solution to reduce the appearance of hyper-functional facial rhytids. Facial wrinkles are caused by repetitive muscle contraction, and a treatment that directly addresses this will be effective. Injections of Botox temporarily relax or paralyze these muscles. This paper will briefly discuss the bacterial basis of Botox and its development. It will explain how the mechanism of action of BTX can be used therapeutically and cosmetically as well. The formula and development of Botox and similar drugs will be explained as well as the relevant facial anatomy. A detailed analysis of case studies and comparisons of different formulations are required before determining if Botox is an effective treatment. The side effects and risk factors involved will be assessed, concluding if Botox is a safe as well as effective treatment.

INTRODUCTION

CLOSTRIDIUM BOTULINUM

Clostridium botulinum, a gram-positive, spore forming, anaerobic bacteria, is found in the soil but easily isolated (Arnon et al, 2001). C. botulinum is best known for its neurotoxin produced by growing cells, botulinum toxin, BTX. BTX is the most poisonous of all known poisons and causes the disease botulinum which is characterized by flaccid muscle paralysis (Sharma and Shukla, 2005).

C. Botulinum was first discovered in the 1820s by Justinus Kertner, a Bavarian. Kertner collected data and published two monographs on 230 cases of botulism, giving a complete and accurate description of botulism including symptoms, duration and physical findings. Symptoms include: tear fluid disappears, the pupils dilate, eye muscles are paralyzed, mucous and saliva secretion is suppressed, skin is dry and the skeletal and gut muscles are paralyzed. Cognition is retained throughout all this (Scott, 2004).

Seventy-five years later, Van Ermengem, a professor of bacteriology correctly described the bacterial basis of botulism after an outbreak among 34 individuals who had attended a funeral and eaten raw partially salted ham. Of these, 34, 23 became paralyzed and 3 died. Van Ermengem found the ham was toxic to lab animals, producing a paralytic disease. He isolated the anaerobic bacteria from the ham and the spleen of one man who died. He grew it, named it, characterized its culture requirements and described its toxin (Scott, 2004). Botulinum is from the Latin *botulis*, meaning sausage, from the first incidences of botulism caused by sausages (Boni, Burg, and Kreydan, 2000).

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BOTULISM

Botulism is a rare disease with four naturally occurring syndromes: foodborne, airborne, infant and wound. The bacteria cannot penetrate unbroken skin, so it must be ingested, inhaled, or colonized in broken skin (Arnon et al, 2001). The most common form is foodborne botulism from improperly stored food containing the bacteria. The spores are resistant to heat and survive when foods are not processed correctly. Once ingested, the bacteria colonize in the GI tract and secrete BTX. Inhalation botulism is caught via airborne BTX that is inhaled and absorbed through the lungs. Wound botulism generally occurs in deep wounds associated with IV drug use. Infant botulism is caused by the ingestion on the bacteria spores that colonize in the GI tract and secrete toxin that is absorbed from the lumen. Infants have an immature gut physiology and an inadequate development of gut flora so they are particularly susceptible to infection (Sharma and Shukla, 2005).

Because of its toxicity, the use of botulism as a biological weapon presents a dangerous threat. A single gram of crystalized toxin, evenly dispersed and inhaled, could kill more than one million people (Arnon et al, 2001). However, because of its potency and unique method of action, BTX also has many therapeutic uses as will be discussed later (Sharma and Shukla, 2005).

BOTULINUM TOXIN (BTX) AND MECHANISM OF ACTION

There are seven distinct serotypes of C. botulism, A-G, that are characterized by immunological differences in their toxin (Sharma and Shukla, 2005). All toxins have a similar structure of a large single polypeptide and block the release of acetylcholine at the neuromuscular junction, causing acute flaccid muscle paralysis (Sobel, 2005).

BTX is synthesized as a single-chain polypeptide with a molecular weight of approximately 150kDa. The bacteria possess a protease that nicks the molecule to create a dichain structure, consisting of a heavy chain (100kDa) linked to a light chain (50kDa) by a disulfide bond. The dichain molecule is the active form of the toxin that blocks cholinergic transmission (Simpson, 2004). BTX is extremely potent because it is enzymatic (Arnon et al, 2001). The potency is remarkable because the toxin goes through a lengthy and complex chain of events until it reaches the action site, the peripheral cholinergic nerve endings (Simpson, 2004).

The toxin is released from the bacteria as part of a non-covalent complex together with auxiliary proteins. These auxiliary proteins have no meaningful role at the site of toxin action. Their function is to make the toxin resistant to the harsh conditions in the gut like low pH and proteolytic enzymes. Once the toxin enters the lumen of the gut or airway, it must cross membrane barriers to reach the general circulation. The toxin does not cause cell death; therefore, it does not kill the cells in its path. Rather, it uses transmembrane and transcellular processes to reach its target. A likely mechanism of action has been proposed: the toxin binds to the apical surface of the epithelial cells, undergoes receptor-mediated endocytosis and transcytosis and is delivered to the basolateral surface of the cells. The toxin can therefore reach blood and lymph (Simpson, 2004).

Once the toxin enters the general circulation, it needs to exit the vasculature to reach the extracellular space near the cholinergic nerve cells. There is no single study that describes this exit from the vasculature, but it could be an active transcellular process of para-cellular movement. Since BTX does not penetrate the blood-barrier, it has little ability to impair central cholinergic transmission of intact organisms (Simpson, 2004).

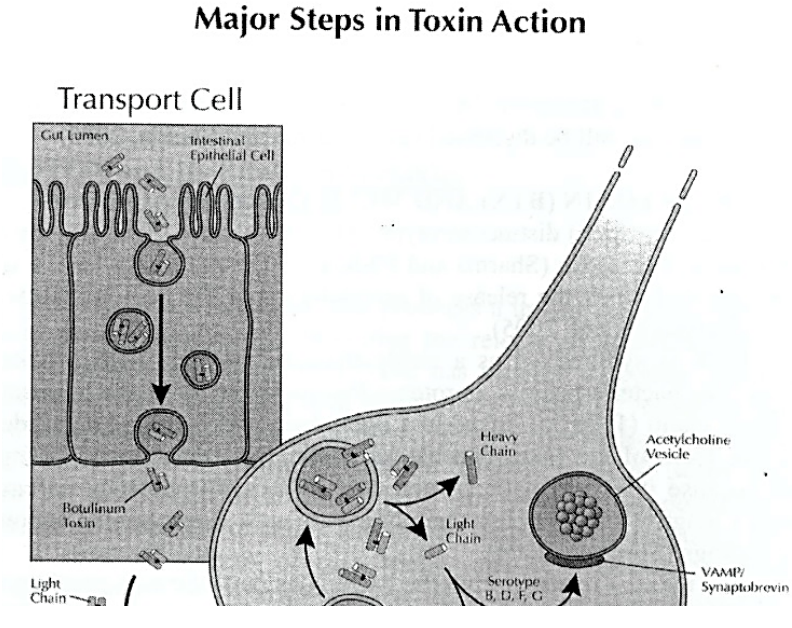
When the toxin reaches the peripheral cholinergic nerve endings, there is a sequence of membrane penetrating events. [Figure 1]

1. The toxin binds to the surface of the plasma membrane
2. This is followed by receptor mediated endocytosis and pH induced translocation across the endosome membrane
3. When the toxin reaches the cytosol, it acts as a zinc-dependent endoprotease to cleave polypeptides that are essential for exocytosis
4. This blockade of the transmitter release accounts for the flaccid paralysis that is characteristic of botulism. Without release of acetylcholine, the muscle cannot contract.

BTX acts preferentially on cholinergic nerve endings, but can block exocytosis from other nerve endings, such as norepinephrine and serotonin, when the concentration of the toxin is increased. While BTX can block exocytosis at all peripheral cholinergic sites, the neuromuscular junction has received the greatest amount of research and clinical attention (Simpson, 2004). No one has yet determined how the toxin is eliminated from the nerve endings. However, the nerve terminals regenerate slowly (Sobel, 2005), and new motor axon twigs sprout to reinervate the paralyzed muscle fibers (Arnon et al, 2001). This process takes several weeks or months, and in the United Staes, 96% of those affected eventually fully recover (Sobel, 2005).

Figure 1

Most of the steps in BTX action occur at two sites. Epithelial cells are transport cells; they bind the toxin and carry it from the lumen of the gut to interstitial fluid and general circulation. Peripheral cholinergic nerve endings like those at the neuromuscular junction are target cells for toxin action. BTX binds to these cells and once internalized into the cytosol attacks polypeptides that are essential for transmitter release (Simpson, 2004).



When BTX is exposed to striated skeletal muscle, it prevents the release of the acetylcholine (ACh) at the neuromuscular junction (Matarasso, 1998). ACh is the major neurotransmitter involved in parasympathetic nerve transmission at the post ganglionic synapse. In skeletal muscles, calcium activates the release of ACh from the presynaptic membrane into the synapse. ACh then binds to the nicotine receptors on the post synaptic membrane. When the nicotine receptors are activated by ACh, they allow transport of sodium and potassium ions across the post synaptic cell membrane. The entry of sodium into the cell causes depolarization of the cell membrane and generation of an endplate potential. The endplate potential initiates propagation of an action potential along the cell membrane of the skeletal muscle, ultimately causing skeletal muscle contraction (Boni, Burg, and Kreydan, 2000). [Figure 2]

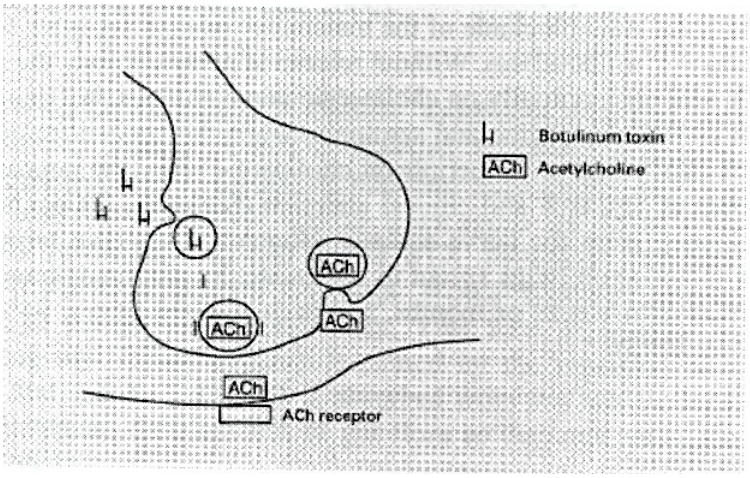


Figure 2

BTX is composed of a heavy chain and a light chain linked by disulfide bonds. Once internalized into the synapse, the light chain dissociates and cleaves the target protein, thus blocking ACh release (Boni et al, 2000).

The receptor at the neuromuscular junction has not been unequivocally identified. The major distinction between serotype A and serotype B are that they attack different substrate. Each serotype cleaves only one peptide bond in its substrate even though the sequence may be repeated in the substrate. Serotypes C and E are similar to serotype A and types D and F are similar to type B. (Simpson, 2004). BTX-A cleaves to a 25-kd synaptosome associated protein (SNAP-25) and BTX-B cleaves to a vesicle-associated membrane protein (VAMP or synaptobrevin) (Carruthers and Carruthers, 2005). Although they both block ACh release, BTX-A blocks the frequency of the spontaneous release by greater magnitude than BTX-B (Simpson,

2004). This difference may be responsible for differences in clinical effects of the two toxins, as discussed later (Carruthers and Carruthers, 2005).

HISTORY OF THE THERAPUETIC AND COSMETIC USES OF BTX

BTX is also used therapeutically and cosmetically. When injected, it produces a temporary chemo-denervation of muscle, resulting in a localized reduction of muscle activity (Carruthers and Carruthers, 2005). When used to treat conditions where there is excessive and uncontrolled cholinergic nerve activity, BTX causes temporary relaxation of muscle where normal function is attained (Simpson, 2004). These potential uses for BTX were not realized until the 1970s, long after it was considered a biological terror threat. In the 1940s, Drachman of Johns Hopkins used small doses of toxin to paralyze the hind limbs of chicks. At that time, alternative therapies were being sought to treat strabismus, such as injections of different anesthetics, alcohols and snake neurotoxins. Techniques to accurately inject extraocular muscle with local anesthetics to determine their function in eye movement had already been developed (Scott, 2004).

The first experimentation on humans began in the 1960s and gained acceptance by the 1990s. Scott investigated therapeutic uses of the toxin first in monkeys and then on humans with strabismus and blepharospasm (Cote et al, 2005). A few picograms of the toxin injected into the target muscle induced a paralysis confined to that muscle with long duration and no side effects. By 1982, the eye muscles were injected for retraction, hemi-facial spasm, blepharospasms and the limbs and neck for dystonia. At first there was a strong aversion to using BTX for these indications, but as there was no adequate alternatives to treat many motility disorders, treatment became more popular (Scott, 2004). In 1989, the FDA approved using BTX-A for treating strabismus and blepharospasm and in 2000, expanded approval to include cervical dystonia (Cote et al, 2005). Other conditions were also treated with BTX in the mid-1990s, such as hyperhidrosis and Frey's syndrome (Scott, 2004).

Cosmetic use of BTX was pioneered by Alistair and Jean Carruthers. Many patients treated for blepharospasm would joke at their 3 and 4 month follow up visits that they were there to "get the wrinkles back out" (Scott, 2004). Carruthers first tested BTX to treat glabellar lines in 1992 and later tested it on selected facial muscles to lift the brow and flatten folds (Carruthers, 2005). In 2002, the FDA approved BTX-A for cosmetic use for temporary improvement to the appearance of glabellar lines. These lines are the only FDA approved rhtyids for BTX treatment, but many physicians use it for off-label purposes as well. The most common injection sites are the glabellar frown lines, crow's feet, wrinkles, the forehead and neck bands (Cote et al, 2005).

BOTOX

BTX-A is available in two formulas: from Allergan in the United States and Canada as Botox, and as Dysport in Britain, France, and Germany. BTX-A is sold in a lyophilized form and needs to be reconstituted with saline before use. BTX-B is available as Myobloc only in the Unites States and is sold in an aqueous solution. The dosages of all three vary greatly and one needs to make sure that the correct dosage for the specific product is used as well as adhering to the manufacturing guidelines (Mantell, 2004). All treatments are temporary and serial doses are needed to maintain the desired results (Mendez-Eastmen, 2000).

Botox is available through Allergen and the toxin is supplied in a 100-unit multi-dosed crystallized complex. The crystalline form must be stored in the freezer at temperatures of -5°C or lower and should be reconstituted immediately before injection (Mendez-Eastmen, 2000). Both Dysport and Botox are reconstituted either with preservative free solutions or with normal saline. When reconstituted with preservative free solutions, the formula should be used within 4 hours, but normal saline retains its potency when refrigerated for 4 weeks. The preservative free saline is refrigerated at 2°-8°C for a maximum of 4 hours to ensure it remains sterile. Many physicians use the reconstituted form 4 hours after reconstitution, but the toxin could denature and jeopardize its sterility (Mendez-Eastmen, 2000). Many patients reported less pain with the injection after using a saline containing preservative. Higher dosages in smaller volume keeps the toxin and its effects more localized and allows for more precise placement and little spread (Mantell, 2004). The mode of measuring toxin strength is paralytic activity in the mouse. One unit is the amount of toxin that kills 50% of a standardized mouse model when injected intraperitoneally (Klein, 2004). Patients can be treated in a sitting or lying position, but sitting is recommended to avoid the toxin spreading to underlying muscles (Boni, Burg, and Kreydan, 2000). The administering physician needs to understand the relevant facial anatomy and only a physician should administer the injection. Local anesthesia is unnecessary. Patients can resume normal activity post injection and may take Tylenol if they experience pain. Nonsteroidal anti-inflammatory agents should be avoided for 7 days before injection as it could exacerbate the symptoms of side effects (Klein, 2004). The FDA approved treatment only for glabellar lines, but Botox is often used for off-label uses as well. The decision to inject Botox in an off-label site should be the physician's and he should follow the recommended dosage and be careful not to give too large of a dose (Mendez-Eastmen, 2000).

Dysport, also from BTX-A, is produced via fermentation and then recovered and dissolved in aqueous solution. It is provided as an air dried powder and is reconstituted and diluted with normal saline before use, similar to Botox. It is available in the UK and used therapeutically for blepharospams, torticollis, pediatric cerebral palsy spacity as well as for cosmetic purposes to treat facial lines. It is not directly comparable to Botox, but there is a ratio of about 1:3 or 1:4 units of Botox to Dysport (Markey, 2004). Myobloc, BTX-B, has the FDA approval to treat cervical dystonia. It is sold in a highly purified liquid formulation from fermented BTX-B. It contains very little protein or inactive toxin because this could increase the risk of antibody formation. It is slightly acidic with a pH of 5.6, so a painful, burning sensation is experienced upon injection. There is no dose determined for cosmetic uses, but a ratio of about 1:125 units of Botox to Myobloc is used (Flynn, 2004).

There are several differences between BTX-A and BTX-B. BTX-B has an increased radius of diffusion than Botox, creating a more uniform effect. The rate on onset of Myobloc is also slightly faster, by about a day. Duration of effect is does dependent, but studies comparing the two show that Myobloc has a shorter effect than Botox. Additionally, Myobloc is very stable even at room temperature because it is not reconstituted (Flynn, 2004).

Injection into the correct part of the muscle is important to achieve maximum benefits. The toxin works best when injected into the muscle belly and are usually not superficial because it easily penetrates muscle (Klein, 2004). Injection by EMG (electromyogrpahic) guidance helps achieve accurate placement by locating the most active part of the muscle responsible for a

particular facial line. It determines which muscles are contracting and contributing to the frown. A combines EMG injection needle is used which requires a larger needle. However, some physicians feel there is no benefit because the anatomy of the glabella is so reliable. Some use the EMG needle only for reinjection, but perhaps reinjection would not be necessary if one was used initially. Additionally, the pattern of muscle activity varies greatly from patient to patient (Mantell, 2004). When EMG injection is used, the area is cleaned with alcohol and allowed to evaporate completely. This is because the alcohol can denature the toxin. The patient then frowns, squints and raises the eyebrows to activate the targeted muscles. A needle is connected to the EMG unit is inserted. After placement, when the muscle is activated, a sound should be heard from the EMG machine. The needle is reinserted if no sound is heard (Mantell, 2004).

FACIAL RHYTIDS

Lines and wrinkles in the face are caused by muscle action and contraction. Treatments such as surgery and implants are only partially effective as they do not address the underlying cause of excessive lines, glabellar lines and crow's feet (Boni, Burg, and Kreydan, 2000). [Figure 3]

Frown lines are caused by the contraction of the frontalis muscle (Boni, Burg, and Kreydan, 2000). The frontalis is the elevator of the brow. This thin muscle covers a large portion of the forehead and is not attached to bone. The action of this muscle is to elevate the brow and wrinkle the forehead. The horizontal creases on the forehead are a direct result of the frontalis action (Vigliante, 2005). 50-60 units of Botox are used to erase these creases (Matarasso, 1998).

Glabellar frown lines are caused by the contraction of the musculus corrugator superciliaris at the medial end of the eyebrow (Boni, Burg, and Kreydan, 2000). This is a deep muscle located against the bone beneath the frontalis and orbicularis oculi muscles. The action draws the eyebrows medially downward, producing vertical glabellar wrinkles. These lines give the characteristic appearance of anger, frustration and negative emotions. Known as the "frowning muscle," it is the principle muscle used in the expression of suffering (Vigliante, 2005). 30-40 units are used to treat the lines (Matarasso, 1998). This muscle is usually stronger in men, so a slightly higher dose is necessary (Boni, Burg, and Kreydan, 2000).

The procerus muscle marks a horizontal groove at the base of the nose (Boni, Burg, and Kreydan, 2000). The primary action of this muscle is to draw the medial angle of the eyebrows which creates the transverse wrinkles at the bridge of the nose (Vigliante, 2005). Generally 30-40 units are needed to improve this furrow (Matarasso, 1998).

The squeezing action of the musculus orbicular oculi causes crow's feet, deep horizontal and oblique furrows at the temporal aspect of each eye (Boni, Burg, and Kreydan, 2000). This muscle a broad, flat muscle, and is the chief muscle surrounding the orbit. Is it the depressor of the brow and eyelid and forceful contraction induces concentric folds. In childhood, these lines are seen only in dynamic situations, such as laughter or squinting in the sunlight. However, in adulthood they are visible even in facial repose. These lines increase with years of sun exposure and dynamic expression (Vigliante, 2005). 20-30 units are needed to treat them (Matarasso, 1998).

The platysma, a broad sheet of muscle, is located in the neck, but is primary function is on the face

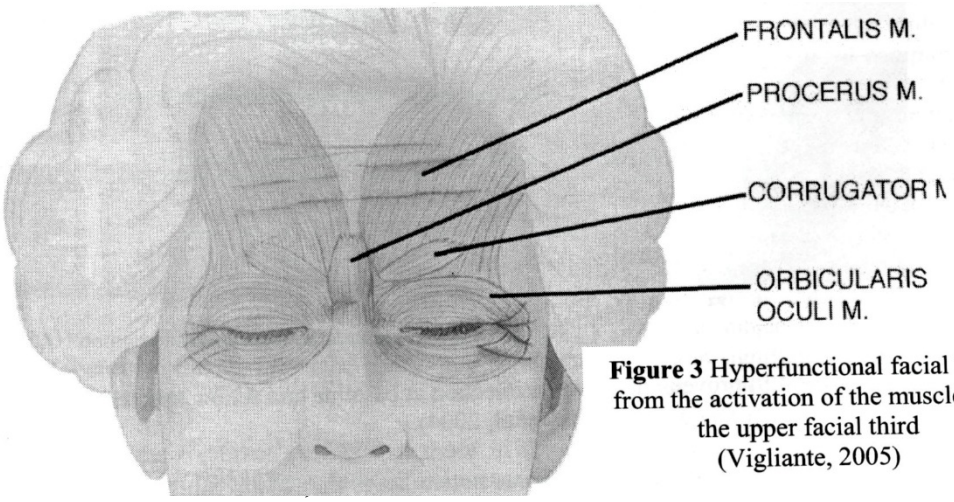


Figure 3 Hyperfunctional facial lines from the activation of the muscles of the upper facial third (Vigliante, 2005)

and mand the mandible, increasing the diameter of the neck and causing hyper-functional bands in the neck. The muscle also acts to draw the lower lip and corner of the mouth laterally and inferiorly, partially opening the mouth as in an expression of surprise or horror (Vigliante, 2005). These bands can be treated with multiple injections (Matarasso, 1998).

CASE STUDIES

THE EFFECTS OF BOTOX ON CROW'S FEET

As mentioned above, repetitive contraction of underlying muscles and their action on the skin produce wrinkles on non-fatty facial areas. Jean Luc Levy, Jean-Jacques Servant and Elisabeth Jouve of France conducted and experiment to determine the duration of a defined dose of BTX-A on crow's feet wrinkles. An objective evaluation of duration of the results of a single injection was provided. The treatment area was the crow feet wrinkles and adults with bilateral symmetric crow's feet were eligible, but those patients who had undergone prior cosmetic surgery, had health conditions, were breastfeeding or were potentially pregnant were excluded. The study included 25 female patients from age 31-65, with a mean age of 48. They were injected at the baseline with 12 units of BTX-A and evaluated at 3, 6 and 9 month intervals. Clinical photographs were taken at the baseline and during the follow up visits with the eyes closed at rest. Patients were positioned in front of the VisioFace special measurement bench with a 3D sensor. 3D microtopography of the skin was recorded with a DermaTop optical 3D in vivo scanner (Levy et al, 2004).

Patients were asked to evaluate their photographs at baseline (T0) and 9 months (T3) to score wrinkle reduction. Observers assessed post treatment and pretreatment photographs. They compared the photographs from the baseline with those from 3 (T1), 6 (T2) and 9 (T3) months after the injection. The result from these assessments by the patients, the 3D profilometry and the independent observers all were similar and showed improvement. Only one patients had a side effect of edema of the lower lid for a month following treatment. The conclusion from this study was that BTX-A is a safe and effective method for treating crow's feet with clear improvement shown at 6 months (Levy et al, 2004). [Figure 4]

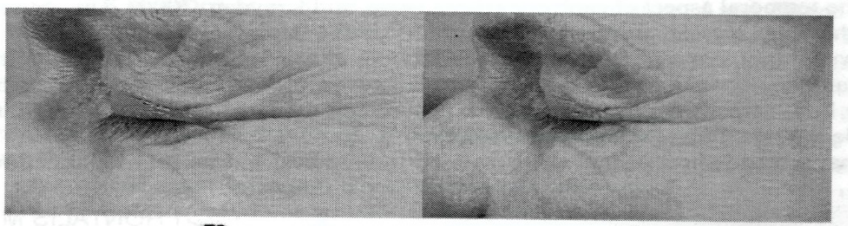


Figure 4: Improvement after one injection at baseline and follow up visits (Levy et al, 2004).

Although the study group for this trial was not so large, the results can be considered objective because of the impartial observers and 3D photography. While the results were positive with few reports of side effects, one should remember that the Crow's feet wrinkles are not a FDA approved injection site.

COMPARING THE EFFECT OF BOTOX AND OTC CREAMS ON GLABELLAR RHYTIDS

Another experiment was conducted by Kenneth Beer to compare the safety and efficacy of BTX-A with topical creams for treating moderate to severe glabellar rhytids. These lines are the only conditions approved by the FDA for Botox treatment. These over the counter (OTC) topical products advertise that they are more effective at treating wrinkles than Botox. This study, a single-center, randomized, investigator blinded parallel study consisted of five treatment groups: Botox, a placebo saline injection, StriVectin-SD, Wrinkle Relax and HydroDerm. Females with clinically diagnosed moderate to severe glabellar lines at maximum frown from the ages of 18-65 were eligible for participation. Criteria for exclusion were pregnancy, previous eyebrow surgery, use of retinoids, hydroxyl acids or products containing vitamins A, C and E, 77 participants were randomly placed into one of the treatment groups. Frowns were assessed both by the patients and a principle investigator, and photographs were taken of the glabellar region of each subject at maximum frown and rest. The first phase was a masked phase with follow up visits at 4, 8 and 12 week intervals. This was followed by an open-label phase where all subjects received open-label Botox injections with a 4 week post injection follow up (Beer, 2006).

All participants were instructed to maintain their standard facial care for the duration of the study. Those subjects in the placebo and Botox groups received injections by a staff member rather than the investigator to maintain the blind. Those assigned to use the topical creams were told to apply it in the morning and evening, massaging the cream gently over the bridge of the nose and eyebrows until absorbed completely. At baseline and each follow up visit, subjects assessed the change in appearance of the wrinkles, the investigator assessed them and photographs were taken. Patients also completed a facial line outcomes (FLO) questionnaire related to self-perception. The primary efficacy measure was the investigator's assessment of glabellar lines at maximum contraction at the follow up visits. Secondary was the subjects'

assessment. Botox treatment resulted in significantly reduced wrinkle severity over the topical creams and placebo. Subjects who received Botox treatment were also more satisfied with the treatment results than those in the other groups and 90% were satisfied with treatment during the open-label phase. Side effects were only reported by 3 participants in the StriVectin-SD group (Beer, 2006). [Figure 5]

This trial was the first that compared the efficacy of Botox to OTC topical creams. The results indicated do not support the advertising claims made by manufacturers of these creams. While they may be a more economic choice, their treatments of hyperfunctional lines were not very different than the placebo. However, the study was not long enough to see the long term effects and duration of treatment. Additionally, the investigator is associated with Allergen, the company that manufactures Botox.

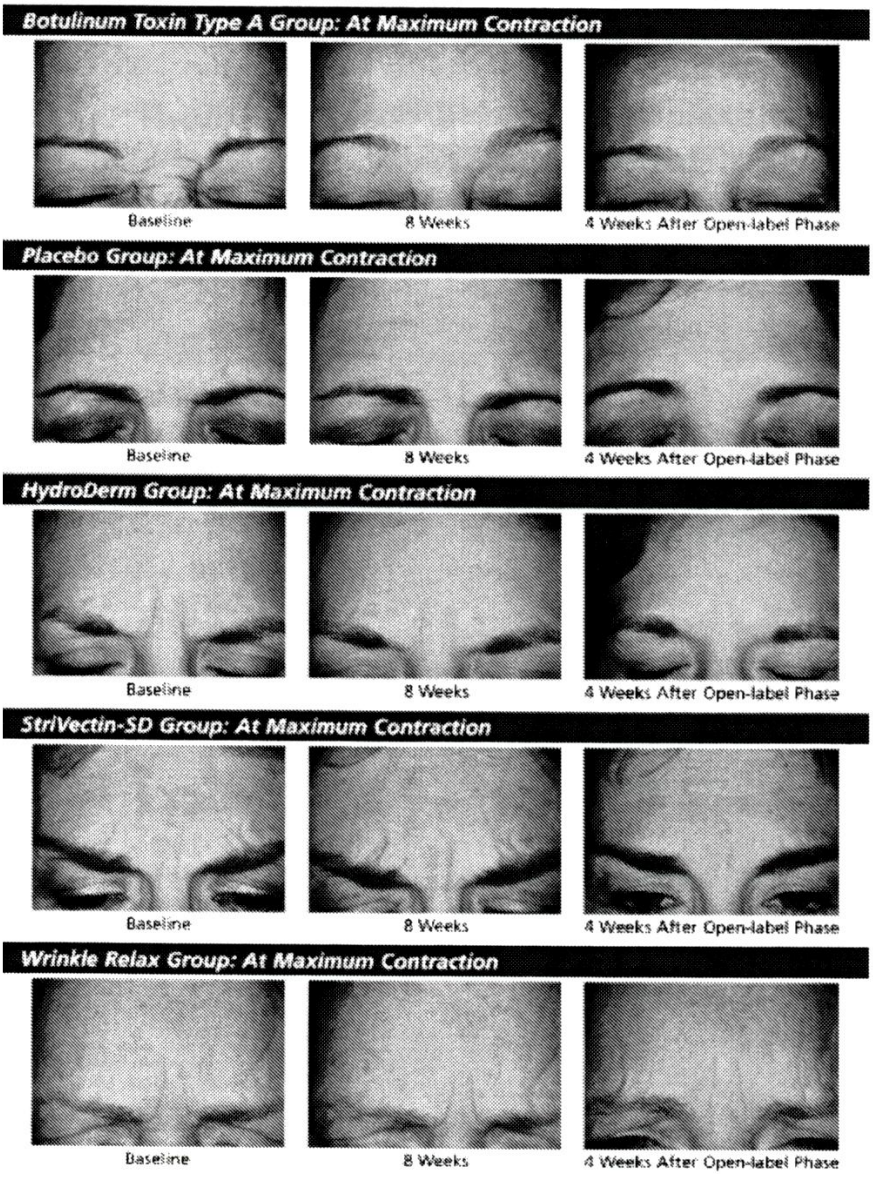


Figure 5: Photographs comparing treatment at baseline, 8 weeks and after the open-label phase. (Beer, 2006)

COMPARISON OF BOTOX AND DYSPORT ON GLABELLAR LINES

There has been little research directly comparing the two formulations of BTX-A which may behave differently, Botox (BTX-A1) and Dysport (BTX-A2). The results and dosages of both formulations are different and cannot be compared. Phillipa Lowe, Rickie Patniak and Nicholas Lowe designed a study to compare the results of both formulations. Patients between 18-55 years of age with moderate to severe glabellar lines at maximum contraction as determined by an investigator were eligible to enroll. Women of childbearing age were required to have a negative urine pregnancy test result. Criteria for exclusion included: facial cosmetic surgery planned during the study, visible scars, prior cosmetic surgery that could interfere with evaluation, history of facial nerve palsy, alcohol abuse, infection at the injection site. 62 patients were randomly assigned to the two groups (59 completed the study). The mean age was 41 and they were predominately Caucasian and female. The mean age of the Botox group was higher, but there were no other major demographic differences (Lowe et al, 2006).

The BTX-A1 group received a dose of 20 units (the FDA approved dose) and the BTX-A2 received a dose of 50 units (reported to be the optimal dose). The dose was divided between 5 injection points. Patients could continue their normal face care regimen, but should not apply creams for 4 hours before the follow up visits. Patients were photographed using the modified

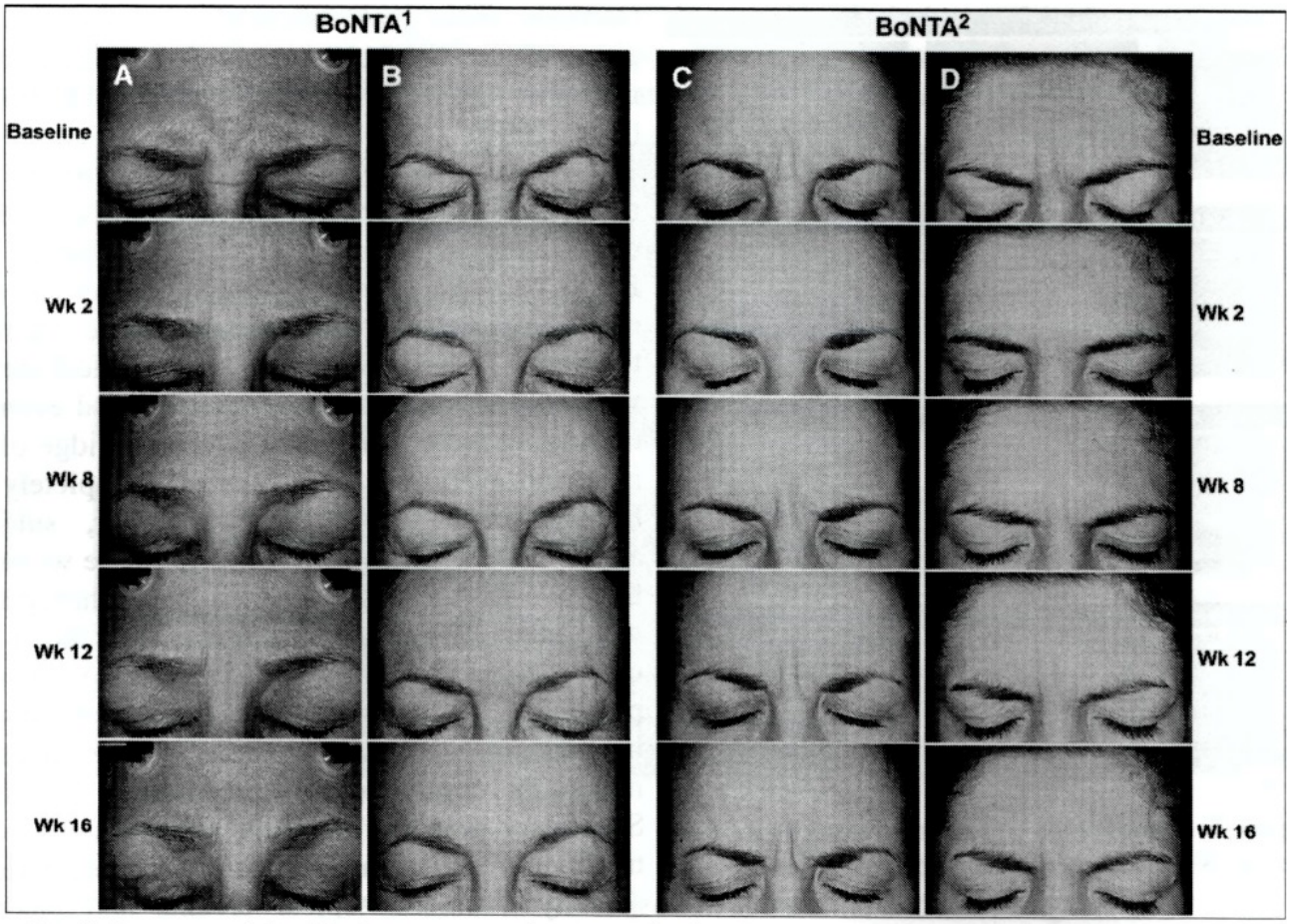


Figure 6: More prolonged duration of effect at week 16 with BTX-A1 (BoNTA1) than with BTX-A2 (BoNTA2) (Lowe et al, 2006).

Canfield system during maximum contraction at each visit. Severity of the lines was graded as none, mild, moderate or severe. The primary outcome measure was improvement of at least 1-grade at week 16. Other outcome measures included patients whose lines were graded as none or mild and incidence of relapse. Using masked assessment of the standard photographs demonstrated that BTX-A1 offers a more prolonged efficacy than BTX- A2. Both groups peaked at week 8 for at least a 1-grade improvement. However, the duration of improvement was generally more prolonged with Botox than with Dysport at week 16. Patient satisfaction was also higher with Botox than with Dysport. Both products were well tolerated and there was no difference in treatment related adverse effects between the two groups (Lowe et al, 2006). [Figure 6]

This study was supported by an unrestricted research grant from Allergen. Furthermore, the age difference between the two groups is considerably significant as younger patients have stronger muscles and may require a higher dosage. While Botox may have a longer duration than Dyspoty, both formulas appear to be effective. Costs cannot be compared because Dysport is not available in the US and Botox is not in the UK.

SIDE EFFECTS AND COMPLICATIONS WITH BTX-A TREATMENT

Botox is a safe, effective treatment without serious side effects. When it is used properly, the complications are a few with mild severity. No irreversible clinical effects have been reported from cosmetic uses. Different treatment areas are associated with different side effects. The most common complication is ptosis of the upper eyelid from injection into the glabellar area, but this could be resolved with eye drops. Eyelid ptosis can be avoided with more accurate placement and lower volume. The most significant complication from treating the frontalis is brow ptosis. The brow shape may also be changed because the muscle responsible for brow elevation is relaxed. To avoid this, the forehead and glabella should not be treated in the same session. Furthermore, the boundaries of the forehead should be defined and injections should not be above the middle brow. This treatment also works best on younger patients (20-45 years of age) and older ones should be injected more cautiously. Reported complications from treatment of Crow's feet include diplopia, drooping lateral lower eyelid and an asymmetric smile from injections placed too low, and this takes longer to resolve than lid ptosis. Strabismus is a very rare side effect, and the patient should be referred to an ophthalmologist for appropriate care. High doses injected into the platysma can produce weakness and dysphagia. Patients with severe over treatment of the neck can have trouble holding their necks erect because the muscles are so weakened. Therefore, injection in any area should be administered carefully in the correct dosage, in the appropriate location and by an experiences physician. (Klein, 2004).

As part of its mission to improve patient and consumer safety, in 2005, the FDA funded Cote et al (2005) to conduct a study reviewing the adverse effects (AE) reported to the FDA after BTX-A treatment. The FDA received reports of AEs through the MedWatch system. Although clinicians are encouraged to report incidences of AEs, it is voluntary, so the number reported is actually only a subset if the incidences that occur. It is very difficult to accurately classify the

AEs into serious and non-serious reports as MedWatch often lacks complete information. AEs that met the US Code of Federal Regulations 600.80 such as: "death, a life threatening adverse drug experience, in-patient hospitalization or prolongation of existing hospitalization, a persistent or significant disability/incapability, or a congenital anomaly/birth defect" were categorized as serious. All other were referred to as non-serious (Cote et al, 2005).

Cote et al (2005) attempted to review all reported cases, both from therapeutic and cosmetic use. Serious AEs were reviewed from December 1989 and May 2003. Due to the large number of non-serious reports, the review was limited from December 2001 to November 2002. 1437 AEs were reviewed, 406 from therapeutic cases and 1031 in cosmetic instances. The proportion of reports classified as serious was higher for therapeutic cases than those cosmetic uses. 217 serious AEs were reported for therapeutic cases that included 28 deaths and 17 seizures. For cosmetic cases, there were 36 serious reports and no deaths. The AEs reported were both known and unknown and for both on-label and off-label uses. The most common reactions were: dysphagia, muscle weakness, allergic reactions, flu-like syndromes, injection site trauma, arrhythmia and myocardial infarction. Non-serious reactions included ptosis and headaches for cosmetic uses. The AEs reported for therapeutic uses were different than those for cosmetic uses since doses are higher for therapeutic purposes and patients have serious underlying diseases that increase the risk of an adverse reaction. Many of the AEs in cosmetic cases were related to improper technique, storage or dilution, higher doses than recommended and injection in sites that are not FDA approved (Cote et al, 2005).

As in any treatment, there are always side effects involved. However, when used properly as advised in the approved labeling, the AEs experienced are generally non-serious and plausible as BTX blocks muscle contraction, so ptosis and dysphagia are likely. Incidences of AEs also decrease when the optimal dose of 20 units is used (Cote et al, 2005).

There is the possibility of developing resistance to BTX because of antibodies produced against the toxin. The likelihood of developing immunoresistance is associated with high doses and greater frequency of treatment (Klein, 2004). All BTX preparations (Botox, Dysport, Myobloc) have complex mixtures of various proteins and excipients such as human serum albumin, lactose, NaCI or buffers. Theoretically, all non-human proteins in the mixture can act as antigens to stimulate formation of antibodies. The antibodies can be formed against the toxin or non-toxin. Only the antibodies against the toxin interfere with the activity of the toxin and there may be partial or complete therapy failure (Dressler and Hallett, 2006). The newer batches of Botox have a decreased amount of protein so chances of antibody production are reduced. The only effect of these antibodies is that BTX-A is no longer effective, but no hypersensitive reaction develop (Klein, 2004). Patients who develop antibodies against BTX-A can be treated with BTX-B as an alternative since the antibodies are antigen specific (Scott, 2004).

CONCLUSION

Botox is a safe, effective and minimally invasive procedure used to reduce the appearance of hyper-functional facial lines. Although it successfully treats a large part of facial lines, the only FDA approved injection site is the glabellar frown lines. There are still ongoing trials to determine the safest and most optimal dosage for the longest duration of effect, but until then, serial injections are needed to maintain the results. Caution should be used with repetitive injections as this increases the risk of side effects and developing immunoresistance. Individuals

should understand the risks involved with Botox injection and consult a physician to determine if this procedure is right for them.

SUMMARY

As this paper has shown, Botox is an effective treatment to temporarily reduce the appearance of facial wrinkles. It is processed from diluted bacterial exotoxin, BTX. BTX acts on the peripheral cholinergic nerve endings to inhibit acetylcholine release and induce flaccid muscle paralysis. Botox uses this same method to temporarily relax targeted muscles in the face responsible for wrinkles. Numerous case studies done over the past several years have proven that Botox is an effective, albeit temporary, alternative to reconstructive surgery to treat and reduce the appearance of these lines. As with any treatment, there are always risk factors involved. By adhering to the recommended FDA guidelines of storage, dosage and procedure, many of these risks are avoided. Patients who opt to receive Botox injections should make sure to choose an experienced physician to ensure a safe and quality result.

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