

Craniofacial Applications of Botulinum toxin: An Update

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Abstract

Various human gene products produced by genetically engineered microorganisms such as insulin, growth hormone, interferons and regulatory peptides are being used therapeutically in humans. Nowadays neuroparalytic toxin of Clostridium botulinum has become an unlikely addition to the range of therapeutic microbial products. Botulinum toxin (BT) has been shown to be useful for the treatment of many clinical conditions. It is a combination of proteins one of which is a botulinum neurotoxin and rest is auxiliary proteins like hemagglutinins (HA) and a nontoxin, nonhemagglutinin (NTNH). When BT is injected into a target tissue, the heavy chain of the botulinum neurotoxin selectively binds to glycoprotein structures specifically found on cholinergic nerve terminals. In cytosol, the light chain binds with high specificity to the SNARE protein complex. The toxin is injected through a monopolar hollow-bore, teflon coated electromyographic needle connected to an EMG amplifier. Purpose of this article is to present an overview of Botulinum toxin(BT) and its clinical implications in craniofacial region.

Keywords: Human Gene Products; Botulinum Toxin; Therapeutic Uses of Botox.

Introduction

Various human gene products produced by genetically engineered microorganisms, such as insulin, growth hormone, interferons and regulatory peptides are being used therapeutically in humans. Nowadays neuroparalytic toxin of Clostridium botulinum has become an unlikely addition to the range of therapeutic microbial products. Botulinum toxin (BT) was seen as danger to mankind for centuries until its use to study spinal cord physiology came to light in 1970's [1]. In the early 1980's, perception for BT changed completely when its therapeutic potential suddenly became apparent. Over the past 20 years, BT has been shown to be useful for the treatment of many clinical conditions. Amongst many serotypes

of BT, BT-A is most commonly used. Here we are presenting an overview of BT and its clinical implications.

History

Sometime between 1817 and 1822, the German physician Justinus Kerner published two monographs on this toxin. He called it 'sausage poison' as it is found in improperly heated canned meat products [2]. After 50 years, Muller Latinised sausage into "botulus" after which the disease got its name botulism. The causative bacterium, Clostridium botulinum, was first cultured by van Ermengem in 1897 [3] and neuromuscular blockade, as the mechanism of action was elucidated in 1949 [4]. It was also used as a bioterrorism and biological weapon with CIA's (Central Intelligence Agency-USA) continuous efforts of eliminating Fidel Castro by impregnating his Cohiba cigars with BT-A [5]. First extra-somatic use of BT-A was reported by Park and Bushara for autonomic chemodeneration in the treatment of hyperhidrosis [6]. Alan Scott was entitled the father of medical BT-A, for first using it for the treatment of strabismus in humans [7]. He further used it for the treatment of blepharospasm in 1985 [8]. With his wife Carruthers, he carried out first scientific study on BT-A for blepharospasm [9].

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Structure

It is a product of *Clostridium botulinum*, *Clostridium baratii*, and *Clostridium butyricum* [10]. *Clostridium botulinum* synthesizes all seven known serotypes (A to G), whereas *C. baratii* and *C. butyricum* produce only one serotype each (F and E respectively)¹¹. Botulinum neurotoxin has a chemical formula $C_{6760}H_{10447}N_{1743}O_{2010}S_{32}$ with a heavy and a light chain with a single disulfide bond between them. It is produced as a relatively inactive 150 kD single chain polypeptide and is activated by proteolytic cleavage into 100 kD heavy chain and 50 kD light chain. It is a combination of proteins one of which is a botulinum neurotoxin and rest is auxiliary proteins like hemagglutinins (HA) and a nontoxin, nonhemagglutinin (NTNH). These proteins make the toxin resistant to the harsh conditions of low pH and proteolytic enzymes in the gut [11].

Mode of Action

When an action potential depolarizes the axon terminal, acetylcholine is released from the cytosol into the synaptic cleft via a transport protein, the soluble N-ethylmaleimide-sensitive factor attachment protein receptor (SNARE) complex. When BT is injected into a target tissue, the heavy chain of the botulinum neurotoxin selectively binds to glycoprotein structures specifically found on cholinergic nerve terminals. In the cytosol, the light chain binds with high specificity to the SNARE protein complex [11]. The target proteins vary amongst the BT serotypes. BT-A cleaves synaptosomal-associated proteins of 25 kDa (SNAP-25) [12] while BT-B cleaves vesicle-associated membrane protein (VAMP or synaptobrevin II). The light chain associated proteolytic cleavage of the SNARE protein complex restricts the attachment of the acetylcholine vesicle on the inner surface of the cellular membrane. The inhibition of acetylcholine exocytosis by BT is terminated by restoration of the SNARE protein complex turnover [13]. The action of botulinum toxin on the various target tissues include following:

Botulinum Toxin Action on the Striate Muscle

When BT is injected into a striate muscle, paresis occurs after two to five days and lasts from two to three months before it gradually starts to wear off. When BT is injected into a hyperactive muscle, the induced paresis produces a reduction of the diameter of the target muscle. With increasing BT dilutions, the tissue diffusion of BT can be increased, thus affecting the therapeutic effect and the side effects of a BT therapy [14].

Botulinum Toxin Action on the Spinal Stretch Reflex

BT produces different effects on the muscle spindle organs. Rosales et al [15] observed atrophy in both extrafusal and intrafusal muscle fibres in an experiments study. Filippi et al [16] observed reduction of the Ia and II afferent signals from the muscle spindle organs and the muscle tone by blocking gamma motoneuron terminals. The antidystonic effect of BT may therefore be caused not only by target muscle paresis but also by spinal reflex inhibition.

Botulinum Toxin Action on the Central Nervous System

When BT is injected into a target tissue, it is almost completely bound to the axon terminal [17]. However, when BT-A is applied to treat cervical dystonia, small fractions of the applied BT are distributed systemically and can be detected by increase of neuromuscular jitter in non-injected muscles [18]. Botulinum neurotoxin with its size of 150 kD cannot penetrate the blood brain barrier. BT could reach the CNS by retrograde axonal transport [19].

Botulinum Toxin Action on Pain

Botulinum toxin is used to treat painful muscle hyperactivity disorders by reducing muscle hyperactivity. However, formalin-induced pain in animals can be reduced by BT direct analgesic effect [20]. Substance P (SP), a neuropeptide involved in pain perception, vasodilation and neurogenic inflammation, can be blocked by BT along with acetylcholine [21] as well as in cultured dorsal root ganglia neurons [22]. BT has also been shown to suppress the release of glutamate, another neurotransmitter involved in nociception, in the periphery and in the dorsal horn [23]. Another possible mechanism of pain alteration is neuroplastic changes in CNS [24].

Preparation and Method of Application of BT-A

C. botulinum cultures are fermented under anaerobic conditions. After 72 hours, toxin is precipitated by acidification. After purification through subsequent redissolving and precipitation, purified toxin is diluted with lactose and human serum albumin containing solutions and freeze dried¹². BT-A should be diluted with preservative-free saline and the preparation should be used within 4 hours of reconstitution. Conditions for stability of the toxin in solution include pH 4.2–6.8 and temperature less than 20°C. The large molecule is very fragile and is inactivated easily in solution by shaking

[25]. 1 IU of botulinum toxin is defined as LD₅₀ in mice. The LD₅₀ in humans is over 3000 IU. The toxin is injected through a monopolar hollow-bore, teflon coated electromyographic needle connected to an EMG amplifier. The needle is injected through the skin into the muscle in the area of the facial line (after instructing the patient to accentuate that particular facial expression). Toxin is injected into the most electrically active part of the muscle, which is read through an EMG signal [1,26].

Clinical Applications

Facial Rejuvenation

One of the most common implications of BT is its usefulness for the treatment of facial wrinkles [13,27-29]. Three main areas of upper third of the face with hyperfunctional movement are glabella frown lines, horizontal forehead lines, crow's feet, middle third includes malar smile lines, malar projection, infraorbital hollow (arcus marginalis), nasojugal fold (tear trough, which is the medial arcus marginalis), nasolabial folds and nasal dorsum and tip [26] while in the lower third, the orbicularis oris muscle activity contributes to the formation of outwardly radiating perioral lines (lipstick lines). Downward radiating lines, called as marionette lines are formed from the oral commissures due to the actions of the depressor anguli oris [16]. Peau d' orange affect or excessive wrinkling on anterior portion of the chin occurs due to hyperactive mentalis muscle and platysma muscle bands in the neck [1]. Initially facial rejuvenation is viewed from a two-dimensional aspect for correction of hyperdynamic facial lines and immobilization of corresponding muscles. Now, focus has shifted to three-dimensional aspects of facial aging particularly movement control, recontouring and volume restoration [26]. It became possible by combining botulinum toxin with hyaluronic acid dermal fillers [30]. Native hyaluronic acid has a short half-life of only about 1 to 2 days in tissue. By the process of cross-linking, hyaluronic acid results in larger, more stable molecules that have biocompatibility and viscoelastic properties similar to those of the naturally occurring substance. Cross-linking causes the ordinarily hygroscopic gel to become less water-soluble thereby increasing product stability in tissue [15]. The type B formulation, commercially available as Myobloc has limited cosmetic use, because of discomfort on injection due to its acidity (pH approximately 5.6) and its shorter duration of effect [31-32].

Gummy Smile

Gummy smile is a problem in dynamic relationship of the lips to the teeth. When the gingival display is increased more than 2 mm it is referred as gummy smile [33]. Possibility of gummy smiles is two times more in females as compared to males [34]. The use of Botox temporarily reduces gummy smile for 3-6 months by decreasing the activity of the elevator muscles of the upper lip [35-36]. According to a study use of 2.5 U of botox per side at the levator labii superioris, 2.5 U per side at the zygomaticus minor, and 1.25 U in the orbicularis oris is recommended [36].

Masseteric Hypertrophy

BT-A may be used to treat facial widening of muscular origin [30]. To perform the injection, have the patient bite down so that the anterior and posterior borders of the masseter can be felt. Injections should be low, just above the mandible with one to two sites per side. Start with 5 U per site. Doses can be increased to as much as 25 to 50 U as needed and as tolerated [26]. Several small but well-documented clinical trials showed sustained reduction of masseter hyperactivity [37-39].

Temporomandibular Disorder (TMD)

Temporomandibular Disorder (TMD) is a collective term which includes a group of clinical conditions affecting the stomatognathic system in particular the muscles of mastication and the Temporomandibular joints (TMJ) which is characterized by a group of commonly reported symptoms: fairly localized pain, limited or asymmetric mandibular movements and TMJ noises (crepitations or clickings) [40]. TMD is of multifactorial etiology. Possible causes for TMD are bruxism, mandibular muscle hyperactivity, facial growth and also other systemic, postural, metabolic, structural, traumatic, psychological, social and behavioral influences which have been identified as possible predisposing, initiating and maintaining factors for TMD [20-21]. Masseteric pain is relieved by injecting 5 U into muscle below an imaginary line from the tragus of the ear to the corner of the mouth, temporalis pain is alleviated by injecting 7.5 U into anterior vertical fibres bilaterally while in severe pain, inject 2.5 U in middle and posterior fibres. Tendon pain is relieved by multiple injections of 2.5 U in temple area at equal distances [25]. Many other studies show improvement in jaw function and pain relief in patients suffering from TMD and bruxism [41-43].

Dental Implants and Surgery

In patients with parafunctional habits, to decrease the failure rate of dental implants and to increase the healing of maxillofacial fractures following fixation, botox injections are given which reduce the strong masticatory forces. Kayikvioglu et al injected botox in 5 patients of zygoma fracture preoperatively and found out reduction in the need for number of miniplates fixation. They got similar results in mandibular condyle fractures [44-46]. It also acts as pharmaceutical splint in clencheders to improve healing and reattachment of gums and formation of new bone in trauma [22].

Mandibular Spasm

It is a condition when the mandibular closing musculature remains semi contracted or in spasm resulting in restricted mouth opening. This type of muscular spasm limits completing the basic oral hygiene necessary to prevent oral disease and places restrictions on dental treatment. BOTOX treatment to the masticatory musculature diminishes the effects of hyper-functional or spastic muscles that can significantly improve function and mouth opening and effectively decrease pain and tenderness to palpation [22,47,48]. Several case reports have been published, describing the effectiveness of botox in patients with hemi- masticatory spasm [34,41,49,50].

Headache, Migraine and Trigeminal Neuralgia

Botox 25"75 U injected into pericranial muscles relieves headache by relaxing the over active muscles by blocking nerve impulses that trigger contractions. For migraines, there is no muscle component involved. It is believed that BOTOX works by blocking the protein that carries the message of pain to the brain and relief typically takes effect in 2"3 weeks after injection. The longer the treatment duration, the better the pain relief [47,51]. According to Elcio, excruciating pain in head and face associated with inflammation of the trigeminal nerve can be substantially relieved by injections of BOTOX[52]. According to Lawrence Robbins, BOTOX actually is an anti-inflammatory substance decreasing or antagonizing the inflammatory (neuronal/brain) effects of W (Calcitonin gene-related peptide) [53].

Myofacial Pain and Neck Pain

Jennifer Warner, reported pain relief in 25 patients with chronic neck pain, after a single injection of Botox, delivered to the affected neck muscle combined with standard physiotherapy [54].

Incisor Eruption

Alfonso et al have demonstrated increase in incisor eruption rates after injecting BT in masseter muscle thereby reducing bite force [55].

Side Effects

- Development of antibodies to the toxin may make the patient resistant to further treatment¹. Antibody responses against botulinum neurotoxin A are observed to be reduced by synthetic monomethoxy polyethylene glycol peptide conjugates [56].
- When used in the treatment of cervical dystonias, dysphagia was a commonly reported event [57].
- Paresis of the zygomaticus major was reported in 4 of 15 patients in Nixdorf's study [58] with resulting in smile asymmetry.
- If neurotoxin diffuses when injecting the corrugator supercillii, the upper eyelid may droop. This can be avoided by digital pressure on the orbital rim to limit toxin diffusion and avoiding post-injection massage [59].
- BT-A injection in masseter muscle may decrease mandibular plane angle, ramus height and total mandibular length [60].

Contraindications

- History of sensitivity to toxin [1].
- Neuromuscular disorders such as myasthenia gravis or Lambert Eaton syndrome [1].
- Patients with known allergy to albumin.
- Patients using medications such as amino glycosides and patients using anticholinesterase or other agents interfering with neuromuscular transmission.

Emerging Trends

Since, most of the effects of the botulinum toxin are temporary, further studies need to be done to increase its long term effectiveness. BT-A along with laser treatment increases the extent and longevity of improvement in facial rejuvenation [17,18,19].

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