

Expert Opinion

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Botulinum toxin therapy for pain and inflammatory disorders: mechanisms and therapeutic effects

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Botulinum toxin (BTX) injections are a well-recognised therapeutic modality for the treatment of regional involuntary muscle disorders and recently BTX has been used for treatment of pain and inflammatory disorders. The primary purpose of this review is to discuss the mechanism of action of therapeutic BTX in light of both the traditional understanding of BTX pharmacological effects as well as new observations. The review will deal with clinical observations and relevant animal experimentation. The data and hypotheses presented are not only relevant to botulinum toxin technology but will certainly prove important in the basic mechanisms of some of the diseases where botulinum toxin has been successfully applied. BTX used clinically comprises botulinum neurotoxin (BoNT) complexed with non-toxic proteins. The non-toxic components of the BTX complexes stabilise the labile BoNT during purification and formulation as a therapeutic. The complex proteins may also have unrecognised clinical significance such as slowing diffusion in tissues or imparting stability. The mechanisms of BTX formulations acting on SNARE proteins are briefly reviewed providing a basis for BTX clinical applications. The potential for design of improved botulinum toxins and formulations is addressed.

Keywords: blepharospasm, botulinum toxin, dystonia, facial pain, inflammation, Meige syndrome, migraine headache, movement disease, myofascial pain, tension headache, torticollis, trigeminal neuralgia, toxin formulation, toxin quality

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1. Introduction

Botulinum toxin has become a major therapeutic tool for the treatment of a number of ophthalmic and non-ophthalmic diseases, including primary pain syndromes. Despite applications for many conditions, full understanding of the basic mechanism of BTX tissue effects and pharmacology remains to be fully understood. Recent observations involving the effectiveness of BTX in treating pain [1,2] and other sensory phenomenon require an explanation beyond the usual neuromuscular tissue bioeffects. Non-neuromuscular bioeffects may prove essential in the future development of the BTX therapeutic technology as well as explaining the full nature of the benefit achieved with its use. These aspects are addressed in detail following the description of the nature of BTXs and their catalytic activities.

2. Composition and structure BTXs and their proteolytic activity on SNARE complexes

Botulinum neurotoxins (BoNTs) are produced by various species of neurotoxicogenic *Clostridia* [3]. Seven serotypes (A-G) of BoNTs are known to be produced that are distinguished by their neutralisation of toxicity by serotype-specific antibodies [4]. BoNTs are proteins of about 150 Mr, which exist naturally as components of progenitor toxic complexes: as the M complex (ca. 300 Mr) consisting of BoNT associated with a non-toxic non-haemagglutinin (NTNH) protein of about 150 Mr; or they can exist as the L and LL complexes (about 450 and 900 kDa respectively) in which the M complex associates with hemagglutinin protein(s) [5]. The non-toxic proteins in the complexes have been demonstrated to provide protection during manipulations in drug formulation and lyophilisation. They may also have other unknown functions relevant to their use as a drug such as slowing diffusion or stabilising BoNT in tissues. Since the isolated neurotoxin component of the complexes is quite labile, the complexes have been used primarily as therapeutic agents [6,7]. Type A is the primary serotype of BTX used therapeutically, although serotypes B, C and F have also been used [7].

BoNTs are produced as single chain protein molecules of ca. 150 Mr which achieve their characteristic high toxicities (~ 10⁸ mouse LD₅₀ per mg) by post-translational proteolytic cleavage to form a dichain molecule comprising a light chain (LC; ~ 50,000 Mr) and heavy chain (HC; ~ 100,000 Mr) linked by a disulphide bond [5]. BoNTs consist of three functional domains:

- Catalytic domain with endopeptidase activity resides in the LC
- Translocation domain resides in the N terminal region of the HC
- Receptor binding domain is located in the C-terminal region of HC

The gene and amino acid sequences, structure and known pharmacology of BoNTs have been thoroughly reviewed recently [8,9]. BoNTs comprise a unique group of zinc proteases with certain unusual properties compared with other metalloproteases. BoNTs enter nerves by a four step mechanism [8,9]:

- Multi-step binding to polygangliosides and to unidentified protein receptors
- Endocytosis into internalised vesicles
- Entry of the LC into the nerve cytosol by an unknown transmembrane crossing event
- Specific cleavage of proteins of the SNARE complex

The SNARE hypothesis was proposed in 1993 to explain the trafficking of lipid vesicles carrying neurotransmitters and other substances to the cytoplasmic membrane of cells, where the vesicles fuse with the membrane and release their contents into the extracellular milieu [10]. According to the hypothesis, transmembrane proteins on the vesicle and target membranes pair in an anti-parallel manner to dock with cell membranes.

The trans-SNARE complex consists of the proteins syntaxin and a SNAP-25 family member on the target membrane and a VAMP-synaptobrevin family protein member on the vesicle [10,11]. SNARE complex formation and exocytosis is triggered by an influx of Ca²⁺ ions [12]. BoNTs were discovered to cleave the SNARE proteins SNAP-25, VAMP-synaptobrevin and syntaxin at specific peptide bonds, thus disrupting membrane fusion of the vesicle and inhibiting exocytosis [8,9]. BoNTs require the recognition of the SNARE structural motif for proteolysis [8,9]. Many different types of cell systems in eukaryotes including neuronal, endocrine, certain immunological and other types probably use the SNARE mechanism for membrane trafficking and release of neurotransmitters, hormones and other signalling molecules.

Current formulations of therapeutic BoNTs use the large 'crystalline' type A complex or the type B large complex (personal communication, RH Whitlock) [6,7,13,14] and are provided as lyophilised (type A) or liquid formulations (type B). Since BTXs are relatively new drugs and were approved by the FDA in 1989 (type A) or 2000 (type B), there appear to be many opportunities for improvements in existing toxin formulations and changes in molecular structure of the toxin molecules to prevent unwanted side effects such as excessive diffusion and acquisition of immunological resistance [14,15]. There are also opportunities through these approaches to increase the specific activity, duration of action and to create toxin products with long shelf-life and room temperature stability. Lastly, as the mechanisms of action of botulinum toxins are revealed, it will be possible for therapeutic treatment of diseases other than the those modulated by excessive cholinergic activity such as segmental dystonias. The basis for treatment of pain and other disorders is addressed in the subsequent sections of this review.

3. Neuromuscular effects

Botulinum toxin has been long known to cause its toxicity by neuromuscular blocking effects. Similar effects have been well documented in the therapeutic literature after botulinum injection within muscular region level [16-19]. The regional tissue effects created by the therapeutic injections involve a process termed chemodenervation, which has been characterised by:

- Reduction of muscular tone and contractility within a regional muscular area
- Muscular fibre atrophy (neurogenic) on histological analysis which is reversible over a 3 - 5 month period
- An axonal sprouting at the neuromuscular junction occurring 2 - 3 weeks after injection [20]
- Spread of acetylcholinesterase staining over muscle fibres which persists for 6 - 8 weeks after muscular injection
- ATPase staining reflecting Type 1 and Type 2 fibre groupings suggestive of anatomic motor denervation of muscle [21,22]
- Complete reversibility of the tissue effects after 12 - 16 weeks

Prior to therapeutic use, BTX had been known to medical professionals as a deadly poison, capable of causing diffuse flaccid neuromuscular paralysis and respiratory failure. At that time, the use of an LD₅₀ seemed to be the most reasonable end point for biological activity measurement because it simulated the clinical condition (death) and the measurement was definitive as an end point (death of a mouse *versus* survival) without the pitfalls of subjective interpretation [13,23,24]. Toxins are often measured using a lethality index; however, the therapeutic botulinum intra-muscular injections are essentially low dose biological quantities intended to confine the effects to a specific anatomic region to insure safety and limit undesirable regional weakness or systemic spread. The distinguishing property of BTX relates to its ability to induce a regional chemodenervative effect without spreading to remote points from the injection site. Stated another way, the dose necessary to produce a regional bioeffect is at a concentration much lower than the dose necessary to create a lethal systemic effect. That is, the ED₅₀/LD₅₀ = therapeutic index = small.

Despite this property, containment of the neuromuscular bioeffect is still a problem in practice. Ptosis and diplopia for the treatment of blepharospasm and strabismus, dysphagia for the treatment of cervical and jaw dystonia and certain forms of facial weakness associated with cosmetic applications result from undesirable diffusion from the injection site. Efforts have been made to quantify the given diffusion property of the LD₅₀ dose unit of BTX using animal models. The *longissimus dorsi* muscle of an albino rabbit back was used for diffusion measurements of varying doses of BTX at a point injection of the dorsal process of the scapulae. Histological assessment using computerised fibre diameter variability (F ratio) and intensity of acetylcholinesterase staining allowed the construction of a nomogram showing varying diffusion for a LD₅₀ given dose, that is, the estimate of the anatomic field in which a neuromuscular effect can be expected [17,18]. A graphic representation of this concept is demonstrated by a reduction of forehead wrinkles after point injections (Figure 1a). Wrinkles can be noted to be reduced in a circular geometric pattern surrounding the injection sites. From the anatomic and structural models of diffusion, efforts to standardised BTX using regional denervation animals bioassays such as a mouse paralysis hindlimb model or a rabbit ptosis models emerged [23,24]. Such animal models demonstrated varying denervation potencies per LD₅₀ unit between various immunotypes [24] and varying duration of action between varying immunotypes at similar LD₅₀ units. Further clinical work now has now clearly shown vast differences in dose response relationships among the various immunotypes of BTX and among toxin derived from varying strains of the same immunotypes. For instance, cervical dystonia is effectively treated with between 150 - 250 LD₅₀ units using BOTOX (type A) botulinum toxin. Yet 2500 - 10,000 units are needed to achieve effective clinical result with one preparation of immunotype B (Athena Neurosciences-Elan, South

San Francisco, CA, USA). When the ophthalmologist applies botulinum toxin type B for the treatment of resistant cases of blepharospasm or other indications, substantial dosing corrections will need to be made. The impact on such large doses on complication rates for use of type B immunotype (ptosis, diplopia, dry eye) remains to be determined.

4. Autonomic bioeffect

In addition to blocking acetylcholine release in striated muscle, BTX has long been known to block cholinergic neurotransmission within the autonomic nervous system, at the both the effector and ganglionic level. Such properties have been monitored in the past with laboratory preparation using intra-orbital injections and monitoring pupillary response to assess mydriasis as a measurable autonomic ganglionic block [25,26]. Most recently, BTX injections have been used to induce a cholinergic suppression of the lacrimal gland to reduce reflex tearing in the syndrome of aberrant facial nerve regeneration [27]. In animals, blocked saliva production in canine animals models [28] and blocked sweat emission [29] have also been demonstrated over the years as explainable autonomic effects of injected BTX. Such effects are currently being utilised in the clinical setting for patient benefit.

Another, perhaps even more significant observation regarding BTX autonomic effects involves heat release and vasodilatation after exercise. Laser Doppler studies have demonstrated that reflex vasodilatation can be blocked after exercise [30]. Using non-specific anticholinergics (atropine), similar effects could not be demonstrated, indicating that this unusual property of BTX can not be readily explained by its well know effect on cholinergic neuromuscular synaptic transmission or even cholinergic autonomic transmission. Such effects of BTX suggest, but do not necessarily prove, another transmitter or co-transmitter may operate in the biological effect of BTX within the denervation field created by point injections. Some authors have suggested vasoactive intestinal peptide (VIP), or other related compounds, may be effectively blocked by the botulinum [30].

Botulinum toxin is well known to exist as immunotypes A through G and varying clinical observations have been made for each of these immunotypes. For instance, poisoning occurring with type A generally results in a longer duration of paralysis compared with other immunotypes, particularly types B and E [7,31-34]. Type A, B and E are the most common types associated with intoxication in humans [35]. Horse botulinum intoxication more commonly occurs with type B. Types C, D most commonly occur in birds [35]. In veterinary practice, relative to autonomic bioactivity activity, horses generally demonstrate much more autonomic symptoms with type B *versus* other immunotypes, particularly type A, indicating a potential differential in autonomic activity between the various immunotypes of BTX [36]. Consistent with this observation is the notable dry mouth complication more common when botulinum type B is used to treat

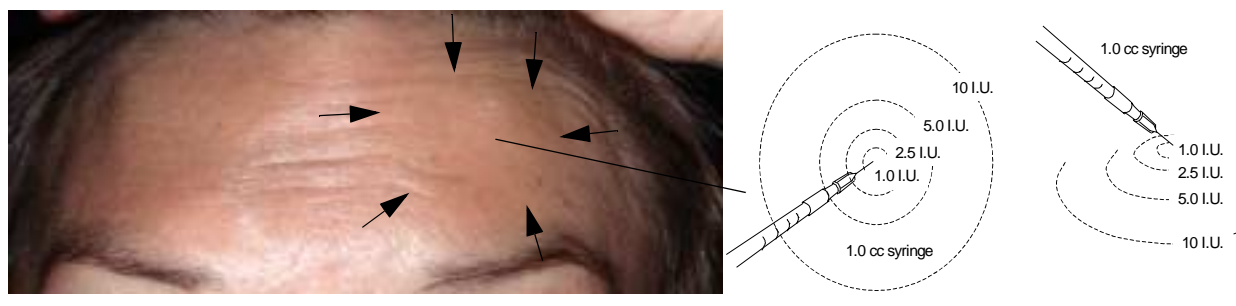


Figure 1a. Diffusion fields (depicted in the clinical setting). Botulinum toxin when used therapeutically effects a definable geometric field of tissue. This field can be seen as a circular area of forehead wrinkle reduction in a patient three weeks after point injections in a patient with forehead tendon transplants as a treatment for oculo-motor nerve paralysis (with ptosis). The graphic emphasises this diffusion is dose-dependent and such diffusion kinetics have been measured using cholinesterase staining and fibre size variability statistics along the *longissimus dorsi* muscle of the albino rabbit back [16].

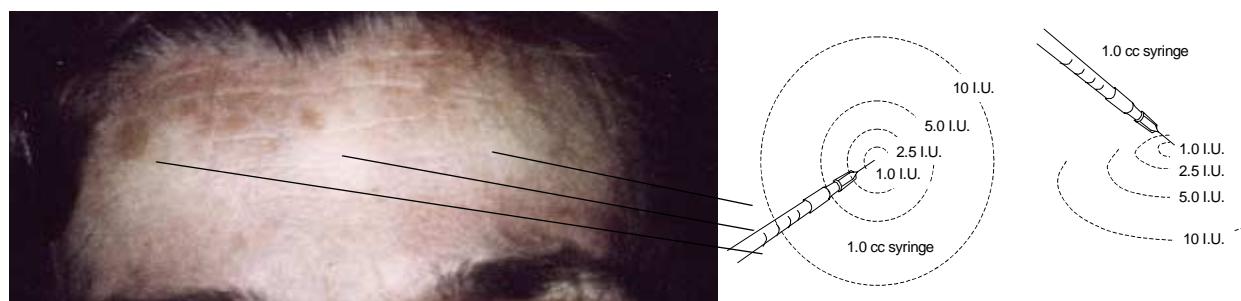


Figure 1b. A new bioeffect (anti-inflammatory) of botulinum toxin is viewed in a patient with cholinergic urticaria. This syndrome causes eruption of small fine hives after moderate exercise (seen here as blotches of red facial discoloration). Pale confluent circular areas are noted in the forehead surrounding four-point injection of the forehead. Three points are depicted, the fourth point was between the brows. This syndrome's pathophysiology (release of preformed inflammatory mediators) and botulinum toxin result confirms a new bioeffect heralded by earlier observations involving botulinum toxin's analgesic properties, with relief of erythema and oedema in post-operative myositis and primary non-surgical myofascial pain syndromes. This bioeffect occurs in a geometric region conforms to the known diffusion field of as measured by neuromuscular weakening (**Figure 1a**), microanatomic changes and autonomic nerve suppression. A similar finding is seen in the peri-ocular region of a patient with cholinergic urticaria with blepharospasm after exertion.

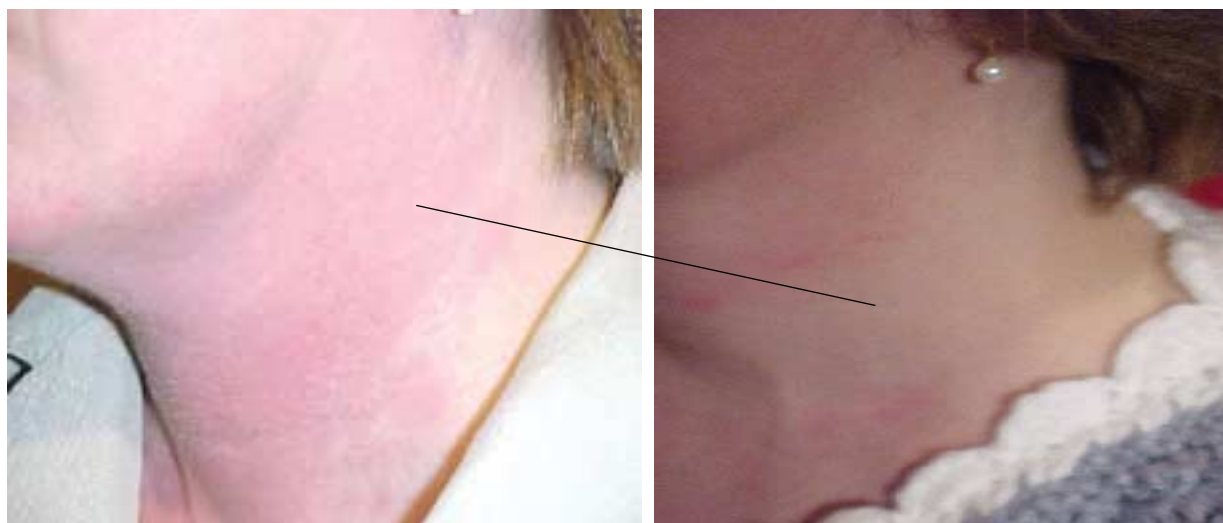


Figure 1c. An erythematous region of a patient with myofascial pain-dystonia, suppression of the erythematous reaction can be seen within the diffusion region two weeks after injection into the region (digitally enhanced).

cervical dystonia, which is rarely observed when botulinum type A toxin is used to treat the same condition [37]. The difference in this complication may result from either differing autonomic nerve reactivity between the immunotypes or perhaps differing diffusion potential.

Assessing the rate of diffusion-related complications associated with botulinum type B in the treatment of neurological forms of blepharospasm should further the understanding of pharmacological differences between the two available immunotypes. Ptosis results from diffusion of BTX into the levator palpebra superioris muscle located deep within orbital tissues from superficial injections. This complication rate should reflect the diffusion potential of a pharmaceutical preparation.

5. Botulinum toxin for the treatment of pain disorders

Botulinum toxin has become an important tool in pain management over the past three years. The first notable observation which led to implementation of this technology came from its use in patients with adult onset spasmodic torticollis (cervicocervical dystonia), a condition associated with pain and discomfort [37-41]. Torticollis is often a hereditary syndrome consisting of pain, increased cervical muscle tone, posture deformity, muscle hypertrophy-hardness, tremor and other involuntary motion. This regional form of dystonia is chronic with fluctuation in symptoms over a lifetime. During periods of exacerbation, there can be severe pain usually localised in the posterior anatomic triangle of neck on the side of shoulder elevation. Botulinum toxin injections for this condition have proven most effective for relieving the sensation of pain over other components of the syndrome (96% pain *versus* decreased range of motion, involuntary movement, posture deformity, χ^2 $p < 0.05$) [2,19]. The reason pain is the most responsive component and responds at a rate significantly higher than other components of the syndrome has never been adequately explained.

The torticollis observations lead to the notion that BTX could be used for primary pain syndromes, particularly those that have a muscle based component [1]. In 1991, BTX was injected in several cases of myofascial pain syndrome and tension headache, a syndrome characterised by pain within the head and neck region without dystonic posture, tremor, muscle hypertrophy or other features of regional dystonia [1]. The reason myofascial pain of cervical musculature was chosen is that it bears some resemblance to torticollis. Both conditions are associated with trigger points (focal areas of exquisite tenderness to palpation by the examiner) and both conditions often involve similar pain distribution within the head and neck region. Myofascial pain of the cervical and head region often involves the same muscles afflicted with the torticollis syndrome (splenius capitis, levator scapulae, scalenes, temporalis, frontalis). Myofascial pain may even have a degree of involuntary movement associated with the

syndrome. Microfasciculations are sometimes noted and indicate some element of hyperactive muscle contraction by Travel *et al.* [42]. Myofascial pain also has been associated with the phenomenon of dermatographism and/or subtle hyperaemia indicating autonomic and/or inflammatory component of the syndrome [42]. In the setting of primary head and neck myofascial pain, a small series of patients with headache and neck pain experience substantial relief in an open label type trial [1,45]. Further experience with BTX for this condition proved useful in both open label and blinded trials involving various forms of headache syndromes [43-46].

From primary myofascial pain, the application was taken further in the treatment of myofascial pain following craniotomy for the removal of acoustic neuroma. A series of three patients (including a dentist and a physician) developed a new onset chronic neck pain and headaches meeting the criterion for chronic surgical wound pain around incisions and muscle flaps following transoccipital craniotomy for the removal of acoustic neuroma. Each was successfully treated with im. BTX (type A, 100 - 150 LD₅₀ units) which relieved the pain for a period of 6 - 12 weeks. Palpable trigger points and perincisional locations were selected as the injection points. After this successful experience, treating the development of chronic myofascial head and neck pain and headache following acoustic neuroma surgery and craniotomy has become more commonplace at our institution. Another small series of patients were also successfully relieved of pain, muscle spasm, oedema and diminished joint mobility following temporal mandibular joint surgery. Such observations on chronic post-operative pain has led to an expanded application following sinus, orbital, facial plastic and reconstructive flaps, fracture repair and dental surgical procedures.

Additionally, BTX was successfully applied to patients with temporal mandibular joint disease and dysfunction who had not undergone prior surgery for this condition. This condition is thought to represent another form of myofascial pain [42]. After treatment, patients noted loosening and increased excursion of jaw opening. Even more remarkable was the analgesia associated with BTX application.

As the analgesic properties of BTX became appreciated, the authors made an interesting observation relative to botulinum mechanism of action in patients with torticollis. The presence of red skin blotches on the neck region could be seen in lightly pigmented individuals usually during periods of pain and worsening of the involuntary movement disease. This discoloration bears some resemblance to the cutaneous descriptions of dermatographism associated with myofascial pain well described in the classic textbook by Travell and Simmons [42]. Even more significant was the suppression of the erythema noted in the skin following BTX, in an anatomic area and radius consistent with known diffusion fields of botulinum dosages (Figure 1c). This observation indicated a bioeffect not easily explained by the well-appreciated neuromuscular effects of the toxin. The presence of subtle erythema and its suppression precisely within the

known diffusion field of BTX suggests inflammation in part may be important in the genesis of spasmodic torticollis. The enlargement and induration of cervical musculature characteristic of this syndrome may not just be the result of increased tone and muscle fibre hypertrophy but may be in part caused by an inflammatory process. Muscle hardness has also been implicated as reflective of the degree of pain with essential headache and myofascial pain.

In summary, it appeared that BTX has a direct effect on the sensory nervous system in relieving pain [1]. This sensory effect involved the depression of pain as well as erythema, as demonstrated by the depression in torticollis patients, post-operative temporal mandibular joint inflammation as well as a number of other post-operative states.

6. Essential blepharospasm, Meige syndrome and pain

Blepharospasm is a syndrome not usually associated with pain [47], but can be associated with a sensation of eye irritation. The most consistent abnormal sensory experience suffered by patients with benign essential blepharospasm and Meige syndrome is photophobia [48]. Photophobia often causes patients with this syndrome to use sunglasses, even indoors. Photophobia is usually a symptom associated with ocular inflammatory conditions. For instance, secondary forms of blepharospasm can be caused by keratitis or uveitis. An eye examination is usually revealing as to the cause of the photophobia and/or blepharospasm in such clinical conditions. The nosologists of previous generation used the term 'essential' to indicate the absence of explainable causes of blepharospasm hence indicating lack of understanding of basic disease mechanism. In fact, the descriptions of blepharospasm in many written works indicated an emotional cause [47]. Unfortunately, the notion of conversion hysteria erroneously often became linked with the understanding of the condition [47]. Although such explanations are no longer accepted, the aetiology of essential blepharospasm remains elusive.

In postulating the possible cause of essential blepharospasm, it will be necessary to explain all common components of the syndrome. Such components include [48]:

- Involuntary eyelid spastic closure without obvious cause
- Strong hereditary nature of the condition
- Association with involuntary movements of the face and head and neck region
- Relief by 'geste antagonist' that is frequent touching of the face to suppress the involuntary movements
- Presence of photophobia seen in over 90% of patient with this condition

Photophobia stands out as the only non-motor component of the syndrome and is often relieved by percutaneous injections of BTX indicating another non-muscular direct effect of the toxin [48].

Another significant phenomenon is an exacerbation of

blepharospasm caused by allergy and other forms of inflammatory ocular surface conditions. When concomitant lid and conjunctival allergy is experienced by patients with essential blepharospasm, the intensity and severity of the involuntary movements are much worse. The traditional explanation for the cause of essential blepharospasm suggests an abnormality in the CNS, causing not only involuntary eyelid closure but also other involuntary movements within the head and neck region. Although no structural lesions are common when neuroradiographic studies are performed [49], positron emission tomography has demonstrated abnormal brain emission to sensory stimuli. Although such studies are enlightening, there still is uncertainty as to the location of the pathology.

Botulinum toxin clearly has become the most significant contemporary contribution for the treatment of essential blepharospasm and Meige syndrome. Efficacy of BTX for the treatment of this condition is so strong that the agent's tissue reactions may be useful in constructing pathophysiological theory. The most conventional understanding of BTX is that the toxin binds to receptors of the preterminal axons at the neuromuscular junctions by its heavy chain, with transport through the cell membrane, dissociation of the light chain into the cytoplasm with catalytic cleavage by the light chain of proteins in the SNARE complex involved in exocytosis [50]. These target proteins SNAP-25, syntaxin and VAMP-synaptobrevin are critical to the movement of organelles throughout the cytoplasm [8,50]. The tissue effects of inactivating these proteins include causing muscle fibre atrophy and fibre size variability over a dose-dependent defined region from the injection point [8,16]. Spread of acetylcholinesterase on post-synaptic membrane is identifiable, similar to the effect of surgical motor denervation [16].

Certain clinical phenomenon can not be explained by chemodenervation of muscle such as blocked reflex tearing associated with lacrimal and salivary gland injections of BTX [27,28], blocked sweat formation in patient with hyperhidrosis syndrome [29] and pupillary dilation after orbital apical injections [26]. These applications and observed results can be explained by cholinergic blockage at the level of the autonomic nervous system.

Despite a sound understanding of the effect of BTX on muscle, this explanation for drug mechanism may be incomplete in explaining its remarkable efficacy for the treatment of blepharospasm. In 1996, BTX was demonstrated to substantially reduce photophobia in patients with benign essential blepharospasm and Meige syndrome [48]. Based on an analysis of 26 patients using a visual analogue and multiple choice questionnaire, a significant but not complete reduction in light sensitivity was reported comparing questionnaire performance before botulinum injections and 2 - 4 weeks after injection ($p < 0.05$, Wilcoxon, Figure 2). This result is actually counter-intuitive to an expected result, as some exposure keratitis created by lagophthalmos and perhaps some decreased tear production from the injections should worsen

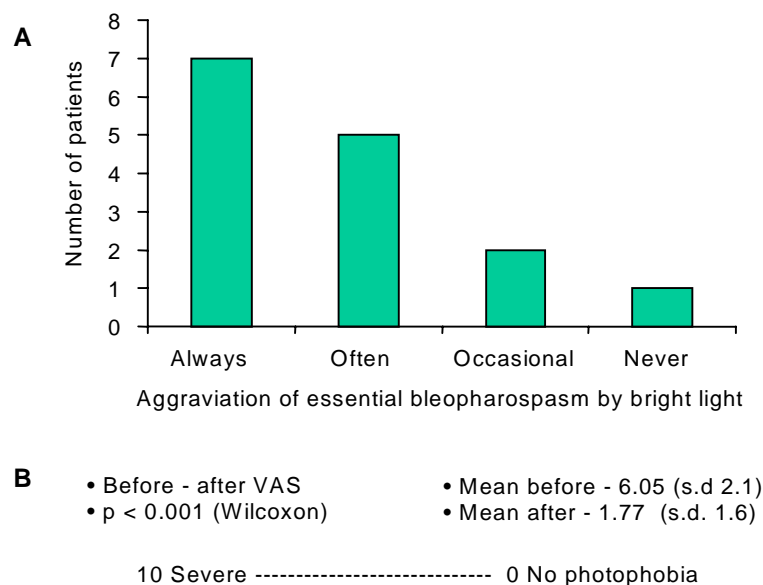


Figure 2. A: Essential blepharospasm aggravation by bright light (n = 15) **B:** Effect of botulinum toxin on photophobia. Photophobia is a common complaint in patients with essential blepharospasm, with Meige syndrome occurring in about 90% of patients (**A**). Visual analogue testing of photophobia showing botulinum relieves the experience of photophobia in patients with essential blepharospasm and Meige syndrome (**B**) (n = 26). Photophobia is also one of the most common complaints experienced with intra-ocular inflammation and its presence in essential blepharospasm (Meige syndrome) suggests that a derangement in non-cellular inflammatory pathways may be playing a role in these syndromes.

photophobia, not improve the symptom. From these observations it seems that BTX is affecting more than the neuromuscular synaptic transmission in essential blepharospasm and a direct effect on the sensory nervous system or effectors of the sensory nervous system is postulated.

7. The migraine story and forehead wrinkles

Beyond tension headaches and post-operative essential headaches, observation have been made recently that BTX is efficacious for the treatment of migraine headaches (personal communication, W Binder) [52]. Actually, many if not most of the original patients treated for migraine suffered from concomitant tension-myofascial headaches [53], which rendered plausibility to the initial migraine observations. Migraine sufferers also frequently suffer from chronic myofascial-tension type essential headaches and the pathophysiology of both conditions has been thought to be linked *via* mast cell and inflammatory releases of possible neuro-effector substances [54-65]. Migraine is a common pain syndrome characterised by photophobia, phonophobia, severe throbbing pain, pain worsening on movement, nausea, occasional vomiting and confined regional location within the head (e.g., hemicrania). During injections of middle aged women seeking cosmetic wrinkle reduction, an observation was made that premenstrual headaches and migraine headaches were reduced in frequency and intensity. In an open label trial consisting of 103 patients with essential

headaches, improvement was noted in 80% with respect to severity and intensity of the pain (Table 1). Another important observation was that a number of patients described a regional relief of pains, sometimes feeling throbbing pain at the borders of the injection field indicating a segmental response consistent with the known diffusion property of the toxin [16,18]. The duration of beneficial effect was 2 - 4 months, consistent with the usual duration of efficacy encountered with BTX injections.

This observation supported the concept that BTX has a direct effect on the afferent sensory nervous system and that pain relieving capability of BTX has generic properties effective between a number of headache syndromes (i.e., tension headache, migraine headache, temporomandibular joint syndrome, post-surgical headache, myofascial pain syndrome associated with headache and photophobia associated with essential blepharospasm). Double blind placebo-controlled trial using botulinum toxins have shown efficacy based on frequency and intensity of headaches, reduction in oral medication [52]. Controlled trials in tension headache syndrome are continuing to demonstrate BTX efficacy [66,67]. Furthermore, in a retrospective review of 97 patients with benign essential blepharospasm, Meige syndrome and hemifacial spasm, 17 patients were found to have concomitant essential headache, with 14 achieving reduced headache complaints and six patients noting that return of worsening blepharospasm is often heralded by return of tension or migraine headache [67].

Table 1. Essential headache experience in 97 patients with blepharospasm. 17 patients were identified with both conditions, 14 felt the frequency and severity of the headaches were reduced with BTX. In open label experience 81% of 127 patients with various forms of essential headache disorders felt there was a beneficial response over a 3 month period.

A: Retrospective evaluation-facial spastic disease[†]

- 97 Patients questioned
- 17 Experienced migraines
- 14 Felt headaches were at least somewhat better since using BTX
- 4 Felt headaches were worse at the end of injection cycle

B: Open label experience with botulinum toxin for migraine and combined essential headache disorders^{††}

- 81% responded (103 of 127 treated)
- 51% requested further injections
- Complications: 1 case of transient ptosis

[†]G Borodic (1996); ^{††}Data-pooled: Dr Gary Borodic, Dr William Binder, Dr L Schenrock, Dr Andrew Blitzer

Essential headache disorders share two symptoms with Meige syndrome and benign essential blepharospasm:

- Photophobia
- Tactile relief ('geste antagoneste')

Both syndromes often cause patients to touch their heads or faces to relieve symptoms. The physiological explanation for tactile relief is unclear but may involve alterations in autocoid or neurotransmitter release within the forehead or peri-ocular tissues and/or an effect on descending inhibition.

The current theories on migraine headaches involve a chemical disorder of the afferent sensory nervous system, resulting from abnormal release of autocooids from mast cells or afferent sensory nerve tips (C mylinated nerve fibres), either through antidromic stimulation or another mechanism [53,56-59]. Preformed autocooids include substances such as histamine, nitric oxide, substance P, bradykinin, neurotensin, prostaglandins, leukotrienes, serotonin, antitumour necrosis factor, VIP, platelet aggregation factors, calcitonin gene-related protein, as well as other possible preformed mediators.

8. Evidence of the nature of the sensory effect of BTX

The theory that BTX blocks autocoid release and suppresses dermatographism and subtle erythema in myofascial pain and torticollis is further supported by more compelling evidence and application coming from observed effects of BTX on cholinergic urticaria (Figure 1b). In patients with both facial movement disorders headache disorders and cholinergic urticaria, BTX was shown to block both exertional-induced urticaria both of the forehead and peri-ocular region in a pattern consistent with known diffusion geometry causing

neuromuscular weakening for specific doses used (Figure 1a,b). For instance, the region of suppressed urticaria shown in Figure 1b compared to the weakening effect of botulinum on frontalis muscle in patient shown in Figure 1a who received equivalent doses in the same region. Each patient shows two separate bioeffects of botulinum over an equivalent region. This phenomena of suppressed cholinergic urticaria has been observed by the authors in patients with essential headache, torticollis and myofascial pain (Figure 1c).

Cholinergic urticaria is a condition characterised by redness of the face and body and a small pinpoint type hive, associated with itching and oedema. Occasionally asthmatic symptoms can occur. The condition has been associated with increased release of histamine and mast cell degranulation has been histologically demonstrated [68,69]. Oedema, erythema and altered sensation are rapidly reversible without evidence of cellular based inflammation. This syndrome represents a pathological extreme in continuum of the physiological vasodilatation after exertion. Past studies have failed to show that typical anticholinergics such as atropine can block this physiological response and anticholinergics have been shown to have limited therapeutic utility in managing cholinergic urticaria [50]. Given these observations, it seems that the sympathetic nervous system appears to be involved in the post-exertional erythema and probably in some way, the urticaria associated with exercise in this syndrome. It appears that BTX is capable of blocking both post-exertional physiological vasodilatation and frank neurogenic inflammation provoked from mast cell degranulation and/or neuro-effector substances following exertion in patients with cholinergic urticaria syndrome.

9. Botulinum toxin as an anti-inflammatory medication

In the first century AD, Celsus described the cardinal physical signs of inflammation as dolor (pain), rubor (erythema), tumor (oedema) and calor (heat). Seventeen centuries later, loss of function was added to these physical signs. Botulinum toxin has demonstrated a physical effect consistent with an anti-inflammatory in a number of disease states within the known anatomic diffusion field:

- Pain: torticollis, essential headache, facial pain, post-operative chronic pain syndromes, temporal mandibular joint disease, myofascial pain. Each of these syndromes has been tied to some inflammatory basic mechanism.
- Oedema: cholinergic urticaria, torticollis, early post-operative pain relief
- Erythema: cholinergic urticaria, torticollis
- Decrease heat emission: cholinergic urticaria
- Loss of function: TMJ, dystonia, blepharospasm, torticollis

Furthermore, depression of irritation and photophobia in blepharospasm patients further led credence to an anti-inflammatory bioeffect. The utility of BTX as an anti-inflammatory medication will need to await further clinical trials.

10. Inflammation, mast cells and BTX bioeffects

Inflammation involving autonomic nerves and sensory nerve tips have been a subject of interest in both pain [60-64] and allergy literature in recent years [65,70-73]. Neurogenic type inflammation has been evoked as an explanation for myofascial pain syndrome [42,75-77] temporal mandibular joint syndrome [75,76], various forms of arthritis [75-81], atopic allergy [82-85], migraine and tension headache [54-58], reflex sympathetic dystrophy [84], asthma [85] and has been thought to play an initial role in the general inflammatory response.

The triple response of Lewis is a classic example of rapid physiological inflammatory response and is probably governed by histamine and other autocooids. Many autocooids, such as histamine, are unable to effect a cellular response (non-cytokines). Such regulators are in effect unable to play a sustained role in the full cellular response attracting neutrophils and lymphocytes, characteristic of acute and chronic inflammation. Hence, certain autocooids although having a role in the early stages of inflammation need cytokines to effect a cellular response. However, the fast phase non-cellular component to the inflammatory response is regulated by pre-formed mediators, which is reversible within minutes. A classic example is urticaria which is a rapidly reversible form of inflammation. Allergic conjunctivitis also is commonly associated with photophobia and, at times, secondary blepharospasm, reversible with resolution of the inflammation. The role of fast acting autocooids on the inflammatory response has been a subject of extensive literature with respect to vascular pain in migraine but has not been discussed to any extent in the cause of benign essential blepharospasm or Meige syndrome. The strong genetic predisposition based on pedigree analysis in 269 families within the New England region and the presence of identical twins with essential blepharospasm and Meige syndrome suggests possible hereditary factors may be playing an aetiologic role. The possibility exists that autocooid regulation-based sensory nerve and proprioceptive nerve adaptation may be operative in these conditions and that such defects have a genetic basis. Such ideas need to be seriously considered as a basic mechanism of the disease.

If non-cellular-based inflammatory mediators are important in explaining photophobia in essential blepharospasm and perhaps other regional movement disorders and BTX may well block such autocooid release, then a nexus is established relating autocooids to muscular spasm, in certain pain and movement disorders. Inflammation is frequently involved in many clinical circumstances where muscle spasm is a key clinical feature: (e.g., meningitis-cervical spasm, peritonitis-abdominal rigidity and spasm, arthritis-muscle spasm, bronchitis-bronchospasm, cystitis-bladder spasm with incontinence). Although conjectural, the rapidly acting inflammatory autocooids could be playing a role in proprioceptive adaptation as well as sensory nerve regulation.

Such theories may be helpful in explaining the alteration of blepharospasm with concomitant seasonal allergy, anxiety, humidity changes, tactile facial stimulation ('geste antagoniste') or other conditions which cause peripheral nerve adaptation or response in tissues. Such theories may also be useful in explaining the influence of humidity, anxiety, emotional state, tactile stimulation, vibratory stimulation, exertional stimulation and skin puncture on urticarial reactions. Mast cells and/or sensory nerve tip autocooids can be released pathologically as part of a deranged system which influences ocular light sensitivity, urticarial eruptions, muscle tension, vibratory adaptation and possibly other sensory phenomenon such as proprioception. The skin and ocular surface tissues are very pressure reactive with erythema resulting after sustained tactile pressure, with pressure related urticaria being a pathological extreme of the usual reaction. The weak efficacy of antihistamines in treating regional movement diseases might be explained in part by a peripheral effect of the antihistamine on peripheral nerve proprioceptive adaptation or similar effects on muscle spindles.

11. Neurogenic inflammatory mediators and allergic inflammation

Recently investigations in models of asthmatic bronchospasm [85], vernal and atopic conjunctivitis [70-74] and allergic rhinitis [70,83] have implicated the mast cell and sensory nerve structures as playing a role in these forms of allergic inflammatory reaction. The effect of bradykinin, capsaicin and substance P on plasma extravasation in the guinea-pig conjunctiva and respiratory system has been studied [83-85] and suggested to contribute part of the pathogenesis of the allergic conjunctivitis. Bradykinin, capsaicin and substance P caused a dose-dependent increase in plasma extravasation with the following order of potency: substance P > bradykinin = capsaicin [85]. In one set of experiments [85], the effect of capsaicin and substance P was abolished by the tachykinin NK₁ receptor antagonist, CP-99,994. The role of neurogenic mechanisms lead to the selection of conjunctival allergy utilising the Bartley guinea-pig [85] as an experimental model for the measurement of non-neuromuscular effects of BTX on the inflammatory response.

12. The effect of BTX in an animal model of allergic-based inflammation

An attempt to scientifically study the effect of BTX on ocular irritation and allergic-based inflammation was accomplished by sensitising guinea-pig conjunctiva to pollen spores over a period of two weeks [86]. This sensitisation was followed by injection of botulinum type A toxin to the peribulbar area and conjunctiva of one eye, followed by re-exposure of the guinea-pig conjunctiva to pollen spores. The Type 1 hypersensitivity reaction causes ocular discomfort

Table 2. Use of botulinum toxin for chronic facial pain[†].

		Patients (n)	Responding	Fisher
Total treated		38	27 (69%)	
Diagnosis:	TMJ	9	7	ns
	Essential headache	9	7	ns
	Neuralgia-trigeminal	10	7	ns
	Post-surgical	10	8	ns
Pain quality:	Neurogenic	10	7	ns
	Myofascial pain	14	11	ns
	Neurogenic/myofascial	14	10	ns
Inflammatory signs:	Present	25	21	p < 0.03
	Absent	12	5	p < 0.03

[†]Acquadro and Borodic. Response rates with four types of facial pain (temporal mandibular joint syndrome, essential headache, trigeminal neuralgia and chronic pain after surgical procedure). The ability of BTX to relieve pain and irritating experience in multiple disease entities suggests strongly that BTX is acting at a fundamental generic neurological process involved in pain emanation.

and irritation associated with rapid onset of conjunctival oedema, vasodilation, flame haemorrhages and epiphora. This animal model has been qualified as a measure of mast cell degranulation based on histological assessments and is used as a method for evaluating topical anti-inflammatory allergy directed pharmaceuticals [85].

In a series of 24 eyes in 12 animals, scratch time was reduced by peri-bulbar injection of botulinum type A toxin in the peri-bulbar region. Reduced conjunctival oedema and injection was noted in the first 15 min after exposure to the allergen. Reduced animal scratching behaviour was also diminished during a comparable period. It should be noted that BTX did not completely block the inflammatory response particularly after 15 min of antigen exposure. It is postulated that BTX blocks a portion of preformed mediators of inflammation or one or more of the preformed mediators.

13. Facial pain syndromes

Recently, BTX has been applied for the treatment of chronic forms of facial pain (Table 2), including temporal mandibular joint syndrome, myofascial pain, facial pain following sinus intra-cranial, ophthalmic and dental procedures and most significantly typical and atypical trigeminal neuralgia. Temporal mandibular joint syndrome and myofascial pain have loosely been characterised as a disorder involving inflammatory mediators effecting sensory nerves [75,76]. Trigeminal neuralgia likewise may involve an inflammatory process probably emanating from trigeminal nerve irritation provoked by aberrant arteries at the base of the brain [87-90]. Such irritation may lead to antidromic impulses and alterations in nociceptive signal processing *via* alterations in nerve receptor alterations or transcriptional changes by signal molecules [91,92]. Whatever the exact mechanism, the presence of small degrees of oedema and/or erythema sometimes visualised in lightly pigmented patients with trigeminal

neuralgia and other forms of facial pain suggests an inflammatory component to nerve sensitisation associated with the syndrome. Although inflammation is loosely related to the mentioned syndromes, there is no question that inflammation is associated with wound pain, as histopathology of wound heading is a well characterised process. Tissue findings associated with trigeminal neuralgia, myofascial pain and essential headaches are not well characterised.

In a pilot series of 31 patients with chronic facial pain not responsive to conventional medications or surgical procedures, 21 patients responded partially or completely to injections of BTX (Table 2). The duration of pain relief appeared consistent with the known duration of action of botulinum, with pre-existing oedema and erythema having predictive value for a beneficial outcome.

The basic and clinical pain literature has established great importance in the discovery and recognition of mechanisms causing pain. Indeed a number of disease aetiologies share common mechanisms of pain. It is the fundamental importance of understanding the mechanism of pain, which dictates pain treatment. Knowing the aetiology may often help prevent, alter, or cure a disease process, but the mechanisms of the pain may at times be independent of whether or not the aetiology is even known or can be corrected [91,92]. The ability of BTX to relieve certain forms of pain will certainly be a subject for future study.

14. Movement disease and inflammatory mediators

If inflammatory mediators are playing a role in movement disease why isn't there more obvious signs of inflammation in these patients on the ocular surface or internal structures? As demonstrated in several patients in this paper, dermatographism and subtle erythema noted in patients with cervical dystonia (torticollis) often associated with the painful

interval of that disease does suggest inflammatory mechanisms could be modulating the spasm intensity as well as contributing to the painful sensory experience. In fact, the prominent spastic hypertrophied and indurated sternocleidomastoid so characteristic of torticollis may not only be caused by tonic spasm but also a degree of inflammatory reaction as previously mentioned. The link between photophobia and essential blepharospasm may also be *via* an inflammatory mechanism. Common mediators influencing both movement and inflammation may explain the worsening of involuntary upper facial movements by concomitant allergic symptoms. The association of reflex sympathetic dystrophy and dystonic movements is still another example suggesting a mechanistic connection.

The nexus between inflammatory changes and human movement disorders needs future study. An explanation may relate to selective effects and regulation of various discovered or even undiscovered autocoids on sensory nervous system. Certainly histamine may play on role and antihistamines do have a mild mitigating benefit, however, the cutaneous reaction and oedema formation associated with a large efflux of histamine should be apparent which is certainly not the case in these conditions.

In spite of the success of the toxin in therapy, there are significant imperfections in the scientific and clinical

technology of toxin use. Considerable improvements could be made in the quality of the toxin for human use. The use of preparations of higher specific toxicity than those in current use could decrease the incidence of resistance by the patient and reduce side reactions such as diffusion of the toxin to neighbouring muscles.

15. Expert opinion

Botulinum toxin certainly is a potent regional muscular relaxant with unique capability to cause a 3 - 4 month reversible effect at the level of the neuromuscular junction, with attendant reversible neurogenic muscle fibre atrophy. The effect on pain in both movement disorders and primary pain syndrome (essential headache, temporal mandibular joint syndrome, facial pain syndromes, myofascial pain syndromes, postoperative pain syndromes) has heralded a new era of clinical applications for this technology in which the sensory side of the peripheral nervous system appears to be altered by regional injection. The nature of the sensory effect appears to be generic rather than disease specific and probably relate to autocoid release (histamine and related preformed mediators) which play an intrinsic role in sensory adaptation relating to pain, itching and possibly other forms of sensory experiences.

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