INVITED REVIEW

ABSTRACT: Botulinum toxins are among the most potent neurotoxins known to humans. In the past 25 years, botulinum toxin has emerged as both a potential weapon of bioterrorism and as a powerful therapeutic agent, with growing applications in neurological and non-neurological disease. Botulinum toxin is unique in its ability to target peripheral cholinergic neurons, preventing the release of acetylcholine through the enzymatic cleavage of proteins involved in membrane fusion, without prominent central nervous system effects. There are seven serotypes of the toxin, each with a specific activity at the molecular level. Currently, serotypes A (in two preparations) and B are available for clinical use, and have been shown to be safe and effective for the treatment of dystonia, spasticity, and other disorders in which muscle overactivity gives rise to symptoms. This review focuses on the pharmacology, electrophysiology, immunology, and application of botulinum toxin in selected neurological disorders.

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BOTULINUM TOXINS IN NEUROLOGICAL DISEASE

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Botulinum toxin (BTX) has become a standard therapy for many neurological disorders and is being investigated for many more (Table 1). In this study we focus on the unique aspects of BTX pharmacology and electrophysiology and review those disorders in which it has been used and studied most extensively.

BOTULINUM TOXIN: HISTORY AND PHARMACOLOGY

Botulinum comes from the Latin word "botulus," meaning sausage. Botulism was originally called "sausage poisoning" because it occurred following the ingestion of poorly prepared blood sausages. Justinus Kerner (1786–1862) described the clinical features of botulism in 1820. Kerner went on to collect additional cases and conduct animal experiments using the toxin.[73,74] In 1895, Van Ermengem isolated the bacillus from the remnants of a meal that sickened the members of a musical party, and killed three musicians in Ellezelle, Belgium.[185] The characteristic clinical picture of botulism is a descending flaccid motor paralysis with prominent cranial involvement, including blurred vision, mydriasis, diplopia, ptosis, dysphagia, and dysarthria. Mental functioning remains intact.[9,12] The progressive flaccid paralysis leads to respiratory failure and death in the absence of life-support measures.

Botulinum toxin is produced by the bacteria *Clostridium botulinum*. Other species of *Clostridium* may also produce toxin. There are seven antigenically distinct serotypes of botulinum toxin, A–G. In 1946, BTX A was the first to be crystallized. The other serotypes have been characterized subsequently. The serotypes most associated with human disease are BTX types A, B, and E.[195]

Botulinum toxins are produced as single-chain polypeptides. The molecular weight of purified BTX is approximately 150 kDa. Enzymatic nicking of the polypeptide by bacterial proteases results in the activated di-chain molecule, consisting of a heavy chain and a light chain linked by a disulfide bond. The heavy chain mediates binding to presynaptic cholinergic nerve terminals and internalization of the

Abbreviations: BTX, botulinum toxin; BTX A, botulinum toxin serotype A; BTX B, botulinum toxin serotype B; CD, cervical dystonia; EDP, extensor digitorum brevis; EMG, electromyography; FTA, frontalis type A test; HFS, hemifacial spasm; IPA, immunoprecipitation assay; MPA, mouse protection assay; OO, orbicularis oculi muscle; SCM, sternocleidomastoid muscle

Key words: blepharospasm; botulinum toxin; cervical dystonia; dystonia; spasticity

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Table 1. Disorders that have been treated with botulinum toxin.

Dystonia
Blepharospasm
Oromandibular dystonia
Spasmodic dysphonia
Cervical dystonia
Limb dystonia
Tardive dystonia
Other motor disorders
Hemifacial spasm
Bruxism
Stuttering
Motor tics
Strabismus
Painful legs moving toes
Reflex sympathetic dystrophy
Thoracic outlet syndrome
Tremor
Essential tremor
Dystonic tremor
Head tremor
Voice tremor
Rest tremor
Autonomic disorders
Frey's syndrome
Hyperhydrosis
Sialorrhea
Hyperlacrimation
Pain
Low back pain
Migraine headache
Tension headache
Fibromyalgia
Myofascial pain
Painful muscle spasm
Spasticity
Post-hemiplegic
Cerebral palsy
Multiple sclerosis
Other causes
Non-neurological disorders
Urogenital disorders
Gastrointestinal disorders
Cosmetic uses

toxin into the cell. The light chain is responsible for the toxic effects, acting as a zinc-endopeptidase, cleaving specific proteins responsible for membrane fusion (SNARE complex).[153]

Each serotype of botulinum toxin binds to the serotype-specific acceptor sites on the presynaptic nerve terminal.[15] The specificity of botulinum toxin for cholinergic neurons is due to the high affinity of the acceptor sites on these nerve terminals for the heavy chain. Internalization of the toxin into the cytosol of the neuron is a receptor-mediated endocytotic process also arising from the heavy chain. The light chain is the neurotoxic fragment of BTX and acts as a zinc-dependent endopeptidase,[50] selectively cleaving specific proteins within the cytosol. The proteins, VAMP, SNAP-25, and syntaxin, are part of the SNARE complex proteins that are involved in vesicle fusion.[44] The light chain of each serotype cleaves these specific proteins involved in membrane fusion. BTX A, C, and E cleave SNAP-25 (synaptosomal-associated protein 25), a membrane-associated protein. Serotypes B, D, F, and G cleave vesicular-associated membrane protein (VAMP), also called synaptobrevin. Type C additionally cleaves syntaxin, a membrane protein.[17,191] Each BTX serotype cleaves its targeted protein at a unique amino acid sequence even when acting on the same protein. The enzymatic action of the light chain disrupts the process of membrane fusion within the cell, and prevents the release of acetylcholine into the synaptic cleft. The overall effect of botulinum toxin at the neuromuscular junction is to interrupt neuromuscular transmission and, in effect, denervate muscle.[153] BTX also has activity at other peripheral cholinergic synapses, causing a reduction in salivation and sweating. Once the toxin has been bound to the acceptor sites on the neuron, and the process of translocation into the cytosol begins, the process is not reversible. Antitoxins are therefore not effective in reversing toxin effects once the process has begun, and are only able to neutralize unbound toxin.

Edward Schantz and Alan B. Scott pioneered the use of botulinum toxin as a therapeutic agent in the 1970s.[190] In 1979, Shantz and colleagues at the University of Wisconsin prepared 200 mg of twice-crystallized botulinum toxin serotype A, which was approved for human use in the United States in 1989 for the treatment of strabismus, blepharospasm, and other disorders of the seventh cranial nerve. In December 2000, BTX A and BTX B were both approved by the Food and Drug Administration for use in cervical dystonia. The number of disorders currently treated with botulinum toxin has expanded greatly, now numbering over 50.[125,155]

ELECTROPHYSIOLOGICAL EFFECTS OF BOTULINUM TOXIN

The electrophysiological effects of BTX have been investigated in systemic human botulism. The major effect is a reduction in the amplitude of compound muscle action potentials (CMAPs) following both single and repetitive supramaximal nerve stimulation. There is maximal reduction in CMAP amplitude following repetitive stimulation at 2 Hz, and variable facilitation following brief maximal exercise. The duration of this effect can exceed 3

months.[104,105] The extent of muscle paralysis from BTX is enhanced by proximity of the injection site to the motor endplate,[193,194] and may be increased further by muscle activation with electrical stimulation or exercise immediately following injection.[71] Once injected into the muscle, BTX diffuses from the injection site, with higher doses and volumes of injection increasing toxin spread.[21] BTX is able to penetrate through muscle fascia, but spread is reduced by approximately 20–25%.[193] Taken together, the electrophysiological data suggest that, to obtain a maximal effect of BTX, injection in proximity to the motor endplate is the major determinant. Targeting injection into the muscle belly using a higher concentration of BTX solution reduces diffusion outside the muscle. The electrophysiological effects of BTX are sustained and tend to outlast the clinical benefit.[158] Although muscle atrophy occurs following injection, repeated injection does not cause irreversible change or muscle fibrosis and does not reduce the effect of subsequent injections.[201]

There is documented evidence of systemic spread of botulinum toxin following regional injection into facial or cervical muscles. Single-fiber electromyographic (EMG) studies have shown increased jitter in muscles distant from the injection site.[83,88,89,140,141,159,189] For example, Olney et al. showed that patients given injections into the neck muscles for cervical dystonia had increased jitter and fiber density in the biceps brachii muscle. These abnormalities returned to normal after approximately 3–6 months.[159]

Other evidence of systemic spread of BTX following local injection is the occurrence of subtle changes in cardiovascular reflexes and blood pressure.[89] The systemic effects of localized injection are rarely of clinical importance. However, there have been case reports of generalized weakness with electrophysiological and pathological changes suggestive of clinically significant effects of localized intramuscular BTX injections.[3,9,12,68]

IMMUNOGENICITY AND SECONDARY NONRESPONSE TO BOTULINUM TOXINS

Botulinum toxin is a protein that serves as an antigen when injected into humans. The development of an antibody response to an antigen depends on several factors. These include the presence of an adjuvant (a substance that increases the immune response), the persistence of the antigen in the tissues, and the frequency of exposure to and quantity of the antigen.[55] In addition, genetic factors may increase the susceptibility for that individual to develop antibodies to a particular antigen. Antibody formation in response to botulinum toxin is a desirable event in people with frequent exposure to the toxin and risk of contracting botulism. Hence, in endemic areas and in laboratory personnel, vaccinations using toxoid are administered. However, in patients whose clinical condition responds to botulinum toxin, the formation of neutralizing antibodies results in a clinical resistance to the beneficial effects. The factors that predispose to development of antibodies have not been identified. Large doses of botulinum toxin ($\geq$250 U botulinum toxin A, Botox), larger cumulative doses, and injections administered at less than 3-month intervals ("booster" injections) have been identified as possible risk factors for the development of resistance.[97,121,233] Botulinum toxin resistance occurs primarily in patients receiving higher doses of toxin for the treatment of disorders such as cervical dystonia, and is rare in those with disorders treated with lower doses, such as blepharospasm or hemifacial spasm.

Initially, BTX resistance was considered uncommon. Early reports suggested a frequency of approximately 5% for resistance among cervical dystonia patients receiving repeated injections.[130] This observation was based on retrospective assessments of patients at a single location. However, the clinical trials of botulinum toxin serotypes A and B, published as package inserts for the drugs when FDA approval was obtained, revealed antibody formation to be much more frequent than previously thought, affecting approximately 20% of subjects treated.[165,166] Prospective studies of both BTX A (Botox) and BTX B (Myobloc) are underway to assess the frequency and factors associated with resistance.

Antibodies to botulinum toxin may be detected using various methods.[94] The mouse protection assay (MPA) is the "gold standard" method. This assay evaluates the ability of increasing dilutions of a patient's serum to protect mice from lethal test doses of botulinum toxin. Recently, the mouse hemidiaphragm model with the phrenic nerve intact has also been described. The immunoprecipitation assay (IPA) is a simple, rapid technique for detecting antibodies against botulinum toxin. IPA has been found to be both sensitive and specific.[61,169] Compared to the MPA, the IPA is more sensitive in detecting botulinum toxin antibodies and shows a positive result earlier, suggesting that it may predict future unresponsiveness.[106,107] However, both of these in vitro assays are expensive and neither has been shown to predict future clinical resistance.

There are clinical tests proposed to ascertain whether there is resistance to the effects of toxin.

The FTA (frontalis type A) test, the SCM (sternocleidomastoid) test, and the EDB (extensor digitorum brevis) test have all been explored as sensitive methods to detect clinical resistance (but not antibody titers) in patients reporting the secondary failure of BTX to improve symptoms. The FTA was proposed by Borodic et al. and involves injections of a small amount of botulinum serotype A (20 U) into the frontalis muscle.[26] Two weeks following injection, the presence of paralysis of the frontalis muscle in the area of injection is evaluated by assessing the symmetry of muscle contraction during voluntary forehead wrinkling. If there is asymmetry of forehead wrinkling, with effacement in the area of the injection, the patient is clinically sensitive to the effects of BTX; if there is no effacement of the forehead wrinkles, the patient is clinically resistant to BTX. Others have advocated using the corrugator muscle (frown line) in a similar fashion because it is easier to interpret and less cosmetically apparent if effacement occurs.[107] Single injections into the sternocleidomastoid and extensor digitorum brevis muscle with electrophysiological evaluation for denervation after 2–4 weeks are additional methods for ascertaining resistance to the effects of BTX in the office setting.[14,60,129,200]

Treatment of the BTX-resistant patient is problematic. Depletion of the neutralizing antibodies through plasma exchange or immunosuppression using drugs such as mycophenolate has been suggested.[65,156] Because each serotype of botulinum toxin is immunologically distinct, replacing one serotype with another may be effective,[4,19,39] although the long-term sensitivity of the patient to an alternate serotype of BTX has not been evaluated prospectively.[39,48,100,101,111,186,196,215]

Until more information is available regarding the predictive factors for the development of BTX resistance to any serotype, the generally accepted guidelines for patient treatment include strategies to minimize exposure to BTX and inactivated BTX (Table 2). Because BTX injections are viewed as a long-term treatment for chronic neurological conditions, it is necessary to educate the patient as to the need to limit treatment dose and frequency as much as possible to provide prolonged benefit over the course of repeated injections.

Table 2. Guidelines to reduce development of clinical resistance to BTX.

1. Preparation of BTX to reduce or prevent its degradation:
   (a) Gentle mixing and careful handling of BTX.
   (b) Injection of BTX within 4 hours of removal from freezer or refrigerator.
   (c) Avoidance of heating or shaking of BTX vials or syringes.
2. Administration of BTX injections at the longest interval possible:
   (a) BTX treatments separated by at least 3 months.
   (b) In patients with suboptimal outcome, avoid supplemental injections ("booster" injections)
3. Injection of the minimal dose of BTX required for clinical improvement.

BTX, botulinum toxin.

CERVICAL DYSTONIA

Cervical dystonia (CD), also known as spasmodic torticollis, is defined as a focal dystonia involving the cervical musculature.[76] The clinical manifestations of CD result from asymmetric, involuntary muscle spasms in the neck causing turning, tilting, flexion, or extension movements of the head. These movements may be combined with elevation or anterior shifting of the shoulders. Intermittent spasms may also occur, resembling head tremor. Pain is present in up to 60% of patients, and is sometimes the most disabling feature of CD.[46] Although transient remission of symptoms may occur, typically CD is a lifelong disorder that waxes and wanes in severity.

A variety of oral medications have been used to treat CD, including anticholinergic agents, baclofen, and benzodiazepines.[99,139] Unfortunately, any benefit that results from oral medications is usually obscured by the occurrence of side effects from these agents. Botulinum toxin treatment is the most effective approach to CD, providing relief for up to 85% of patients. This improvement is for head posture, pain, and range of motion.

The initial report of botulinum toxin in 12 patients was single-blinded, using electromyographic guidance and a total maximum dose of 200 U of botulinum toxin serotype A (Oculinum, Smith-Kettlewell, San Francisco, CA). Improvement occurred in 92% of patients, lasting 4–8 weeks, with transient neck weakness reported by 25%.[217] A double-blinded, placebo-controlled crossover study of 21 patients using 100 U confirmed these early results.[218] Subsequent studies showed that from 65% to 90% of CD patients improved with botulinum toxin. Improvement occurred in head posture and CD-related pain.[16,24,32,34,45,85,115,143,206,210,216,219] Benefit was enhanced in patients injected at multiple sites in each muscle.[24,25]

Patients most improved by injections were those with muscle involvement moving the head in one direction (ipsilateral splenius and contralateral sternocleidomastoid).[216] However, in most patients CD manifests as a complex movement, with a combina-

tion of rotation, anterior flexion or extension, lateral flexion, and shifting.[211] A uniform approach to injection in all patients would be ineffective. A study of 30 patients with CD in which the same muscles were injected with a fixed total dose of 150 U did not find objective improvement, which highlights the need to assess each patient individually for dose and muscle involvement.[135]

The duration of benefit from BTX treatment has been assessed both prospectively and retrospectively. A prospective assessment of 37 CD patients treated with Dysport showed continued benefit with repeated injections. The duration of benefit was a mean of 95 days.[173] A retrospective chart review[193,194] from two research centers that included 60 CD patients treated for at least 1 year showed a mean clinical response lasting 15.6 weeks, with a range of 12.2–24.3 weeks. Duration of benefit tended to last the longest in patients experiencing moderate symptoms.[33] Another study in CD patients treated with Dysport for six or more injection series showed that the greatest degree of improvement occurred after the first injection series, although improvement was sustained following subsequent injections. Closer scrutiny of the data shows that this "first treatment" effect could be attributed to patients' unwillingness to allow the benefit from BTX to completely dissipate prior to returning for repeat treatment, thus receiving their next injection while still experiencing residual benefit from the previous injection.[130] Approximately 20% of patients treated at least one time chose not to continue long-term treatment with botulinum toxin. The most common reason for discontinuing BTX was an unsatisfactory response or an absence of effect. The occurrence of adverse events, most frequently dysphagia, was the second most common reason for discontinuation. In addition, 15% reported sustained improvement of symptoms, suggesting a coincident partial or complete remission of CD, although the possibility that botulinum toxin treatment may increase the chances of remission in CD has been proposed.[87]

In a similar survey study, 155 CD patients receiving BTX treatment at a single center were asked to complete a questionnaire related to their decision to continue or discontinue treatment. The response rate was high, with 86.6% of patients returning the survey. Of these, 21.8% discontinued treatment, with the major reasons cited being lack of efficacy, high cost of treatment, or no advantage over oral medications.[31] In this series, adverse events from treatment were not cited as a major reason for treatment cessation.

In addition to the occurrence of primary BTX treatment failure (patients never benefiting from injection), secondary BTX failure may also occur. These patients, although receiving benefit from initial injections, find that they fail to respond to subsequent injections. The prevalence of secondary nonresponse has not been investigated systematically in prospective studies. Retrospective studies suggest that secondary BTX failure affects approximately 10–15% of CD patients. One study found that 9.9% of patients reported a secondary nonresponse,[130] having lost the benefit initially obtained. A second study of 242 patients with adequate follow-up showed that 16% were nonresponders and, of these, 35.7% were found to have antibodies to botulinum toxin by the mouse neutralization assay.[122] Interestingly, the pivotal clinical trials included in the package inserts for commercially available BTX suggest that, even in patients who reported continued benefit with BTX A, 17% were found to have neutralizing antibodies and did not show objective clinical benefit following treatment.[165] The extrapolated occurrence of neutralizing antibody to BTX B was similar, with neutralizing antibodies estimated to occur in approximately 18% of patients after 18 months.[166] These observations are currently being investigated in prospective studies.

In patients who are resistant to BTX serotype A, other serotypes have been used successfully. Serotype F has been shown to improve CD symptoms in patients who are secondary nonresponders. The duration of benefit using serotype F was shorter than serotype A, with clinical benefit lasting only 1 month.[100,101,144,196] Increasing the dose of serotype F was subsequently shown to prolong the duration, but at the price of increased adverse effects.[111] With repeated injections, however, approximately 33% of these patients also developed resistance to serotype F.[48,100] Patients who had never benefited from injections of serotype A (primary nonresponders) did not improve with serotype F.[48,196]

Similar findings of efficacy in patients resistant to BTX serotype A–resistant patients have been found using serotype B.[39,215] A randomized, placebo-controlled study evaluated 77 such patients treated with either 10,000 U of serotype B or placebo. The active drug group showed significant improvement following a single treatment.[39] Whether there is a continued response to repeated injections of BTX serotype B in these BTX serotype A–resistant patients remains unknown.

Electromyography (EMG) has been utilized in several roles in BTX treatment for CD. It has been used to evaluate the pattern of muscle activation, the

effective dose of botulinum toxin in an injected muscle, and the effects of local injections of BTX on muscles remote from the treatment area.[172]

In untreated CD, EMG studies have shown long-duration bursts of activity, with cocontraction of agonist and antagonist muscles and involvement of multiple muscles.[211] Following BTX injection, both spontaneous and voluntary muscle activities are reduced in dystonic muscle.[211] Quantitative EMG has been used to evaluate the effect of varying doses of botulinum toxin in a single dystonic muscle. A single dose of botulinum toxin as Botox or Dysport into a dystonic sternocleidomastoid (SCM) muscle has been assessed in two studies. These investigations demonstrated that doses of 20 U of Botox or 100 U of Dysport markedly reduced muscle activity,[42,62] suggesting that the usual doses administered in clinical practice may be higher than needed. Quantitative EMG has been utilized to evaluate the duration of BTX effect. Reduction in turns per second, mean amplitude, and turns per amplitude ratio were maximal at 6 weeks after injection[41] and gradually returned toward baseline, with turns per second reaching pretreatment values at approximately 30 weeks.[82] In contrast, patients' perceived benefit only lasted from 10 to 16 weeks, suggesting that the electrophysiological measures of BTX effect are of longer duration than its clinical effects. EMG has also been utilized to assess muscle recovery following BTX injection, demonstrating that BTX-induced muscle denervation was transient, without any permanent effects.[75]

The utility of EMG guidance in identifying dystonic muscles in CD and targeting muscles for injection has been tested.[27,53,66,161,162] In a study of 52 consecutive CD patients randomized to injection with or without EMG assistance, and assessed by an examiner blinded to patient grouping, a greater magnitude of improvement and a larger number of patients with a marked improvement were found in the EMG-assisted group at equivalent BTX doses.[53] The role of EMG in conjunction with clinical examination in improving outcome probably relates to the improved ability to identify the muscles involved in the dystonic posture[64,86,161,223] or to the more accurate targeting of injections into overactive muscle.[1,78,204] This may be of particular relevance in injecting deep posterior cervical muscles, including the semispinalis capitis, longissimus capitis, and muscles of the suboccipital triangle. The deep cervical muscles have been shown to be spontaneously overactive in 68% of consecutive CD patients.[161] Clinical assessment of these muscles is problematic without EMG.

Speelman and Brans showed that clinical placement of a needle into dystonic muscle is frequently inaccurate. Without EMG, needle placement by clinical examination was accurate only 83% of the time in the superficial SCM, and only 47% of the time in the more deeply located levator scapulae.[204] Although diffusion of BTX may compensate for injections done outside the muscle belly, there is evidence that BTX diffusion is limited. In animals, the diffusion gradient of a single injection showed a marked attenuation of BTX effect at between 30 and 45 mm from the injection site.[21] By extrapolation, if EMG guidance is used, it may be possible to reduce dystonic activity in a muscle by reducing the role of diffusion and injecting directly into the muscle. This has been demonstrated indirectly in a study showing that, with EMG, the dose of BTX was substantially reduced without loss of clinical benefit.[30] However, more direct controlled studies are needed to substantiate this hypothesis.

The adverse effects of botulinum injection for CD include pain at injection site, neck weakness, a flu-like syndrome, hoarseness, dry mouth, and dysphagia.[130] Dysphagia is the most frequent complication following injection, reported in 25–90% of patients injected with either Botox or Dysport.[16,32,85,98,117,154,205] Women are more likely than men to experience dysphagia, as are patients receiving injections into the sternocleidomastoid muscle.[23,122] Although usually mild and transient, in some patients, the severity may require a change in diet to soft or pureed foods and, rarely, nasogastric feedings.[130] Radiological evaluations of BTX-related dysphagia have shown paralysis of vocal cords and the pharyngeal muscles.[54,132] Although up to 22% of CD patients may have radiological evidence of pharyngeal peristaltic abnormalities prior to treatment, as many as 50% develop new radiological abnormalities following treatment, with 33% developing new symptoms of swallowing difficulty, primarily related to coarse solids.[54]

Other rare complications of botulinum toxin injections for dystonia include cervical radiculopathy,[57] polyradiculoneuritis, and brachial plexopathy.[90,112,188,207] These adverse effects have been attributed to several possible mechanisms: a secondary, immune-mediated effect of BTX injections; a direct effect of BTX as a chemical irritant; or postural changes occurring after injection, leading to mechanical compression.

One of the ongoing issues related to the clinical use of botulinum toxin is the high cost of the injection.[59] The cost varies depending on the severity of CD, the number of vials needed, the number of treatment sessions per year, and the additional cost

of electromyography. The improvement in functional health measures following BTX treatment, including CD-specific disability and quality of life, may offset the expense of treatment.[28,29,35,103,114]

BLEPHAROSPASM AND HEMIFACIAL SPASM

Blepharospasm is a focal dystonia involving the periocular muscles. Clinical manifestations include increased blinking and spasms of involuntary eye closure. Blepharospasm may cause significant disability through interference with vision that may result in functional blindness in severe cases.[76,77,116,147,175] Hemifacial spasm (HFS) is a nondystonic disorder characterized by unilateral twitching of one side of the face, most prominent in the periocular muscles.[79] The movements are usually intermittent, although more sustained tonic spasms may also occur. The pathophysiology of HFS is thought to be compression of the facial nerve as it emerges from the pons by an aberrant or ectopic artery in the posterior fossa. These disorders are discussed together as the techniques for BTX injection are similar.

Prior to the introduction of botulinum toxin injections, the treatment of blepharospasm was rarely successful. In a retrospective study of oral pharmacological agents that included a limited number of blepharospasm patients, benefit occurred in 23–55% of patients.[99] However, other investigators have observed that the benefit of oral medications tend to be transient and are frequently complicated by the systemic side effects.[96,116] HFS may improve with anticonvulsant medications, but benefit is usually transient. Microvascular decompression may be effective, but surgical complications, such as deafness, occur in up to 10% of patients and are often permanent.[187]

The introduction of botulinum toxin as a therapeutic agent was a major milestone in the effective clinical management of blepharospasm and HFS.[119,124,150] Botulinum toxin injections were introduced as a treatment for blepharospasm in the early 1980s[192] and several subsequent reports confirmed its efficacy.[13,20,68,69,72,123,127,128,137,148,149] These studies were largely uncontrolled, observational studies. A small number of controlled studies confirmed the results of open trials.[123] Long-term follow-up of patients receiving multiple treatments showed sustained improvement, with reduction in spasm intensity.[68] BTX injections were initially used for HFS in 1985.[79] Subsequently, BTX became the treatment of choice for this disorder. The technique of injection for HFS is similar to that for blepharospasm except that it is unilateral and often requires a reduced dose. Clinical experience using BTX for treatment of both blepharospasm and HFS has led to modifications in the technique of injection to optimize benefits and minimize adverse effects (Table 3).

Table 3. Optimizing effects of botulinum toxin for treatment of blepharospasm.

1. Initiate treatment with low doses of botulinum toxin.
2. Avoid injections into orbicularis oris above tarsal plate in the midline.
3. Avoid injections into the medial portion of the lower eyelid.
4. Avoid injections into the midportion of the face (zygomaticus major, zygomaticus minor, orbicularis oris, and levator labii muscles).
5. Routinely use natural tears following injection around the eyes.
6. If side effects occur, consider reduction of dose or altering sites of injection.

In order to effectively treat blepharospasm patients with local chemodenervation, the anatomy underlying the spasms has to be understood. The anatomy of the orbicularis oculi (OO) muscle is complex. The OO muscle forms a subcutaneous layer of concentric muscle fibers around the eye. It acts as a sphincter or protractor of the eyelids. The OO arises from three sites: the nasal part of the frontal bone, the frontal process of the maxilla, and the medial palpebral ligament. The OO muscle is divided into three confluent parts: orbital, palpebral, and lacrimal. The palpebral portion is further subdivided into preseptal and pretarsal portions. All parts of the OO are innervated by cranial nerve VII. The action of the orbital part is the forced closure of the eye. The palpebral portion is involved in closing the eyelids without effort. The lacrimal part of the muscle is thought to dilate the lacrimal sac by pulling on the lacrimal fascia, producing a siphon for tears into the duct.[11]

Other muscles that may also be involved in blepharospasm include the corrugator supercilii (which draws the eyebrows medially and inferiorly to produce vertical ridges in the middle of the forehead when frowning), the frontalis (which lifts the eyebrows, producing transverse forehead furrows), and the procerus (which produces transverse wrinkles over the bridge of the nose).[11,170]

Starting with small doses at the initial injection and increasing the dose as needed at successive treatment visits is a conservative way to maximize benefit and limit adverse effects. EMG is seldom used to guide injections into the upper facial muscles as the injections are administered subcutaneously. Injections into the lower facial muscles, where more precise localization of injection is required to avoid nearby muscles of facial expression, may be refined by use of EMG. As a general rule, injections into the

zygomaticus, the levator labii superioris, and levator anguli oris are not recommended because they often lead to asymmetric facial expressions, drooping of the lip, and inability to form a tight seal while eating or drinking.

Side effects from botulinum toxin injection in the periocular muscles include diplopia, ptosis, dry eyes, photophobia, lid entropion, and epiphora. Adverse events occur in approximately 20–25% of treated patients, with ptosis as the most frequent. Other adverse effects include corneal exposure from incomplete eye closure (dry eyes, tearing, redness, and corneal ulcerations).[68,147,148] Diplopia is a rare side effect, usually occurring following injections into the medial aspect of the lower eyelid, with diffusion of toxin through the orbital septum into the inferior oblique muscle.[230] By omitting injections into this area of the lower lid, diplopia is minimized.[80] The anatomy of the eyelid and face, with the proximity of the levator superioris muscle to the orbicularis oculi muscle, makes ptosis one of the most frequent complications, affecting from 10–20% of patients.[69] The OO muscle is approximately 1 mm thick with a loose connective tissue fascial plane below it. This plane allows for easy diffusion of the toxin. The orbital septum lies deep to this fascial plane. This septum resists diffusion of injected liquid but may be attenuated in older patients or punctured, allowing free access to the levator muscle. In order to avoid ptosis, medial and lateral pretarsal injections (avoiding the midline) are now recommended in place of injections into the orbital part of the OO.[2,5,6]

Dry eyes and epiphora are additional common complications following injection. Because the effect of botulinum toxin is to reduce blink and weaken eye closure, the inferior portion of the cornea is exposed and symptoms of dry eyes, including burning, scratchiness, and excessive tearing, may occur. In more extreme cases, superficial punctate keratopathy and corneal ulcerations also occur. Most clinicians recommend that patients receiving injections should be placed on artificial tears. In symptomatic cases, an evaluation by an ophthalmologist is suggested.

Periocular ecchymosis is an acute complication of injection, relating to the procedure itself that is minimized by using a fine-gauge needle (30 gauge) and applying light pressure to the injection sites. Entropion and ectropion result from laxity of the lower eyelid support, in particular the lateral canthal tendon.[171] Following injection of BTX into the OO, there is a reduction of compound muscle action potentials and motor evoked potentials in the lower facial muscles.[71,88] If pronounced, local diffusion into these muscles produces facial weakness and asymmetry.

Secondary resistance to BTX following long-term treatment for blepharospasm is rare, likely because the doses typically used for the treatment of blepharospasm are low compared to those used for other dystonic disorders. Although reported anecdotally,[63] it seems that resistance is rare even with repeated injections over many years.

Following injections of BTX into the OO muscle, serial EMG examinations of that muscle have shown marked denervation changes, with increased jitter and blocking appearing at 1 week. These changes are associated with clinical improvement. After a mean of 116 days, these denervation changes persisted but at a reduced level, despite a return to baseline clinical status.[18] In a study of muscle pathology following repeated injections, changes in the OO muscle were minimal, with few specimens showing any evidence of fibrosis, indicating that the effects of botulinum toxin injections, although beneficial for months following each injection, did not result in irreversible muscle atrophy or degenerative changes if administered long term.[22] The absence of normalization of blink reflexes indicates that improvement following injection related to the peripheral effects of the toxin and not to alterations in central mechanisms.[88,222]

LIMB DISORDERS: DYSTONIA AND SPASTICITY

Although BTX has not yet been approved in the United States for use in limb disorders, it has been used to treat a variety of conditions, including dystonia, essential tremor, and conditions associated with excessive muscle contraction such as spasticity related to stroke, multiple sclerosis, and cerebral palsy. Taken together, studies on the use of BTX in dystonia, tremor, and spasticity over the past 15 years have demonstrated that BTX injections are efficacious in relieving the spasms, unwanted movements, abnormal postures, and associated pain in some patients with these disorders.[52,118,127,174,176,178,181,198,221,226,228,229] Furthermore, the clinical effects of BTX A and B are localized and reversible, without the central nervous system effects of systemically administered oral pharmacological agents.[159]

The development of different antigenic strains,[101,144] including BTX-A and BTX-B,[142] allows for a greater scope and flexibility in treating dystonia, tremor, and spasticity, even in seronegative patients nonresponsive to BTX A.[100] In the future, high doses of the shorter-acting botulinum

toxin F, although not a good choice for long-term management, may be useful prior to a surgical procedure such as tendon release in patients with dystonia or cerebral palsy, to temporarily relax muscle tension in the surgical site during the immediate postoperative period.

There are no absolute contraindications to using BTX in limb conditions except for the presence of an infection at the proposed injection site or in individuals with a known hypersensitivity to any ingredient in the formulation. However, in patients who are pregnant or breast-feeding[182,220] or those with significant peripheral nerve or muscle disease, particularly disorders of the neuromuscular junction such as Eaton–Lambert syndrome or myasthenia gravis,[155] injections of BTX should be avoided unless the patient is informed of potential complications and the benefit of treatment is considered to outweigh the risk. Similarly, patients on antibiotics, anesthetics or other medications that may affect neuromuscular transmission[7] should be treated cautiously.

Limb Dystonia. The early clinical descriptions of writer's cramp from the late 19th century characterized the altered voluntary activity, stiffness, and pain found in this disorder.[95,203] As originally defined by Oppenheim in the early 20th century,[160] dystonia refers to sustained writhing and contorting movements. Dystonic movements in the limbs, however, may be rapid[77] and resemble tremor or chorea. In some patients, distinguishing dystonia from other movement disorders may be difficult and require additional diagnostic tests, including electrophysiological,, biochemical, or genetic evaluations.[36–38,163]

Focal limb dystonia affects one body area and typically presents as task-specific muscle spasms or "occupational cramps" in which learned or repetitive motor tasks (such as writing or playing a musical instrument) trigger muscle spasms and interfere with practiced motor execution whereas other actions remain normal. Writer's cramp is the most common form of idiopathic limb dystonia,[51,157,197] in which involuntary muscle activity and abnormal postures affect the arms and hands. One classification scheme defines simple writer's cramp as muscle spasms only with writing, and dystonic writer's cramp as spasms with writing as well as other actions.[197] Simple cramps may progress to sustained dystonic cramps.

Patients with writer's cramp typically experience involuntary dystonic spasms that result in altered writing speed and script quality. Dystonic spasms, loss of movement speed, and decreased fluency when performing learned motor skills may occur in musicians, dentists, golfers, and other individuals in whom skilled work activity involves frequent, highly controlled, repetitive movements.

Dystonic movements may be suppressed (or triggered) by sensory input such as postural change, tactile stimuli, and compensatory movements.[102] Studies have shown that the involuntary muscle spasms may be due, at least in part, to abnormal sensory processing of spindle afferent information or changes of recurrent inhibition.[126,133,200,209,229] This may explain the mechanism of sensory "tricks." In addition, alterations in sensory input following BTX injections may also account for the observation that, in some patients, the effect of BTX treatment outlasts the weakness it creates. An additional feature sometimes seen in focal brachial dystonia is the presence of mirror movements. This phenomenon is observed when the patient uses the contralateral asymptomatic limb and triggers the dystonic spasms in the affected limb.[77] The clinical utility of this feature is in identifying the primary dystonic posture of the involved limb without the compensatory muscle activation that can occur during activity of that limb, thus allowing a more accurate selection of muscles for BTX injection.

The role of trauma as a triggering factor for dystonia remains an unresolved issue. Local trauma to a limb may be temporally related to the onset of dystonia in that area. Head trauma may also precede the development of dystonia in the limbs.[56,92,186,208,212,214] Whether the association of trauma to dystonia is causal has not been established.

The only clinical abnormalities on examination in primary dystonia or occupational cramps are the dystonic movements and postures themselves. No other localizing or lateralizing signs should be found. The presence of such abnormalities suggests a secondary cause for the dystonic symptoms[43] or an additional pathological process. In some cases, a radiculopathy or peripheral neuropathy may mimic focal or segmental dystonia.[81] In other cases, the abnormal posture of the dystonic condition may predispose the patient to develop root or peripheral nerve damage. For example, ulnar neuropathy has been associated with focal, occupational dystonia in musicians.[47,183]

EMG may be helpful in corroborating the diagnosis. Nerve conduction studies, short- and long-loop reflexes, and analyses of motor units are normal.[146,184] Ballistic movements, normally triphasic in pattern with alternating agonist–antagonist bursts, may show disrupted patterns with cocontraction of

agonist and antagonist muscles and excessively long EMG bursts in dystonia.[51] However, not all patients have involvement of the same muscles during dystonic movements nor do they demonstrate the same abnormal firing patterns.[51]

Although BTX treatment for many patients with limb dystonia is often the most effective,[120] other approaches include physical and occupational therapy, exercises, and writing aids.[179] In newly diagnosed patients or those with extensive muscle involvement, particularly complicated cases of task-specific dystonia, a trial of oral pharmacological agents, such as anticholinergic drugs, muscle relaxants, or levodopa, may be tried. Although some patients report benefits, the success of these agents is limited and adverse effects are common. In these patients, supplemental use of BTX injections into the most overactive muscles may provide additional relief.

Before injecting BTX for limb dystonia, it is important that the specific goals and limitations of this treatment are established. Involuntary muscle action needs to be distinguished from compensatory activity in order to prevent misdirected injections into nondystonic muscles that activate to reduce the dystonic symptoms. Compensatory activity is defined as voluntary or semivoluntary motor behavior that lessens the effect of the pathological spasms. As an example, in extensor writer's cramp, compensatory activation of the fingers, with hyperflexion of the thumb and index finger (gripping the pen too tightly), may resemble the flexor variant of hand dystonia. To distinguish primary from compensatory involvement of muscle in this example, patients are instructed to write holding the pen with digits 3 and 4 or 4 and 5, allowing the thumb and index finger to move freely. If there is no flexion of the thumb and index finger during this maneuver, it is likely that the flexor actions of these digits are compensatory to overcome the extensor dystonic spasms, and the appropriate extensor muscles are then injected.[177] This approach can be used when defining the role of other sets of cocontracting antagonists. It may require several series of injections before the role of each muscle group is determined.

The assessment of outcome following injection for limb dystonia is primarily focused on the patient's perception of functional improvement, the degree of weakness in the injected muscles, and the absence of BTX effect on nearby muscles. Individual muscle strength should be evaluated at each visit using the Medical Research Council scale[151] in order assess changes that occur following BTX treatment. Monopolar needle EMG can also be performed following BTX treatment to assess for spontaneous activity (positive sharp waves, fibrillation potentials), motor unit forms, and recruitment patterns at minimal and maximal contraction efforts.

If treatment with BTX injection does not result in the expected improvement, reevaluation of the patient is crucial at a 4–6-week follow-up visit when the BTX effect should be maximal.[178] There are several reasons for treatment failure that can be elucidated at this follow-up visit. First, if the muscles injected have not weakened and show no signs of denervation on EMG, the patient may have an immunological resistance to the botulinum toxin serotype used, or the dose of BTX injected may have been inadequate. If an inadequate BTX dose is suspected, reinjection of a larger dose of BTX into the same muscles after a 3-month interval may be successful. A second reason for lack of benefit is the injection of uninvolved, compensatory muscles, or failure to inject an involved muscle. Reassessment of the patient for the pattern of dystonic action and reinjection into appropriate muscles at the next treatment series will likely improve the outcome. When an optimal response is obtained, follow-up injections are usually given at 3–4-month intervals.

A useful manual for locating injection sites for specific muscles from skin landmarks and by joint maneuvers is available.[58] Of additional value is a study depicting three-dimensional renderings of forearm muscles made from actual magnetic resonance image cross-sections of a normal subject.[84] In larger forearm muscles, such as the flexor digitorum superficialis or extensor digitorum communis, individual digital fascicles can be located only by EMG guidance to provide accurate control over specific finger joints involved in the dystonic spasms.

The adverse effects of BTX treatment for limb dystonia and occupational cramps include local ecchymoses and, rarely, a flu-like syndrome with malaise and low-grade fever lasting a few days.[231] Muscle weakness is the direct result of treatment and invariably develops in all injected muscles.[52] However, diffusion of BTX away from the injection site into uninjected muscle may cause unintended loss of strength in the involved region, and impair the patient's ability to perform tasks previously unaffected by dystonia. This is most troublesome in patients requiring the highest degree of motor control, such as musicians, surgeons, or dentists. Excessive weakness due to BTX[176,213] often occurs in finger extension (particularly digit 3 at the metacarpophalangeal joint) after wrist extensors are injected.[178] Usually, when excessive weakness occurs, it tends to be short-lived, lasting only a few weeks, whereas the effects of

BTX in the dystonic muscle may last up to 3–4 months.

BTX treatment for limb dystonia affecting upper and lower extremities has shown variable efficacy, improving symptoms in 35–85% of patients, with most studies showing benefit in 60–70%. Pain is the most frequent symptom showing improvement, regardless of whether motor function responds, and independent of underlying disease.[178]

In most limb conditions, BTX effect is related to muscle size, with the larger muscles requiring higher doses. Forearm flexor muscles require more BTX than forearm extensor muscles. Proximal arm and shoulder muscles require larger doses than hand muscles, and foot and leg muscles require higher doses than either the hand or arm. Specific muscle fascicles, for example, fascicles for digits 3 and 4 in the flexor digitorum sublimis, reveal more susceptibility to BTX than fascicles for digits 2 and 5. In larger forearm, arm, and leg muscles, multiple injection sites are often used, allowing for better distribution of BTX and reducing pain associated with injection by reducing the volume of fluid injected.[178] For leg dystonia, one of the major reasons for reduced efficacy is limitation of the BTX dose, for safety reasons, to no more than 400 U (Botox). Although lower doses are effective in small muscles, the larger proximal arm and proximal and distal leg muscles typically require larger doses and cannot safely be completely treated with the currently available commercial preparations.

Spasticity. Spasticity is characterized by velocity-dependent increases in muscle tone and deep tendon reflexes, and is part of the upper motor neuron syndrome.[138] Spasticity typically presents with impaired movement, weakness, painful muscle spasms, and stiffness. Functional impairments include difficulty dressing and problems with hygiene and other activities of daily living. There can also be disabling limb and trunk postures, decreased range of motion of the joints, and excoriation of the palm due to digit hyperflexion. Severe or long-standing spasticity may lead to contractures and joint ankylosis.

BTX is beneficial for most types of spasticity, although higher doses are usually required than for other limb conditions such as dystonia.[67,113,198,199] As with dystonia, the effect of BTX may be greater in the arms than legs as a result of the lower doses needed to treat the smaller muscles of the arms. In contrast to dystonia, spasticity involving the leg may respond better to BTX treatment.[136,178,202] Although the reduction in tone by BTX treatment may reveal underlying weakness of the leg, or cause additional weakness, in most cases this is not found to occur. It has been hypothesized that the effect of BTX on muscle fiber length may decrease or interrupt the cycle of spasticity as one mechanism of its beneficial effects.[198] It is also postulated that reducing antagonist muscle overactivity in spasticity may uncover functional residual power due to a possible differential effect on extrafusal and intrafusal fibers.[110] Younger patients with leg spasticity may improve more than older patients following BTX treatment because of the smaller muscle size and the use of adjunctive therapies such as physical rehabilitation.[136] Additional investigations are needed to determine the optimal BTX dosing to provide maximal benefit without increasing the potential risk of BTX antibody formation in patients of all ages with spasticity.

The fundamental approach to treating limb spasticity with BTX is very similar to the treatment of limb dystonia, with the key elements being identification of the most involved muscles and the use of the smallest effective doses. Outcome measurements following BTX treatment for spasticity can be problematic. Although reduction in muscle tone using the Ashworth scale and improvement in joint mobility using goniometry is frequently achieved, whether these objective improvements translate to improvement in functional ability has been more difficult to establish for spasticity.[131,180]

TREMOR

Tremor is the most common movement disorder and is characterized by rhythmic oscillations of part of the body around one or more joints.[70,145] Tremor can be associated with numerous underlying causes and is classified by its clinical characteristics into four broad categories: rest tremor, postural tremor, action tremor, and task-specific tremor. BTX injections for the treatment of tremors is not as successful as for dystonia and spasticity, although some patients have moderate to marked benefit.[8,40,91,108,109,134,152,164,167,178,213,225,227] The proportion of tremor patients reported to improve ranges from 35.7% for the rest tremor of Parkinson's disease to 50.6% for cerebellar tremors.[178] In some patients, tremor may even worsen following BTX injection,[177] despite the fact that the effect of BTX on muscles is the same regardless of the underlying disorder.[178]

BTX treatment of kinetic disorders has shown mixed results in restoring meaningful function.[118,213,227] In essential tremor, the amplitude of action and postural tremor may be significantly reduced

without improvement in functional ability. This has been attributed to the weakness in the hand and arm induced by BTX, despite improvement in the tremor.[213] One large study in essential tremor patients found that BTX A was effective at reducing postural components for up to 4 months, but had a limited effect on kinetic tremor and variable improvements in quantitative measurements.[40] The tremors that have been most successfully treated with BTX are voice tremor and head tremor.[49,109,168,224,227] In essential voice tremor, BTX injected into the thyroarytenoid muscles has been shown to reduce tremor amplitude and laryngeal resistance, and also to ease vocal strain when speaking.[224]

PAIN AND HEADACHE

Muscle spasms due to dystonia, spasticity, or other causes are frequently associated with pain. BTX treatment has been found to relieve painful spasms in 82.7% of patients.[178] Several mechanisms have been hypothesized to underlie musculoskeletal pain, including the extremes of posture, excessive tendon and joint tension, and direct muscle fiber injury. BTX therapy has been shown to be useful in alleviating pain in many of the focal dystonias, particularly in torticollis, where up to 88% of patients have reported improvement in pain.[218] BTX treatment for CD may improve pain to a greater degree than it improves the dystonic posture. Similarly, studies of limb dystonia and spasticity have shown that BTX can alleviate pain in 75–100% of cases.[10,93,174,181,198] and, as in CD, pain relief can occur in spasticity even when there is minimal improvement in motor function.

The application of BTX to headache is promising but remains to be established. The current evidence is largely anecdotal and somewhat conflicting.

The mechanism of analgesic action of BTX is unknown. It has been related to still poorly understood effects on sensory nociceptive systems, particularly substance P, or attributed to a reduction in muscle tone rather than a direct analgesic effect.

REFERENCES

1. Ajax T, Ross MA, Rodnitzky RL. The role of electromyography in guiding botulinum toxin injections for focal dystonia and spasticity. J Neurol Rehabil 1998;12:1–4.
2. Albanese A, Bentivoglio AR, Colosimo C, Galardi G, Maderna L, Tonali P. Pretarsal injections of botulinum toxin improve blepharospasm in previously unresponsive patients. J Neurol Neurosurg Psychiatry 1996;60:693–694.
3. Ansved T, Odergren T, Borg K. Muscle fiber atrophy in leg muscles after botulinum toxin type A treatment of cervical dystonia. Neurology 1997;48:1440–1442.
4. Aoki KR. Pharmacology and immunology of botulinum toxin serotypes. J Neurol 2001;248(suppl 1):3–10.
5. Aramideh M, Ongerboer de Visser BW, Brans JW, Koelman JH, Speelman JD. Pretarsal application of botulinum toxin for treatment of blepharospasm. J Neurol Neurosurg Psychiatry 1995;59:309–311.
6. Aramideh M, Ongerboer de Visser BW, Koelman JHTM, Bour LJ, Devriese PP, Speelman JD. Clinical and electromyographic features of levator palpebrae superioris muscle dysfunction in involuntary eyelid closure. Mov Disord 1994; 9:395–402.
7. Argov Z, Mastaglia FL. Disorders of neuromuscular transmission caused by drugs. N Engl J Med 1979;301:409–413.
8. Bakar M, Zarifoglu M, Bora I, Turan F, Sen C, Ogul E. Treatment of hereditary trembling chin with botulinum toxin. Mov Disord 1998;13:845–846.
9. Bakheit AM, Ward CD, McLellan DL. Generalised botulism-like syndrome after intramuscular injections of botulinum toxin type A: a report of two cases. J Neurol Neurosurg Psychiatry 1997;62:198.
10. Barwood S, Baillieu C, Boyd R, Brereton K, Low J, Nattrass G, Graham HK. Analgesic effects of botulinum toxin A: a randomized, placebo-controlled clinical trial. Dev Med Child Neurol 2000;42:116–121.
11. Berkovitz BKB, Moxham BJ. A textbook of head and neck anatomy. London: Wolfe; 1988. p 85–128.
12. Bhatia KP, Munchau A, Thompson PD, Houser M, Chauhan VS, Hutchinson M, Shapira AH, Marsden CD. Generalised muscular weakness after botulinum toxin injections for dystonia: a report of three cases. J Neurol Neurosurg Psychiatry 1999;67:90–93.
13. Biglan AW, May M, Bowers RA. Management of facial spasm with *Clostridium botulinum* toxin, type A (Oculinum). Arch Otolaryngol Head Neck Surg 1988;114:1407–1412.
14. Birklein F, Erbguth F. Sudomotor testing discriminates between subjects with and without antibodies against botulinum toxin A: a preliminary observation. Mov Disord 2000; 15:146–149.
15. Black JD, Dolly O. Interaction of 125I-labeled botulinum neurotoxins with nerve terminals. I. Ultrastructural autoradiographic localization and quantitation of distinct membrane acceptors for types A and B on motor nerves. J Cell Biol 1986;103:521–534.
16. Blackie JD, Lees AJ. BTX treatment in spasmodic torticollis. J Neurol Neurosurg Psychiatry 1990;53:640–643.
17. Blasi J, Chapman ER, Yamasaki S, Binz T, Niemann H, Jahn R. Botulinum neurotoxin C1 blocks neurotransmitter release by means of cleaving HPC-1/syntaxin. EMBO J 1993; 12:4821–4828.
18. Bogucki A. Serial SFEMG studies of orbicularis oculi muscle after the first administration of botulinum toxin. Eur J Neurol 1999;6:461–467.
19. Borodic G, Johnson E, Goodnough M, Schantz E. Botulinum toxin therapy, immunologic resistance, and problems with available materials. Neurology 1996;46:26–29.
20. Borodic GE, Cozzolino D. Blepharospasm and its treatment, with emphasis on the use of botulinum toxin. Plast Reconstr Surg 1989;83:546–554.
21. Borodic GE, Ferrante R, Pearce LB, Smith K. Histologic assessment of dose-related diffusion and muscle fiber response after therapeutic botulinum A toxin injections. Mov Disord 1994;9:31–39.
22. Borodic GE, Ferrante R. Effects of repeated botulinum toxin injections on orbicularis oculi muscle. J Clin Neuroophthalmol 1992;12:121–127.
23. Borodic GE, Joseph M, Fay L, Cozzolino D, Ferrante RJ. Botulinum A toxin for the treatment of spasmodic torticollis: dysphagia and regional toxin spread. Head Neck 1990;12: 392–398.
24. Borodic GE, Mills L, Joseph M. Botulinum A toxin for the treatment of adult-onset spasmodic torticollis. Plast Reconstr Surg 1991;87:285–289.

25. Borodic GE, Pearce B, Smith K, Joseph M. Botulinum A toxin for spasmodic torticollis: multiple vs single injection points per muscle. Head Neck 1992;14:33–37.
26. Borodic GE, Duane D, Pearce B, Johnson E. Antibodies to botulinum toxin. Neurology 1995;45:204.
27. Brans JW, de Boer IP, Aramideh M, Ongerboer de Visser BW, Speelman JD. Botulinum toxin in cervical dystonia: low dosage with electromyographic guidance. J Neurol 1995;242: 529–534.
28. Brans JW, Lindeboom R, Aramideh M, Speelman JD. Long-term effect of botulinum toxin on impairment and functional health in cervical dystonia. Neurology 1998;50:1461–1463.
29. Brans JW, Lindeboom R, Snoek JW, et al. Botulinum toxin versus trihexyphenidyl in cervical dystonia: a prospective, randomized, double-blind controlled trial. Neurology 1996; 46:1066–1072.
30. Brans JWM, de Boer IP, Aramideh M, Ongerboer de Visser BW, Speelman JD. Botulinum toxin in cervical dystonia: low dosage with electromyographic guidance. J Neurol 1995;242: 529–534.
31. Brashear A, Bergan K, Wojcieszek J, Siemers ER, Ambrosius W. Patients' perception of stopping or continuing treatment of cervical dystonia with botulinum toxin type A. Mov Disord 2000;15:150–153.
32. Brashear A, Lew MF, Dykstra DD, Comella CL, Factor SA, Rodnitzky RL, Trosch R, Singer C, Brin MF, Murray JJ, Wallace JD, Willmer-Hulme A, Koller M. Safety and efficacy of NeuroBloc (botulinum toxin type B) in type A–responsive cervical dystonia. Neurology 1999;53:1439–1446.
33. Brashear A, Watts MW, Marchetti A, Magar R, Lau H, Wang L. Duration of effect of botulinum toxin type A in adult patients with cervical dystonia: a retrospective chart review. Clin Ther 2000;22:1516–1524.
34. Brashear A. The botulinum toxins in the treatment of cervical dystonia. Semin Neurol 2001;21:85–90.
35. Brefel-Courbon C, Simonetta-Moreau M, More C, Rascol O, Clanet M, Montastruc JL, Lapeyre-Mestre M. A pharmacoeconomic evaluation of botulinum toxin in the treatment of spasmodic torticollis. Clin Neuropharmacol 2000;23:203–207.
36. Bressman SB, Fahn S, Ozelius LJ, Kramer PL, Risch NJ. The DYT1 mutation and nonfamilial primary torsion dystonia. Arch Neurol 2001;58:681–682.
37. Bressman SB, Greene PE. Dystonia. Curr Treat Options Neurol 2000;2:275–285.
38. Bressman SB, Sabatti C, Raymond D, de Leon D, Klein C, Kramer PL, Brin MF, Fahn S, Breakefield X, Ozelius LJ, Risch NJ. The DYT1 phenotype and guidelines for diagnostic testing. Neurology 2000;54:1746–1752.
39. Brin MF, Lew MF, Adler CH, Comella CL, Factor SA, Jankovic J, O'Brien C, Murray JJ, Wallace JD, Willmer-Hulme A, Koller M. Safety and efficacy of NeuroBloc (botulinum toxin type B) in type A-resistant cervical dystonia. Neurology 1999; 53:1431–1438.
40. Brin MF, Lyons KE, Doucette J, Adler CH, Caviness JN, Comella CL, Dubinsky RM, Friedman JH, Manyam BV, Matsumoto JY, Pullman SL, Rajput AH, Sethi KD, Tanner C, Koller WC. A randomized, double masked, controlled trial of botulinum toxin type A in essential hand tremor. Neurology 2001;56:1523–1528.
41. Buchman AS, Comella CL, Stebbins GT, Tanner CM, Goetz CG. Quantitative electromyographic analysis of changes in muscle activity following botulinum toxin therapy for cervical dystonia. Clin Neuropharmacol 1993;16:205–210.
42. Buchman AS, Comella CL, Stebbins GT, Weinstein SL. Determining a dose–effect curve for botulinum toxin in the sternocleidomastoid muscle in cervical dystonia. Clin Neuropharmacol 1994;17:188–195.
43. Calne DB, Lang AE. Secondary dystonia. In: Fahn S, Marsden CD, Calne DB, editors. Dystonia 2. Advances in neurology, vol 50. New York: Raven Press; 1988. p 9–33.
44. Catsicas S, Grenningloh G, Pich EM. Nerve-terminal proteins: to fuse to learn. Trends Neurosci 1994;17:368–373.
45. Ceballos-Baumann AO. Evidence-based medicine in BTX therapy for cervical dystonia. J Neurol 2001;248(suppl 1):I-14–I-20.
46. Chan J, Brin MF, Fahn S. Idiopathic cervical dystonia:clinical characteristics. Mov Disord 1991;6:119–126.
47. Charness ME, Ross MH, Shefner JM. Ulnar neuropathy and dystonia flexion of the fourth and fifth digits: clinical correlation in musicians. Muscle Nerve 1996;19:431–437.
48. Chen R, Karp BI, Hallett M. Botulinum toxin type F for treatment of dystonia: long-term experience. Neurology 1998;51:1494–1496.
49. Cho J, Chu K, Jeon BS. Case of essential palatal tremor: atypical features and remarkable benefit from botulinum toxin injection. Mov Disord 2001;16:779–782.
50. Coffield J, Considine RV, Simpson LL. The site and mechanism of action of botulinum neurotoxin. In: Jankovic J, Hallett M, editors. Therapy with botulinum toxin. New York: Marcel Dekker; 1994. p 3–13.
51. Cohen LG, Hallett M. Hand cramps: clinical features and electromyographic patterns in a focal dystonia. Neurology 1988;38:1005–1012.
52. Cohen LG, Hallett M, Geller BD, Hochberg F. Treatment of focal dystonias of the hand with botulinum toxin injections. J Neurol Neurosurg Psychiatry 1989;52:355–363.
53. Comella CL, Buchman AS, Tanner CM, Brown-Toms NC, Goetz CG. Botulinum toxin injection for spasmodic torticollis: increased magnitude of benefit with electromyographic assistance. Neurology 1992;42:878–882.
54. Comella CL, Tanner CM, DeFoor-Hill L, Smith C. Dysphagia following botulinum toxin injections for spasmodic torticollis. Neurology 1992;42:1307–1310.
55. Critchfield J. Immunogenecity of botulinum toxin therapy. CNS Spectrums 2000;7(Suppl 6):51–58.
56. Dauer WT, Burke RE, Greene P, Fahn S. Current concepts on the clinical features, etiology and management of idiopathic cervical dystonia. Brain 1998;121:547–560.
57. Defazio G, Lepore V, Melpignano C, Lamberti P, Livrea P, Ferrari E. Cervical radiculopathy following botulinum toxin therapy for cervical dystonia. Funct Neurol 1993;8:193–196.
58. Delagi EF, Perotto A, Iazetti J, Morrison D. Anatomic guide for the electromyographer, 2nd ed. Springfield, IL: Charles C. Thomas; 1981.
59. Dodel RC, Kirchner A, Koehne-Volland R, Kunig G, Ceballos-Baumann A, Naumann M, Brashear A, Richter HP, Szucs TD, Oertel WH. Costs of treating dystonias and hemifacial spasm with botulinum toxin A. Pharmacoeconomics 1997; 12:695–706.
60. Dressler D, Bigalke H, Rothwell JC. The sternocleidomastoid test: an in vivo assay to investigate botulinum toxin antibody formation in humans. J Neurol 2000;247:630–632.
61. Dressler D, Dirnberger G. Botulinum toxin antibody testing: comparison between the immunoprecipitation assay and the mouse diaphragm assay. Eur Neurol 2001;45:257–260.
62. Dressler D, Rothwell JC. Electromyographic quantification of the paralysing effect of botulinum toxin in the sternocleidomastoid muscle. Eur Neurol 2000;43:13–16.
63. Dressler D. Complete secondary botulinum toxin therapy failure in blepharospasm. J Neurol 2000;247:809–810.
64. Dressler D. Electromyographic evaluation of cervical dystonia for planning of botulinum toxin therapy. Eur J Neurol 2000;7:713–718.
65. Duane DD, Monroe J, Morris RE. Mycophenolate in the prevention of recurrent neutralizing botulinum toxin A antibodies in cervical dystonia. Mov Disord 2000;15:365–366.
66. Dubinsky RM, Gray CS, Vetere-Overfield B, Koller WC. Electromyographic guidance of botulinum toxin treatment in cervical dystonia. Clin Neuropharmacol 1991;14:262–267.
67. Dunne JW, Heye N, Dunne SL. Treatment of chronic limb spasticity with botulinum toxin A. J Neurol Neurosurg Psychiatry 1995;58:232–235.

68. Dutton JJ, Buckley EG. Long-term results and complications of botulinum A toxin in the treatment of blepharospasm. Ophthalmology 1988;95:1529–1534.
69. Dutton JJ. Botulinum-A toxin in the treatment of craniocervical muscle spasms: short- and long-term, local and systemic effects. Surv Ophthalmol 1996;41:51–65.
70. Elbe RJ, Koller WC. In: Tremor. Baltimore: Johns Hopkins University Press; 1990. p 143–157.
71. Eleopra R, Tugnoli V, Caniatti L, De Grandis D. Botulinum toxin treatment in the facial muscles of humans: evidence of an action in untreated near muscles by peripheral local diffusion. Neurology 1996;46:1158–1160.
72. Elston JS. Botulinum toxin for blepharospasm. In: Jankovic J, Hallett M, editors. Therapy with botulinum toxin. New York: Marcel Dekker; 1994. p 191–197.
73. Erbguth FJ, Naumann M. Historical aspects of botulinum toxin: Justinus Kerner (1786–1862) and the "sausage poison." Neurology 1999;53:1850–1853.
74. Erbguth FJ. Historical note on the therapeutic use of botulinum toxin in neurological disorders. J Neurol Neurosurg Psychiatry 1996;60:151.
75. Erdal J, Ostergaard L, Fuglsang-Frederiksen A, Werdelin L, Dalager T, Sjo O, Regeur L. Long-term botulinum toxin treatment of cervical dystonia—EMG changes in injected and non-injected muscles. Clin Neurophysiol 1999;110:1650–1654.
76. Fahn S, Bressman SB, Marsden CD. Classification of dystonia. In: Fahn S, Marsden CD, DeLong MR, editors. Advances in neurology, Vol 78. New York: Lippincott-Raven; 1998. p 1–10.
77. Fahn S. Concept and classification of dystonia. In: Fahn S, Marsden CD, Calne DB, editors. Advances in neurology, Vol 50. New York: Raven Press, 1988. p 1–8.
78. Finsterer J, Fuchs I, Mamoli B. Quantitative electromyography-guided botulinum toxin treatment of cervical dystonia. Clin Neuropharmacol 1997;20:42–48.
79. Flanders M, Chin D, Boghen D. Botulinum toxin: preferred treatment for hemifacial spasm. Eur Neurol 1993;33:316–319.
80. Frueh BR, Nelson CC, Kapustiak JF, Musch DC. The effect of omitting botulinum toxin from the lower eyelid in blepharospasm treatment. Am J Ophthalmol 1988;106:45–47.
81. Fry H, Hallett M. Focal dystonia (occupational cramp) masquerading as nerve entrapment or hysteria. Plast Reconstr Surg 1988;82:908–910.
82. Fuglsang-Frederiksen A, Ostergaard L, Sjo O, Werdelin L, Winkel H. Quantitative electromyographical changes in cervical dystonia after treatment with botulinum toxin. Electromyogr Clin Neurophysiol 1998;38:75–79.
83. Garner CG, Straube A, Witt TN, Gasser T, Oertel WH. Time course of distant effects of local injections of botulinum toxin. Mov Disord 1993;8:33–37.
84. Geenen C, Consky E, Ashby P. Localizing muscles for botulinum toxin treatment of focal hand dystonia. Can J Neurol Sci 1996;23:194–197.
85. Gelb DJ, Lowenstein DH, Aminoff MJ. Controlled trial of botulinum toxin injections in the treatment of spasmodic torticollis. Neurology 1989;39:80–84.
86. Gelb DJ, Yoshimura DM, Olney RK, Lowenstein DH, Aminoff MJ. Change in pattern of muscle activity following botulinum toxin injections for torticollis. Ann Neurol 1991;29:370–376.
87. Giladi N, Meer J, Kidan H, Honigman S. Long-term remission of idiopathic cervical dystonia after treatment with botulinum toxin. Eur Neurol 2000;44:144–146.
88. Girlanda P, Quartarone A, Sinicropi S, Nicolosi C, Messina C. Unilateral injection of botulinum toxin in blepharospasm: single fiber electromyography and blink reflex study. Mov Disord 1996;11:27–31.
89. Girlanda P, Vita G, Nicolosi C, Milone S, Messina C. Botulinum toxin therapy: distant effects on neuromuscular transmission and autonomic nervous system. J Neurol Neurosurg Psychiatry 1992;55:844–845.
90. Glanzman RL, Gelb DJ, Drury I, Bromberg MB, Truong DD. Brachial plexopathy after botulinum toxin injections. Neurology 1990;40:1143.
91. Goetz CG, Horn SS. Treatment of tremor and dystonia. Neurol Clin 2001;19:129–144.
92. Goldman S, Ahlskog JE. Posttraumatic cervical dystonia. Mayo Clin Proc 1993;68:443–448.
93. Gooch JL, Sandell TV. Botulinum toxin for spasticity and athetosis in children with cerebral palsy. Arch Phys Med Rehabil 1996;77:508–511.
94. Goschel H, Wohlfarth K, Frevert J, Dengler R, Bigalke H. Botulinum A toxin therapy: neutralizing and nonneutralizing antibodies: therapeutic consequences. Exp Neurol 1997;147:96–102.
95. Gowers WR. A manual of diseases of the nervous system. Philadelphia: P. Bakiston, Son & Co.; 1888. p 23–42.
96. Grandas F, Elston J, Quinn N, Marsden CD. Blepharospasm, a review of 264 patients. J Neurol Neurosurg Psychiatry 1988;51:767–772.
97. Greene P, Fahn S, Diamond B. Development of resistance to botulinum toxin type A in patients with torticollis. Mov Disord 1994;9:213–217.
98. Greene P, Kang U, Fahn S, Brin M, Moskowitz C, Flaster E. Double-blind, placebo-controlled trial of botulinum toxin injections for the treatment of spasmodic torticollis. Neurology 1990;40:1213–1218.
99. Greene P, Shale H, Fahn S. Experience with high dosages of anticholinergic and other drugs in the treatment of torsion dystonia. Adv Neurol 1988;50:547–556.
100. Greene PE, Fahn S. Response to botulinum toxin F in seronegative botulinum toxin A–resistant patients. Mov Disord. 1996;11:181–184.
101. Greene PE, Fahn S. Use of botulinum toxin type F injections to treat torticollis in patients with immunity to botulinum toxin type A. Mov Disord 1993;8:479–483.
102. Greene PE, Bressman S. Exteroceptive and interoceptive stimuli in dystonia. Mov Disord 1998;13:549–551.
103. Gudex CM, Hawthorne MR, Butler AG, Duffey P. Effect of dystonia and botulinum toxin treatment on health-related quality of life. Mov Disord 1998;13:941–946.
104. Gutmann L, Pratt L. Pathophysiologic aspects of human botulism. Arch Neurol 1976;33:175–179.
105. Hamjian JA, Walker FO. Serial neurophysiological studies of intramuscular botulinum-A toxin in humans. Muscle Nerve 1994;17:1385–1392.
106. Hanna PA, Jankovic J, Vincent A. Comparison of mouse bioassay and immunoprecipitation assay for botulinum toxin antibodies. J Neurol Neurosurg Psychiatry 1999;66:612–616.
107. Hanna PA, Jankovic J. Mouse bioassay versus Western blot assay for botulinum toxin antibodies. Neurology 1998;50:1624–1629.
108. Henderson JM, Ghika JA, Van Melle G, Haller E, Einstein R. Botulinum toxin A in non-dystonic tremors. Eur Neurol 1996;36:29–35.
109. Hertegard S, Granqvist S, Lindestad PA. Botulinum toxin injections for essential voice tremor. Ann Otol Rhinol Laryngol 2000;109:204–209.
110. Hesse S, Reiter F, Konrad M, Jahnke MT. Botulinum toxin type A and short-term electrical stimulation in the treatment of upper limb flexor spasticity after stroke: a randomized, double-blind, placebo-controlled trial. Clin Rehabil 1998;12:381–388.
111. Houser MK, Sheean GL, Lees AJ. Further studies using higher doses of botulinum toxin type F for torticollis resistant to botulinum toxin type A. J Neurol Neurosurg Psychiatry 1998;64:577–580.
112. Huag BA, Dresslet D, Prange HW. Polyradicloneuritis following botulinum toxin therapy. J Neurol 1990;237:62–63.
113. Hyman N, Barnes M, Bhakta B, Cozens A, Bakheit M, Kreczy-Kleedorfer B, Poewe W, Wissel J, Bain P, Glickman S, Sayer A, Richardson A, Dott C. Botulinum toxin (Dysport) treatment of hip adductor spasticity in multiple sclerosis: a pro-

spective, randomized, double blind, placebo controlled, dose ranging study. J Neurol Neurosurg Psychiatry 2000;68: 707–712.
114. Jahanshahi M, Marsden. Psychological functioning before and after treatment of torticollis with botulinum toxin. J Neurol Neurosurg Psychiatry 1991;229–231.
115. Jankovic J, Brin MF. Therapeutic uses of botulinum toxin. N Engl J Med 1991;324:1186–1194.
116. Jankovic J, Orman J. Blepharospasm: demographic and clinical survey of 250 patients. Ann Ophthalmol 1984;16:371–376.
117. Jankovic J, Schwartz K. Botulinum toxin injections for cervical dystonia. Neurology 1990;40:277–280.
118. Jankovic J, Schwartz K. Botulinum toxin treatment of tremors. Neurology 1991;41:1185–1188.
119. Jankovic J, Schwartz K, Donovan DT. Botulinum toxin treatment of cranial–cervical dystonia, spasmodic dysphonia, other focal dystonias and hemifacial spasms. J Neurol Neurosurg Psychiatry 1990;53:633–639.
120. Jankovic J, Schwartz KS. Use of botulinum toxin in the treatment of hand dystonia. J Hand Surg 1993;18:883–887.
121. Jankovic J, Schwartz K. Response and immunoresistance to botulinum toxin injections. Neurology 1995;45:1743–1746.
122. Jankovic J, Schwartz KS. Clinical correlates of response to botulinum toxin injections. Arch Neurol 1991;48:1253–1256.
123. Jankovic J. Blepharospasm and oromandibular–laryngeal–cervical dystonia: a controlled trial of botulinum A toxin therapy. In: Fahn S, Marsden CD, Calne DB, editors. Dystonia 2. Advances in neurology, Vol 50. New York: Raven Press; 1988. p 583–591.
124. Jankovic J. Botulinum A toxin in the treatment of blepharospasm. In: Jankovic J, Tolosa E, editors. Advances in neurology: facial dyskinesias, Vol 49. New York: Raven Press; 1988. p 467–472.
125. Jost WH, Kohl A. Botulinum toxin: evidence-based medicine criteria in rare conditions. J Neurol 2000;248(Suppl 1):I39–I44.
126. Kaji R, Rothwell JC, Katayama M, Tomoko I, Kubori T, Kohara N, Mezaki T, Shibasaki H, Kimura J. Tonic vibration reflex and muscle afferent block in writer's cramp. Ann Neurol 1995;38:155–162.
127. Karp BI, Cole RA, Cohen LG, Grill S, Lou JS, Hallett M. Long-term botulinum toxin treatment of focal hand dystonia. Neurology 1994;44:70–76.
128. Kennedy RH, Bartley GB, Flanagan JC, Waller RR. Treatment of blepharospasm with botulinum toxin. Mayo Clin Proc 1989;64:1085–1090.
129. Kessler KR, Benecke R. The EBD test—a clinical test for the detection of antibodies to botulinum toxin type A. Mov Disord 1997;12:95–99.
130. Kessler KR, Skutta M, Benecke R. Long-term treatment of cervical dystonia with botulinum toxin A: efficacy, safety, and antibody frequency. German Dystonia Study Group. J Neurol 1999;246:265–274.
131. Kirazli Y, On AY, Kismali B, Aksit R. Comparison of phenol block and botulinus toxin type A in the treatment of spastic foot after stroke: a randomized, double-blind trial. Am J Phys Med Rehabil 1998;77:510–515.
132. Koay CE, Allun-Jones T. Pharyngeal paralysis due to botulinum toxin injection. J Laryngol Otol 1989;103:698–699.
133. Koelman JHTM, Willemse RB, Bour LJ, Hilgevoord AAJ, Speelman JD, Ongerboer de Visser BW. Soleus H-reflex tests in dystonia. Mov Disord 1995;10:44–50.
134. Koller WC, Hristova A, Brin M. Pharmacologic treatment of essential tremor. Neurology 2000;54:S30–S38.
135. Koller W, Vetere-Overfield B, Gray C, Dubinsky R. Failure of fixed-dose, fixed muscle injection of botulinum toxin in torticolllis. Clin Neuropharmacol 1990;13:355–358.
136. Koman LA, Mooney JF, Smith B, Goodman A, Mulvaney T. Management of cerebral palsy with botulinum-A toxin: preliminary investigation. J Pediatr Orthopaed 1993;13:489–495.
137. Kraft SP, Lang AE. Botulinum toxin injections in the treatment of blepharospasm, hemifacial spasm, and eyelid fasciculations. Can J Neurol Sci 1988;15:276–280.
138. Lance JW. What is spasticity? Lancet 1990;335:606.
139. Lang AE, Sheehy MP, Marsden CD. Anticholinergics in adult-onset focal dystonia. Can J Neurol Sci 1982;9:313–319.
140. Lange DJ, Brin MF, Fahn S, Lovelace RE. Distant effects of locally injected botulinum toxin: incidence and course. In: Fahn S, Marsden CD, Calne DB, editors. Dystonia 2. Advances in neurology, Vol 50. New York: Raven Press; 1988. p 609–613.
141. Lange DJ, Rubin M, Greene PE, Kang UJ, Moskowitz CB, Brin MF, Lovelace RE, Fahn S. Distant effects of locally injected botulinum toxin: a double-blind study of single fiber EMG changes. Muscle Nerve 1991;14:672–675.
142. Lew MF, Adornato BT, Duane DD, Dykstra DD, Factor SA, Massey JM, Brin MF, Jankovic J, Rodnitzky RL, Singer C, Swenson MR, Tarsy D, Murray JJ, Koller M, Wallace JD. Botulinum toxin type B: a double-blind, placebo-controlled, safety and efficacy study in cervical dystonia. Neurology 1997;49:701–707.
143. Lorentz IT, Subramaniam SS, Yiannikas C. Treatment of idiopathic spasmodic torticollis with botulinum-A toxin: a pilot study of 19 patients. Med J Austral 1990;152:528–530.
144. Ludlow CL, Hallett M, Rhew K, Cole R, Shimizu T, Sakaguchi G, Bagley JA, Schulz GM, Yin SG, Koda J. Therapeutic use of type F botulinum toxin. N Engl J Med 1992;326:349–350.
145. Marsden CD. Origins of normal and pathological tremor. In: Findley LJ, Capildeo R, editors. Movement disorders: tremor. New York: Oxford University Press; 1984. p 37–84.
146. Marsden CD, Rothwell JC. The physiology of idiopathic dystonia. Can J Neurol Sci 1987;14:521–527.
147. Mauriello JA, Aljian J. Natural history of treatment of facial dyskinesias with botulinum toxin: a study of 50 consecutive patients over seven years. Br J Ophthalmol 1991;75:737–739.
148. Mauriello JA, Coniaris H. Use of botulinum in the treatment of 100 patients with blepharospasm. N J Med 1987;84:43–44.
149. Mauriello JA. Blepharospasm, Meige syndrome, and hemifacial spasm: treatment with botulinum toxin. Neurology 1985;35:1499–1500.
150. Maurri S, Brogelli S, Alfieri G, Barontini F. Use of botulinum toxin in Meige's disease. Riv Neurol 1988;58:245–248.
151. Medical Research Council. Aids to the examination of the peripheral nervous system. London: HMSO; 1976.
152. Metz L. Multiple sclerosis: symptomatic therapies. Semin Neurol 1998;18:389–395.
153. Montecucco C, Schiavo G, Rossetto O. The mechanism of action of tetanus and botulinum neurotoxins. Arch Toxicol 1996;18(suppl):342–354.
154. Moore AP, Blumhardt LD. A double blind trial of botulinum toxin "A" in torticollis, with one year follow up. J Neurol Neurosurg Psychiatry 1991;54:813–816.
155. National Institutes of Health Consensus Development Conference: Clinical use of botulinum toxin. Arch Neurol 1991; 48:1294–1298.
156. Naumann M, Toyka KV, Mansouri Taleghani B, Ahmadpour J, Reiners K, Bigalke H. Depletion of neutralising antibodies resensitizes a secondary non-responder to botulinum A neurotoxin. J Neurol Neurosurg Psychiatry 1998;65:924–927.
157. Nutt JG, Muenter MD, Melton LJ, Aronson A, Kurland LT. Epidemiology of dystonia in Rochester, Minnesota. In: Fahn S, Marsden CD, Calne DB, editors. Dystonia 2. Advances in neurology, Vol 50. New York: Raven Press; 1988. p 361–365.
158. Odergren T, Hjaltason H, Kaakkola S, Solders G, Hanko J, Fehling C, Marttila RJ, Lundh H, Gedin S, Westergren I, Richardson A, Dott C, Cohen H. A double blind randomized parallel group study to investigate the dose equivalence of Dysport and Botox in the treatment of cervical dystonia. J Neurol Neurosurg Psychiatry 1998;64:6–12.

159. Olney RK, Aminoff MJ, Gelb DJ, Lowenstein DH. Neuromuscular effects distant from the site of botulinum neurotoxin injection. Neurology 1988;38:1780–1783.
160. Oppenheim H. Uber eine eigenartige Krampfkrankheit des kindlichen und jungendichen Alters (dysbasia lordotica progressiva, dystonia musculorum deformans). Neurol Centralb 1911;30:1090–1107.
161. Ostergaard L, Fuglsang-Frederiksen A, Sjo O, Werdelin L, Winkel H. Quantitative EMG in cervical dystonia. Electromyogr Clin Neurophysiol 1996;36:179–185.
162. Ostergaard L, Fuglsang-Frederiksen A, Werdelin L, Sjo O, Winkel H. Quantitative EMG in botulinum toxin treatment of cervical dystonia. A double-blind, placebo-controlled study. Electroencephalogr Clin Neurophysiol 1994;93:434–439.
163. Ozelius LJ, Page CE, Klein C, Hewett JW, Mineta M, Leung J, Shalish C, Bressman SB, de Leon D, Brin MF, Fahn S, Corey DP, Breakefield XO. The TOR1A (DYT1) gene family and its role in early onset torsion dystonia. Genomics 1999; 62:377–384.
164. Pacchetti C, Mancini F, Bulgheroni M, Zangaglia R, Cristina S, Sandrini G, Nappi G. Botulinum toxin treatment for functional disability induced by essential tremor. Neurol Sci 2000;21:349–353.
165. Package insert, Botox. Allergan Pharmaceuticals, December 2000.
166. Package insert, Myobloc. Elan Pharmaceuticals, December 2000.
167. Pahwa R, Busenbark K, Swanson-Hyland EF, Dubinsky RM, Hubble JP, Gray C, Koller WC. Botulinum toxin treatment of essential head tremor. Neurology 1995;45:822–824.
168. Pal PK, Samii A, Schulzer M, Mak E, Tsui JK. Head tremor in cervical dystonia. Can J Neurol Sci 2000;27:137–142.
169. Palace J, Nairne A, Hyman N, Doherty TV, Vincent A. A radioimmuno-precipitation assay for antibodies to botulinum A. Neurology 1998;50:1463–1466.
170. Patrinely JR, Anderson RL. Anatomy of the orbicularis oculi and other facial muscles. Adv Neurol 1988;49:15–23.
171. Patrinely JR, Whiting AS, Anderson RL. Local side effects of botulinum toxin injections. In: Fahn S, Marsden CD, Calne DB, editors. Advances in neurology, Vol 50. New York: Raven Press; 1988. p 493–500.
172. Poewe W, Deuschl G, Nebe A, Feifel E, Wissel J, Benecke R, Kessler KR, Ceballos-Baumann AO, Ohly A, Oertel W, Kunig G and the German dystonia study group. What is the optimal dose of botulinum toxin A in the treatment of cervical dystonia? Results of a double blind, placebo controlled, dose ranging study using Dysport. J Neurol Neurosurg Psychiatry 1998;64:13–17.
173. Poewe W, Schelosky L, Kleedorfer B, Heinen F, Wagner M, Deuschl G. Treatment of spasmodic torticollis with local injections of botulinum toxin. One-year follow-up in 37 patients. J Neurol 1992;239:21–25.
174. Poungvarin N. Writer's cramp: the experience with botulinum toxin injections in 25 patients. J Med Assoc Thai 1991; 74:239–247.
175. Price J, Farish S, Taylor H, O'Day J. Blepharospasm and hemifacial spasm. Opthalmology 1997;104:865–868.
176. Pullman SL. Limb dystonia: use of botulinum toxin. In: Jankovic J, Hallett M, editors. Therapeutic use of botulinum toxin. New York: Marcel Dekker; 1994. p 307–321.
177. Pullman SL, Elibol B, Fahn S. Modulation of parkinsonian tremor by radial nerve palsy. Neurology 1994;44:1861–1864.
178. Pullman SL, Greene P, Fahn S, Pedersen SF. Approach to the treatment of limb disorders with botulinum toxin A. Experience with 187 patients. Arch Neurol 1996;53:617–624.
179. Ranawaya R, Lang A. Usefulness of a writing device in writer's cramp. Neurology 1991;41:1136–1138.
180. Richardson D, Edwards S, Sheean GL, Greenwood RJ, Thompson AJ. The effect of botulinum toxin on hand function after incomplete spinal cord injury at the level of C5/6: a case report. Clin Rehabil 1997;11:288–292.
181. Rivest J, Lees AJ, Marsden CD. Writer's cramp: treatment with botulinum toxin injections. Mov Disord 1991;6:55–59.
182. Robin L, Herman D, Redett R. Botulism in a pregnant women. N Engl J Med 1996;335:823–824.
183. Ross MH, Charness ME, Lee D, Logigian EL. Does ulnar neuropathy predispose to focal dystonia? Muscle Nerve 1995;18:606–611.
184. Rothwell JC, Obeso JA, Day BL, Marsden CD. Pathophysiology of dystonias. In: Desmedt JE, editor. Advances in neurology: motor control mechanisms in health and disease, vol 39. New York: Raven Press; 1983. p 851–863.
185. Sakaguchi G. *Clostridium botulinum* toxins. Pharm Ther 1983; 19:165–194.
186. Sankhla C, Lai EC, Jankovic J. Peripherally induced oromandibular dystonia. J Neurol Neurosurg Psychiatry 1998;65: 722–728.
187. Samii M, Günther T, Iaconetta G, Muehling M, Vorkapic P, Samii A. Microvascular decompression to treat hemifacial spasm: long-term results for a consecutive series of 143 patients. Neurosurgery 2002;50:712–718.
188. Sampaio C, Castro-Caldas, Sales-Luis ML, Alves M, Pinto L, Apolinario P. Brachial plexopathy after botulinum toxin administration for cervical dystonia. J Neurol Neurosurg Psychiatry 1993;56:220–221.
189. Sanders DB, Massey EW, Buckley EG. Botulinum toxin for blepharospasm: single-fiber EMG studies. Neurology 1986; 36:545–547.
190. Schantz EJ, Johnson EA. Botulinum toxin: the story of its development for the treatment of human disease. Perspect Biol Med 1997;40:317–327.
191. Schiavo G, Rossetto O, Catsicas S, Polverino de Laureto P, DasGupta BR, Benfenati F, Montecucco C. Identification of the nerve terminal targets of botulinum neurotoxin serotypes A, D, and E. J Biol Chem 1993;268:23784–23787.
192. Scott AB, Kennedy RA, Stubbs HA. Botulinum toxin injection as a treatment for blepharospasm. Arch Ophthamol 1985;103:347–350.
193. Shaari CM, George E, Wu BL, Biller HF, Sanders I. Quantifying the spread of botulinum toxin through muscle fascia. Laryngoscope 1991;101:960–964.
194. Shaari CM, Sanders I. Quantifying how location and dose of botulinum toxin injections affect muscle paralysis. Muscle Nerve 1993;16:964–969.
195. Shapiro RL, Hatheway C, Swerdlow DL. Botulism in the United States: a clinical and epidemiologic review. Ann Intern Med 1998;129:221–228.
196. Sheean GL, Lees AJ. Botulinum toxin F in the treatment of torticollis clinically resistant to botulinum toxin A. J Neurol Neurosurg Psychiatry 1995;59:601–607.
197. Sheehy MP, Marsden CD. Writers' cramp—a focal dystonia. Brain 1982;105:461–480.
198. Simpson DM. Treatment of spasticity with botulinum toxin. Muscle Nerve 2000;23:447–449.
199. Simpson DM, Alexander DN, O'Brien CF, Tagliati M, Aswad AS, Leon JM, Gibson J, Mordaunt JM, Monaghan EP. Botulinum toxin type A in the treatment of upper extremity spasticity: a randomized, double-blind, placebo-controlled trial. Neurology 1996;46:1306–1310.
200. Sloop RR, Cole BA, Escutin RO. Human response to botulinum toxin injection: type B compared with type A. Neurology 1997;49:189–194.
201. Sloop RR, Cole D, Patel MC. Muscle paralysis produced by botulinum toxin type A injection in treated torticollis patients compared with toxin naive individuals. Mov Disord 2001;16:100–105.
202. Snow BJ, Tsui JKC, Bhatt MH, Varelas M, Hashimoto SA, Calne DB. Treatment of spasticity with botulinum toxin: a double blind study. Ann Neurol 1990;28:512–515.
203. Solly S. Scrivener's palsy, or the paralysis of writers. Lancet 1864;ii:709–711.

204. Speelman JD, Brans JW. Cervical dystonia and botulinum treatment: is electromyographic guidance necessary? Mov Disord 1995;10:802.
205. Stell R, Thompson PD, Marsden CD. BTX in spasmodic torticollis. J Neurol Neurosurg Psychiatry 1988;51:920–923.
206. Tarsy D, First ER. Painful cervical dystonia: clinical features and response to treatment with botulinum toxin. Mov Disord 1999;14:1043–1045.
207. Tarsy D. Brachial plexus neuropathy after botulinum toxin injection. Neurology 1997;49:1176–1177.
208. Tarsy D, Sudarsky L, Charness ME. Limb dystonia following electrical injury. Mov Disord 1994;9:230–232.
209. Tempel L, Perlmutter J. Abnormal vibration-induced cerebral blood flow responses in idiopathic dystonia. Brain 1990; 113:691–707.
210. Therapuetics and Technology Assessment Subcommittee of the American Academy of Neurology. Assessment: the clinical usefulness of botulinum toxin-A in treating neurologic disorders. Neurology 1990;40:1332–1336.
211. Thompson PD, Stell R, Maccabe JJ, Day BL, Rothwell JC, Marsden CD. Electromyography of neck muscles in the treatment of spasmodic torticollis. Motor Disturb 1990;2:289–304.
212. Thyagarajan D, Kompoliti K, Ford B. Post-traumatic shoulder 'dystonia': persistent abnormal postures of the shoulder after minor trauma. Neurology 1998;51:1205–1207.
213. Trosch RM, Pullman SL. Botulinum toxin A injections for the treatment of hand tremors. Mov Disord 1994;9:601–609.
214. Truong DD, Dubinsky R, Hermanowicz N, Olson WL, Silverman B, Koller WC. Posttraumatic torticollis. Arch Neurol 1991;48:221–223.
215. Truong DD, Cullis PA, O'Brien CF, Koller M, Villegas TP, Wallace JD. BotB (botulinum toxin type B): evaluation of safety and tolerability in botulinum toxin type A–resistant cervical dystonia patients (preliminary study). Mov Disord 1997;12:772–775.
216. Tsui JK, Calne DB. Botulinum toxin in cervical dystonia. In: Jankovic J, Tolosa E, editors. Advances in neurology: facial dyskinesias, Vol 49. New York: Raven Press; 1988. p 473–478.
217. Tsui JK, Eisen A, Mak E, Carruthers J, Scott A, Calne DB. A pilot study on the use of botulinum toxin in spasmodic torticollis. Can J Neurol Sci 1985;12:314–316.
218. Tsui JK, Eisen A, Stoessl AJ, Calne S, Clane DR. Double-blind study of botulinum toxin in spasmodic torticollis. Lancet 1986;ii:245–246.
219. Tsui JKC, Fross RD, Calne S, Calne D. Local treatment of spasmodic torticollis with botulinum toxin. Can J Neurol Sci 1987;14:533–535.
220. Tsui JK. Botulinum toxin as a therapeutic agent. Pharmacol Ther 1996;72:13–24.
221. Tsui JKC, Bhatt M, Calne S, Calne DB. Botulinum toxin in the treatment of writer's cramp: a double blind study. Neurology 1993;43:183–185.
222. Valls-Sole J, Tolosa ES, Ribera G. Neurophysiological observations on the effects of botulinum toxin treatment in patients with dystonic blepharospasm. J Neurol Neurosurg Psychiatry 1991;54:310–313.
223. Van Gerpen JA, Matsumoto JY, Ahlskog JE, Maraganore DM, McManis PG. Utility of an EMG mapping study in treating cervical dystonia. Muscle Nerve 2000;23:1752–1756.
224. Warrick P, Dromey C, Irish J, Durkin L. The treatment of essential voice tremor with botulinum toxin A: a longitudinal case report. J Voice 2000;14:410–421.
225. Warrick P, Dromey C, Irish JC, Durkin L, Pakiam A, Lang A. Botulinum toxin for essential tremor of the voice with multiple anatomical sites of tremor: a crossover design study of unilateral versus bilateral injection. Laryngoscope 2000;110: 1366–1374.
226. Wissel J, Kabus C, Wenzel R, Klepsch S, Schwarz U, Nebe A, Schelosky L, Scholz U, Poewe W. Botulinum toxin in writer's cramp: objective response evaluation in 31 patients. J Neurol Neurosurg Psychiatry 1996;61:172–175.
227. Wissel J, Masuhr F, Schelosky L, Ebersbach G, Poewe W. Quantitative assessment of botulinum toxin treatment in 43 patients with head tremor. Mov Disord 1997;12:722–726.
228. Wissel J, Muller J, Dressnandt J, Heinen F, Naumann M, Topka H, Poewe W. Management of spasticity associated pain with botulinum toxin A. J Pain Sympt Manag 2000;20: 44–49.
229. Wohlfarth K, Schubert M, Rothe B, Elek J, Dengler R. Remote F-wave changes after local botulinum toxin application. Clin Neurophysiol 2001;112:636–640.
230. Wutthiphan S, Kowal L, O'Day J, Jones S, Price J. Diplopia following subcutaneous injections of botulinum A toxin for facial spasms. J Pediatr Ophthalmol Strabismus 1997;34:229–234.
231. Yoshimura DM, Aminoff MJ, Olney RK. Botulinum toxin therapy for limb dystonias. Neurology 1992;42:627–630.
232. Yoshimura DM, Aminoff MJ, Olney RK. Botulinum toxin therapy for limb dystonias. Neurology 1992;42:627–630.
233. Zuber M, Sebald M, Bathien N, de Recondo J, Rondot P. Botulinum antibodies in dystonic patients treated with type A botulinum toxin: frequency and significance. Neurology 1993;43:1.