Toxicology 306 (2013) 124–146

Contents lists available at SciVerse ScienceDirect

Toxicology

journal homepage: www.elsevier.com/locate/toxicol

Review

Botulinum toxins: Mechanisms of action, antinociception and clinical applications

Anthony Wheeler[a,1], Howard S. Smith[b,*]

[a] The Neurological Institute, 2219 East 7th Street, Charlotte, NC 28204, United States
[b] Albany Medical College, Department of Anesthesiology, 47 New Scotland Avenue, MC-131, Albany, NY 12208, United States

ARTICLE INFO

Article history:
Received 12 December 2012
Received in revised form 7 February 2013
Accepted 10 February 2013
Available online 19 February 2013

Keywords:
Botulinum neurotoxin
Chronic pain
Muscle spasm
Spasticity
Movement disorders
Dystonia

ABSTRACT

Botulinum toxin (BoNT) is a potent neurotoxin that is produced by the gram-positive, spore-forming, anaerobic bacterium, Clostridum botulinum. There are 7 known immunologically distinct serotypes of BoNT: types A, B, C1, D, E, F, and G. Clostridum neurotoxins are produced as a single inactive polypeptide chain of 150 kDa, which is cleaved by tissue proteinases into an active di-chain molecule: a heavy chain (H) of ~100 kDa and a light chain (L) of ~50 kDa held together by a single disulfide bond. Each serotype demonstrates its own varied mechanisms of action and duration of effect. The heavy chain of each BoNT serotype binds to its specific neuronal ecto-acceptor, whereby, membrane translocation and endocytosis by intracellular synaptic vesicles occurs. The light chain acts to cleave SNAP-25, which inhibits synaptic exocytosis, and therefore, disables neural transmission. The action of BoNT to block the release of acetylcholine botulinum toxin at the neuromuscular junction is best understood, however, most experts acknowledge that this effect alone appears inadequate to explain the entirety of the neurotoxin's apparent analgesic activity. Consequently, scientific and clinical evidence has emerged that suggests multiple antinociceptive mechanisms for botulinum toxins in a variety of painful disorders, including: chronic musculoskeletal, neurological, pelvic, perineal, osteoarticular, and some headache conditions.

Contents

Abbreviations: BoNT, botulinum neurotoxin; BoNT/A, botulinum neurotoxin type A; USFDA, United States Food and Drug Administration; NSF, Soluble N-ethylmaleimide-sensitive factor; NSF, Soluble N-ethylmaleimide-sensitive factor; SNARE complex, adaptor protein receptor complex; Sbr, Synaptobrevin; EHD1, EH (Eps15 homology; SM, (Sec1/Munc18-like) protein; BSA, bovine serum albumin; DAS, digit abduction score; MNA, mouse neutralization assay; MPA, mouse protection assay; MPS, myofascial pain syndrome; ODQ, the Oswestry Disability Questionnaire; TTH, tension-type headache; CDH, chronic daily headache; EM, episodic migraine; PBO, placebo.

* Corresponding author. Tel.: +1 518 262 4461; fax: +1 518 262 2671.
E-mail addresses: ponwheeler@yahoo.com (A. Wheeler), smithh@mail.amc.edu (H.S. Smith).
[1] Tel.: +1 704 372 3714; fax: +1 704 333 4601.

0300-483X/$ – see front matter © 2013 Elsevier Ireland Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.tox.2013.02.006

1. Introduction

Since its introduction in the late 1970s for strabismus, blepharospasm, and the focal dystonias, botulinum toxin type A (NoBTX-A) has been used increasingly in the treatment of numerous other disorders largely characterized by excessive or inappropriate muscle contraction (Table 1) (Brin, 1997, 1998; Jankovic and Hallett, 1994; Jankovic and Brin, 1997). These disorders include each form of focal dystonia; spasticity; inappropriate contraction in most of the body's sphincters, such as those associated with achalasia, anal spasm, and vaginismus; eye movement disorders including nystagmus; other hyperkinetic disorders including tics and tremors; (Brin, 2000; Jankovic and Brin, 1997) autonomic disorders such as hyperhidrosis; (Heckmann et al., 2001; Naumann and Lowe, 2001; Naumann et al., 2002) and cosmetically troublesome hyperfunctional facial lines (glabellar lines, crow's feet, forehead lines) (Brin, 2000; Blitzer et al., 1993; Binder et al., 1998a; Carruthers and Carruthers, 2001a,b). In addition, BoNT/A has been reported to be useful in the treatment of more commonly occuring pain syndromes, including myofascial pain syndrome, migraine and tension headaches (Brin et al., 2002).

Table 1
Therapeutic uses for botulinum toxin.

Focal dystonias-sustained muscular activity producing abnormal and functional disability.
• Blepharospasm[a]
• Cervical (torticollis, anterocollis, laterocollis)[a]
• Laryngeal (spasmodic dysphonia)
• Oromandibular (opening or closing of the mouth/jaw)
• Orolingual (mouth and tongue involved)
• Limb (occupational or task-driven, parkinsonism)
• Tremor (due to dystonia)
Nondystonic disorders of involuntary muscle contraction and movement
• Hemifacial spasm[a]
• Tremor (essential, parkinsonism)
• Myokymia and synkinesis
• Tics
• Myoclonus
• Benign fasiculations
• Bruxism
Disorders of conjugate eye movement (strabismus[a], nystagmus, oscillopsia)
Spasticity (due to stroke, cerebral palsy, multiple sclerosis, brain or spinal cord injury)[a]
Cosmetic disorders (hyperhidrosis[a], undesirable wrinkles caused by hyperkinetic muscles[a], e.g., face, anterior neck)
Disorders of localized muscle spasm
• Sphincter "spasms"
o
Bladder (detrusor-sphincter dyssynergia)
o
Gastrointestinal (achalasia, anismus, cricopharyngeal, lower esophagus, rectal)
• Skeletal muscle (myofascial pain, lumbar paraspinal muscle spasm, post-operative spasms after prostatectomy or hemorrhoidectomy)
Pain Disorders
• Headaches (1°-chronic migraine[a] & tension-type; 2°-TMJD, dystonia)
• Shoulder pain following stoke (caused by spasticity[a])
• Osteoarthritis of large joints
• Pelvic pain (vestibulodynia, pelvic floor muscle spasm, interstitial cystitis)
• Neck pain after dissection surgery/radiotherapy for cancer
• Neuropathic pain (post-herpetic neuralgia, spinal radiculopathy)

Conditions for which botulinum toxin has been shown to have proven or promising experimental results (modified from Wheeler, 1997).
[a] FDA approved indications.

In addition, BoNT/A has been reported to be useful in the treatment of more commonly occuring pain syndromes, including myofascial pain syndrome, migraine and tension headaches (Brin et al., 2002).

BoNT/A injections have several advantages over primary drug and surgical therapies in the management of intractable disease. Systemic pharmacologic effects are rare for botulinum toxin type A; permanent destruction of tissue does not occur. Graded degrees of therapeutic effect can be achieved by varying the dose injected and most adverse effects are transient. If the patient has a strong response to therapy and too much muscle weakness occurs, strength gradually returns. The patient's acceptance is high, and in most cases, botulinum toxin therapy is preferred to alternative pharmacotherapy, although drug therapy can be added as needed.

In the discussion that follows, we will refer to the botulinum toxins by serotype (NoBT/A, NoBT/B, etc.) and, when relevant, trade names. NoBT/A is available in 3 different biological formulations. Under the trade name BOTOX® (approved in 1989, U.S.; prior to 1992 marketed as Oculinum®), NoBT/A is manufactured in the United States by Allergan, Inc. It is licensed worldwide, and the product and its precursors have been successfully utilized in clinical trials since the 1970s. In the United States, BOTOX® is approved for treatment of strabismus, blepharospasm, hyperkinetic facial lines, cervical dystonia and chronic migraine. The United States Food and Drug Administration (USFDA) dictates standards of production, buffering, stability, potency and vial size. The European preparation of botulinum toxin type A has the trade name Dysport® (first approved, 1991 U.K.), and is manufactured in the U.K. and distributed by Beaufour-Ipsen Pharmaceuticals in France. This preparation has been used clinically with success, and is licensed for distribution by the Ministry of Health in England. BTX-B is available in the U.S. as MYOBLOC® (Elan Corporation, Ireland) and the same formulation is available in Europe under the name NeuroBloc®. MYOBLOC® was licensed in the U.S. in December, 2000 for treatment of cervical dystonia.Pharmacology of the botulinum toxins

2.1. General overview

The different strains of the bacteria *Clostridium botulinum* produce seven serologically distinct toxins that are designated A, B, C1, D, E, F, and G (Simpson, 1981). Although these seven neurotoxins are serologically distinct, they possess similar molecular weights and they have a common subunit structure (DasGupta and Foley, 1989; Simpson and DasGupta, 1983). The active toxins have a molecular mass of approximately 150,000 daltons (DasGupta, 1994), and are dichain molecules, in which a heavy chain (~100,000 daltons) is linked by a disulfide bond to a light chain (~50,000 daltons) associated with a single atom of zinc.

Each serotype demonstrates its own varied mechanisms of action, duration of effect, and adverse effects. Each toxin is initially synthesized by the bacteria as a single chain polypeptide. Bacterial proteases then "nick" both type A and type B proteins, resulting in a di-chain structure consisting of 1 heavy and 1 light chain. Type A is nicked more than type B, and there is less than a 50% homology between these two toxins (Settler, 2002). Each of the commercially available botulinum toxins has its own distinct potency and dosing regimen, and the units used to dose each product are not interconvertible. Each formulation and serotype has distinct biological characteristics, which have an impact on the efficacy and adverse event profile in humans.

Only types A and B have been developed for commercial use in routine clinical practice. Three type-A preparations, BOTOX® (onabotulinumtoxinA, product of Allergan, Inc. Irvine, CA), Xeomin® (incobotulinumtoxinA, product of Merz Pharmaceuticals, LLC, Greensboro, NC) and Dysport® (abobotulinumtoxinA, product of Medicis Pharmaceutical Inc., Scottsdale, AZ) have been developed. Currently, Type B is commercially available as MYOBLOC® in the United States. These neurotoxins have a median lethal dose (LD_{50} in the range of 0.1–1 ng per kg, which make them the most lethal poisonous substances known to man (Schiavo et al., 1994)

The primary functional effect of BoNT is thought to be at the neuromuscular junction by chemodenervation of the motor neuron terminal to the associated endplate of the injected muscle. However, there are two other functional effects of the toxin, namely the effect on the afferent limb of the motor system, and the analgesic effects on the sensory system. These will be described below. These latter two effects have been demonstrated in experimental models for BoNT/A only.

3. Mechanisms of botulinum toxin-inhibition of synaptic vesicle exocytosis

Botulinum toxins interfere intracellularly with the process of Ca^{2+} regulated synaptic vesicle exocytosis, and thereby, the releasing of their contents into the synaptic cleft. Critical to this neurotransmitter release is the fusion of the synaptic vesicle to the presynaptic plasma membrane. Different botulinum toxins may specifically interfere with different proteins involved in the docking/attachment and fusion of the synaptic vesicles and the presynaptic plasma membrane (Table 2). Other proteins may help to hold the synaptic vesicles and presynaptic plasma membrane close together and also contribute to triggering the initial processes involved in fusion.

The **s**oluble **N**-ethylmaleimide-sensitive factor (NSF) associated protein **r**eceptor complex (SNARE complex) is thought to play a critical role in forming a protein bridge to promote membrane fusion by bringing two separate lipid bilipid layers into close proximity with subsequent vesicle exocytosis of neurotransmitters into the synapse between neurons. The SNARE complex is composed of synaptobrevin-2, syntaxin-1 and SNAP-25 (Fig. 1).

Table 2
Putative target proteins of botulinum toxin (modified from Huttner, 1993).

Toxin type	Cellular substrate	Target cleavage site
BTX-A	SNAP-25	Gln197-Arg198
BTX-B	VAMP/Synaptobrevin	Gln76-Ph77
BTX-C	Syntaxin 1A, 1B	Lys253-Ala254, lys252-Ala253
	SNAP-25	Arg198-Ala199
BTX-D	VAMP/Synaptobrevin	Lys-Leu60
		Ala67-Asp-68
	Cellubrevin	Unknown
BTX-E	SNAP-25	Arg180-Ile181
BTX-F	VAMP/Synaptobrevin	Gln58-Lys-59
	Cellubrevin	Unknown
BTX-G	VAMP/Synaptobrevin	Ala81-Ala82

Synaptobrevin (Sbr) is not only involved in SNARE complex formation but also binds to Synaptophysin (Syp) and the two complexes are mutually exclusive (Becher et al., 1999). Syp together with synaptoporin belongs to the physin family of tetraspan synaptic vesicle (SV) proteins which are highly abundant on SVs. The Syp/Sbr complex dissociates before membrane fusion (Hinz et al., 2001). Complexin is a protein that normally interacts with the SNARE complex acting as a "clamp" and interfering with synaptic fusion. An action potential opens calcium channels, thereby transiently increasing the local calcium concentration in the presynaptive terminal. This increase in calcium activiates synaptotagmins which may bind calcium through two C2-domains that facilitate synpatotagmin interaction with complexin, resulting in a confimational switch in complexin that promotes synaptic fusion and triggers neurotransmitter release within a few microseconds (Fig. 2).

EHD1 is an EH (Eps15 homology) domain-containing protein involved in endosomal recycling. It works in conjunction with a number of binding partners (e.g., dysbindin-1). Snapin is a soluble protein associated with synaptic vesicles that appears to be one of these binding partners (Ilardi et al., 1999; Salazar et al., 2005; Talbot et al., 2006). Snapin is implicated in diverse neuronal processes (Ilardi et al., 1999; Ruder et al., 2005; Tian et al., 2005; Granata et al., 2008; Pan et al., 2009), but is best known for its role in mediating exocytosis of synaptic vesicles through the soluble N-ethyl-maleimide-sensitive factor attachment receptor (SNARE) complex (Ilardi et al., 1999; Tian et al., 2005; Pan et al., 2009).

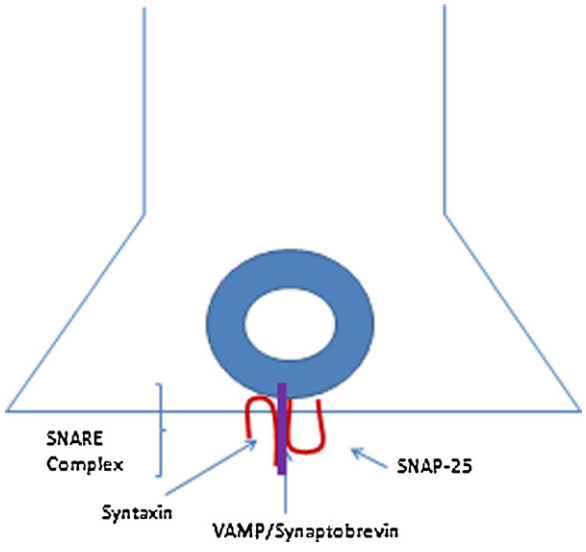


Fig. 1. SNARE complex.

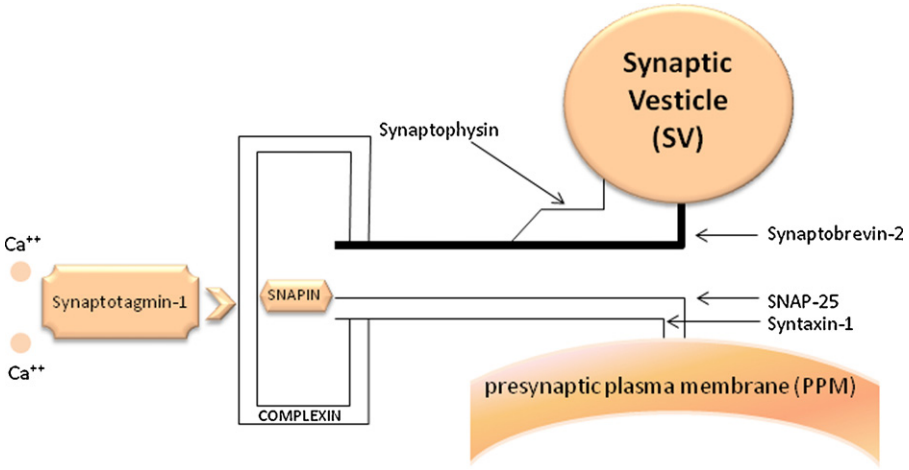


Fig. 2. SV fusion to presynaptic plasma membrane (PPM).

The stabilization of the SNARE complex depends on the binding of SNAP-25 to synaptotagmin-1 (Gerona et al., 2000; Chieregatti et al., 2002; Zhang et al., 2002; Bai et al., 2004; Bhalla et al., 2006; Chicka et al., 2008). Snapin binds to SNAP-25 and promotes the binding of SNAP-25 to synaptotagmin-1 (Ilardi et al., 1999; Chheda et al., 2001; Tian et al., 2005; Pan et al., 2009) and helps to stabilize the binding of synaptotagmin-1 to the SNARE complex before depolarization induced Ca^{2+} influx (Pan et al., 2009). This boosts the number of synaptic vesicles in the readily releasable state (Tian et al., 2005; Pan et al., 2009) and enhances their capacity for efficient, synchronous release (Pan et al., 2009).

The two universally required components of the intracellular membrane fusion machinery, SNARE and SM (Sec1/Munc18-like) proteins, play complementary roles in fusion (Südhof and Rothman, 2009).

Synaptotagmin (the calcium-ion sensor for fusion) cooperates with complexin (the clamp activator) to control the precisely timed release of neurotransmitters that initiates synaptic transmission (Südhof and Rothman, 2009). Synaptotagmin competes with complexin for binding to assembled SNARE complexes, releasing complexin in a Ca^{2+}-dependent manner (Tang et al., 2006), the simplest possible molecular mechanism for Ca^{2+} coupling.

SM proteins are composed of a conserved ~600-amino acid sequence that folds into an arch-shaped "clasp" structure (Misura et al., 2000). SM proteins interact with SNAREs in different ways. Together, these two proteins (complexin and synaptotagmin) account for the precise timing and regulation of the secretion (Südhof and Rothman, 2009). Synaptic and other exocytic SNAREs are first activated and then clamped by complexin (Reim et al., 2001; Giraudo et al., 2006; Tang et al., 2006) and are finally triggered by Ca^{2+} binding to synaptotagmin, which reverses the action of complexin and allows fusion to be completed (Fernández-Chacón et al., 2001; Pang et al., 2006).

Studies on cortical neurons from snapin knockout mice indicate that snapin specifically stabilizes the binding of synaptotagmin-1 to the SNARE complex via SNAP-25 during the fusion clamp stage in priming, which is critical in maximizing the size of the readily releasable pool of vesicles and consequently in maximizing the synchronized release of that pool upon Ca^{2+} influx (Pan et al., 2009).

It is possible that EHD1 competes with SNAP-25 for binding to distinct but overlapping motifs on the C terminus of snapin through steric hindrance. The binding of EHD1 to snapin may decrease snapin's interaction with SNAP-25 and synaptotagmin-1, (i.e., competitive binding of snapin by EHD1 could be the mechanistic basis for the EHD1's ability to negatively impact exocytosis).

4. Primary effects on the neuromuscular junction

The botulinum toxins exert their effect at the neuromuscular junction by inhibiting the release of acetylcholine, and this in turn causes muscle relaxation. There are three steps involved in toxin-mediated relaxation; binding, internalization, and inhibition of neurotransmitter release. The heavy chain is responsible for neuron-specific binding (Evans et al., 1986; Kozaki and Sakaguchi, 1982). Internalization is via receptor-mediated endocytosis (Simpson and DasGupta, 1983; Simpson, 1984; Black and Dolly, 1986). Once internalized and within a vesicle, the light chain translocates across the vesicle membrane and is released into the neuronal cytoplasm.

The light chain is a zinc-dependent protease, whose substrate is one of the fusion proteins responsible for docking and ultimately exocytosis of the acetylcholine-containing vesicle (Dolly et al., 1990; Simpson, 1989; Coffield et al., 1997; Schiavo et al., 1992; Barinaga, 1993). Each serotype light chain cleaves a specific residue of one of the proteins which make up the vesicle docking SNARE complex. Cleavage by botulinum toxin impedes the function or formation of this SNARE complex, and hence, prevents neurotransmitter exocytosis.

Exposure to toxin causes reversible denervation atrophy without fibrosis (Alderson et al., 1991; Borodic et al., 1994), a process recently further elucidated in Oliver Dolly's laboratory using BOTOX (de Paiva et al., 1999). The initial phase of reinnervation occurs through sprouting (Alderson et al., 1991). de Paiva et al. (1999) showed that newly formed sprouts, but not the parent terminal, would elicit muscle contraction with nerve stimulation at 28 days; therefore, only sprouts were responsible for stimulated nerve-muscle transmission during this early phase of recovery. However, a second and distinct phase followed, with a return of vesicle turnover to the original terminals, accompanied by loss of exocytosis activity from the sprouts and gradual elimination of the sprouts. The return of synaptic function to the original neuromuscular junction associated with elimination of the sprouts in this preclinical model required approximately 91 days.

Dolly et al. (2011) produce a recombinant toxin in *E. coli* of two serotypes of botulinum neurotoxin (BoNT/A and BoNT/E), proteins known to block release of transmitters by targeting and entering nerve endings where their proteases cleave and inactivate a protein SNAP-25, essential for Ca^{2+}-regulated exocytosis. $BoNT_E$ acts

transiently, due to lacking these residues, but is a superior inhibitor of TPV1-mediated release of pain peptides from sensory nerves. The advantageous features of each serotype were harnessed by attaching the $BoNT_E$ protease moiety to an enzymically-inactive mutant of $BoNT_A$. The resultant purified composite protein could target motor neurons by binding to the $BoNT_A$ ecto-acceptor and persistently produce $BoNT_E$–truncated SNAP-25. Injection of "recombinant combination" biotherapeutic into the foot pad of rats resulted in an extended amelioration of inflammatory pain (Dolly et al., 2011).

5. Effects on the afferent limb of the BoNT/A

BTX-A may also modify the sensory feedback loop to the central nervous system. Ludlow et al. (1990) and Zwirner et al. (1992) proposed that reduced muscle activity and therefore feedback to laryngeal motoneuron pools may be a primary mechanism of action of BTX-A. Brin et al. (1992) offered the possibility that toxin might have a direct effect on sensory afferents by blocking intrafusal fibers, resulting in decreased activation of muscle spindles. This would effectively change the sensory afferent system by reducing the Ia traffic (Rosales et al., 1996).

Filippi et al. (1993) supported this hypothesis by establishing that local injections of BTX-A directly reduce afferent Ia fiber traffic, and therefore exert a modulatory effect on sensory feedback. This may also account for the clinical observation that injections of BoNT/A have an effect on regional non-injected muscles, most strikingly in spastic limbs (Borg-Stein et al., 1993).

Support for this mechanism derives from the cumulative work of Ryuji Kaji and colleagues (Kaji et al., 1995a,b,c, 1996; Yoshida et al., 1998; Mezaki et al., 1999). They showed that the increase in severity of dystonic writer's cramp associated with enhancement of Ia muscle spindle activity via the tonic vibration maneuver can be decreased by intramuscular injections of dilute lidocaine, which preferentially affects the afferent innervation of the muscle spindle. Both ethanol and lidocaine block sodium channels; however, ethanol blocks the channels for a longer duration than the anesthetic. Kaji has coined the term "muscle afferent block" for this treatment of lidocaine plus ethanol, and has shown an effect in neck, jaw (Yoshida et al., 1998) and limb dystonia (Kaji et al., 1995a,b) and spasticity (Kaji et al., 1996; Mezaki et al., 1999). The benefit for each treatment only lasts a few weeks, and therefore is of limited use in most dystonic and spastic situations. However, this model of blocking Ia afferents supports the proposed mechanism of afferent action with BTX-A in conditions associated with excessive muscle contraction.

6. Analgesic effects of BTX-A on the sensory system

The analgesic effects of BoNT/A were first reported in 1985 in a pilot study of BoNT/A treatment for cervical dystonia, characterized by abnormal, involuntary neck and shoulder muscle contractions and often resulting in significant, disabling musculoskeletal pain. Tsui et al. (1985) described that the most marked benefit of BoNT/A injections was pain relief in all 6 patients who reported severe neck pain due to muscle spasm. In a small, double-blind, placebo-controlled extension of this pilot study, 16 patients treated with BoNT/A experienced significantly reduced pain compared to placebo (Tsui et al., 1986). In subsequent open-label, prospective studies involving larger numbers of patients, we reported pain relief in 74–84% of cervical dystonia patients following BoNT/A injections (Brin et al., 1987; Tsui et al., 1987; Jankovic and Schwartz, 1990; Poewe et al., 1992). Additional double-blind, placebo-controlled studies confirmed the observed effects on pain of BoNT/A in cervical dystonia patients (Greene et al., 1990; Blackie and Lees, 1990; Lorentz et al., 1991; Lu et al., 1995).

In 1992, Memin et al. (1992) reported results from a pilot study of BoNT/A as treatment for spasticity following an upper motoneuron lesion; 5 of 6 patients with pain experienced significant pain relief. Also in 1992, Dengler et al. (1992) reported analgesic effects of BoNT/A among 10 patients treated for spastic foot drop. Later, a larger prospective study of patients with chronic limb spasticity due to various causes observed that 90% of 31 patients with painful flexor spasm or passive stretching experienced at least moderate pain relief and 26% experienced complete pain resolution after BoNT/A injections (Dunne et al., 1995). Another prospective study in Thailand observed joint pain relief in 22 post-stroke spasticity patients (Viriyavejakul et al., 1998). Double-blind, placebo-controlled studies provided further support for the effect of BoNT/A on pain relief in spasticity patients (Grazko et al., 1995; Hyman et al., 2000).

Early in its use as a therapeutic agent, BoNT/A was observed to provide pain relief in disorders other than dystonia and spasticity. Published case reports detail analgesic effects of BTX-A injections for muscle hypertrophy associated with complex repetitive discharges (Nix et al., 1992) and for stiff-person syndrome (Davis and Jabbari, 1993). In a prospective study of 60 achalasia patients, BoNT/A improved chest pain associated with this disease of the esophagus (Fishman et al., 1996). Among 100 patients treated for anal fissure, 78% reported pain resolution within 3 days after initial injection (Jost, 1997).

We have recently reviewed the published reports of BoNT/A for the relief of pain disorders (Brin et al., 2002), which includes pain associated with myofascial pain syndrome (Acquadro and Borodic, 1994; Diaz and Gould, 1999; Cheshire et al., 1994; Porta, 2000a), blepharospasm (Johnstone and Adler, 1998) temporomandibular disorder and bruxism (Girdler, 1994; Van Zandijcke and Marchau, 1990; Ivanhoe et al., 1997; Rijsdijk et al., 1998; Tan and Jankovic, 2000; Freund et al., 2000), back pain (Foster et al., 2001), painful myoclonus (Polo and Jabbari, 1994) prostatic pain/sterile prostatitis (Zermann et al., 2000), and cervicogenic (Freund and Schwartz, 2000a), cluster (Ginies et al., 1996; Freund and Schwartz, 2000b; Smuts and Barnard, 2000), tension-type (Smuts and Barnard, 2000; Zwart et al., 1994; Relja, 1997, 2000; Schulte-Mattler et al., 1999; Porta, 2000b; Carruthers et al., 1999), and migraine headache (Smuts and Barnard, 2000; Binder et al., 1998b, 2000; Mauskop and Basedo, 2000; Silberstein et al., 2000; Brin et al., 2000a). In a double-blind, placebo-controlled study, cerebral palsy patients given BoNT/A for postoperative pain following adductor-release surgery had significantly reduced pain scores, analgesic requirements, and hospital stays compared to placebo (Barwood et al., 2000). BoNT/A has emerged as a promising option for patients suffering from chronic pain disorders.

The association between BoNT/A and pain relief was originally thought to relate only to its effect on muscle contraction. However, several studies suggest that muscle relaxation effects may not directly coincide with pain relief, suggesting alternative mechanisms for analgesic effects of BoNT/A. As noted in this chapter, there is experimental evidence that BoNT/A affects afferent transmission (Filippi et al., 1993; Rosales et al., 1996), which may be a factor in pain relief. There is also evidence that BoNT/A inhibits the release of substance P (Ishikawa et al., 2000) and potentially other neuromodulators. Substance P is a neuropeptide that plays a role in pain perception, vasodilation, and neurogenic inflammation. We have also shown experimentally that BoNT/A relieves formalin-induced pain in laboratory animals (Cui and Aoki, 2000). This is an important observation in understanding the action of BoNT/A on pain because formalin causes pain not through muscle tension, but by first directly stimulating nociceptors and then through

Table 3
Preclinical comparison of the local murine muscle weakness and systemic safety of intramuscular BOTOX® and Dysport®.

	IM ED_{50} (U kg^{-1} body weight)	IM LD_{50} (U kg^{-1} body weight)	Safety margin IM LD_{50}/IM ED_{50}
BTX-A (BOTOX®)	6.2 ± 0.6	81.4 ± 3.5	13.9 ± 1.7
BTX-A (Dysport®)	22.9 ± 3.2[a]	160.8 ± 7.2[a]	7.6 ± 0.9[a]
BOTOX®/Dysport® Ratio	1:3.7	1:1.98	1.8:1

[a] $p < 0.001$.

inflammation. It seems likely that the analgesic effects of BoNT/A relate not only to its well-established effect at the neuromuscular junction, but also to an effect on the nociceptor system (Aoki, 2001a).

7. Additional effects of BTX-A at the neuromuscular junction

BoNT/A may be more effective in blocking the neuromuscular junctions of the most active muscle fibers (Hallett et al., 1994), a topic that was reviewed by Hallett (2002, 2000, 1995). Hughes and Whaler (1962) showed that stimulation of the axon to a frog diaphragm preparation resulted in greater uptake of BoNT/A. Subsequently, Eleopra et al. showed that peripheral electrical stimulation enhanced the effect of BoNT/A on the extensor digitorum brevis muscle (Eleopra et al., 1997a). This concept was supported by the work of Glocker in a hemifacial spasm model (Glocker et al., 1995), and Hesse et al. (1998) and Molteni (1995) in spasticity patients, and finally Hallett's group (Chen et al., 1999) in writer's cramp. These animal and human models suggest that intramuscular BoNT/A therapy will result in a relatively stronger effect on those synapses associated with movement, whether involuntary or volitional.

8. Potency

The potency of commercially available product is determined through in vivo mouse assays. One unit of BoNT is defined as the amount of toxin administered intraperitoneally required to kill 50% (LD_{50}) of a group of 18–20 g female Swiss-Webster mice (Hatheway and Dang, 1994; Schantz, 1964; Schantz and Kautter, 1977; Pearce et al., 1994; Sellin and Thesleff, 1981). This unit is variously referred to as a mouse unit, a mouse LD_{50} unit, or simply a Unit. Numerous factors influence the clinical potency of the preparations (McLellan et al., 1996). Thus, the units are neither clinically equivalent nor interchangeable between products.

9. Pharmacology of botulinum toxin serotypes

9.1. Specific pharmacology: botulinum toxin type A (BOTOX and Dysport)

Both BOTOX® and Dysport® contain botulinum toxin type A (di-chain molecule as previously described) complexed with hemagglutinins (HA) and nontoxin-nonhemagglutinin (NTNH) proteins. The intraneuronal target for cleavage by BoNT/A is the plasma membrane-associated protein known as SNAP-2 (synaptosome associate protein of molecular weight of 25,000 daltons). SNAP-25 is one of several critical proteins whose interaction facilitates rapid vesicle fusion with neuronal plasma membrane and thus neurotransmitter exocytosis (Martin, 1997).

BoNT/A is the only clostridial neurotoxin which is found in the largest complex of 900 kDa, also known as the LL form (Sakaguchi et al., 1984; Melling et al., 1988). During fermentation, the bacteria produce the type A neurotoxin as a single chain polypeptide associated with various proteins to form complexes ranging in size from the LL form to the L (~500 kDa) and M form (~300 kDa) as well as the free neurotoxin (150 kDa). During the later stages of fermentation, endogenous proteases nick the majority (>95%) of single chain into the active di-chain form (DasGupta and Sathyamoorthy, 1984). The bulk toxin (LL form) for both BOTOX® and Dysport® is purified from the other proteins in the fermentation broth. Each product is formulated uniquely and packaged for commercial distribution. The bulk for BOTOX® and Dysport are purified by different methods and thus will have different physiochemical and clinical characteristics. BOTOX is vacuum dried whereas Dysport is freeze dried. Both products require reconstitution with sterile saline for injection prior to use.

Despite the fact that the unit potencies of both products are determined with the mouse assay, there is no equivalence between a unit of BOTOX® and a unit of Dysport®. Reasons for the discrepancy include differences in assay procedures for the two products (McLellan et al., 1996; Hambleton and Pickett, 1994; Pearce et al., 1995; Wohlfarth et al., 1997; Krack et al., 1998; Van den Bergh and Lison, 1998) and different physiochemical properties due to the formulation, bulk toxin and/or final product manufacturing techniques and the dilution protocol during the performance of the mouse LD_{50} assay. The diffusion and side-effect profiles of the two products are distinct as well. Bigalke demonstrated that Dysport® could attain a similar unit potency as BOTOX®, as determined by the mouse phrenic nerve hemidiaphragm preparation, by the addition of extra human serum albumin to the formulation of Dysport® (Bigalke et al., 2001). In further clinical evaluation in the extensor digitorum brevis model, the authors demonstrated that equivalent doses of BOTOX® and Dysport® supplemented with additional human serum albumin produced a similar effect (Bigalke et al., 2001; Rollnik et al., 2000).

The sensitivity of the Dysport® preparation to enhanced unit potency with the addition of albumin was also demonstrated with the digit abduction scoring (DAS) assay (Aoki, 2001b; Peng et al., 1998), and in vivo model that can be used to examine induction of local muscle weakness. BOTOX® and Dysport® were reconstituted with sterile saline (0.9% NaCl) at various dilutions, and compared with each other and with vehicle. The ability of mice to abduct their hindlimb digits was scored with a five point score (0–4) system after a unilateral, intramuscular hindlimb injection. Treated mice were scored daily after a single intramuscular injection of the test article to determine onset of action and dose that produced peak efficacy, and these data were used for identifying the intramuscular effective dose in 50% of the test animals (IM-ED_{50}). A dose-related muscle weakness was observed in the digit abduction score (DAS) for BOTOX® and Dysport. Both products demonstrated onset of action by day 1 and a peak effect by day 3. Average ED_{50} values ± s.e.m., $n = 3$, for BOTOX® and Dysport® were 4.7 ± 1 and 27 ± 5 units/kg, respectively. When nonspecific adsorption was blocked with the use of 0.5% bovine serum albumin (BSA) in saline, the ED_{50} values were 3.4 ± 0.2 and 12.7 ± 5 units/kg for BOTOX and Dysport, respectively. Thus, BOTOX® was approximately six-fold more potent than Dysport when reconstituted with the clinically relevant vehicle, saline, but the apparent loss of Dysport activity when reconstituted in saline was partially prevented with the addition of BSA, while BOTOX efficacy remained consistent regardless of the vehicle utilized.

Further comparison of BOTOX® and Dysport® in the murine DAS assay supports the concept that a simple dose conversion between the two products containing botulinum toxin type A is not possible (Aoki, 2001b). A simple dose ratio implies parallel dose-response curves for both efficacy and safety. The local muscle effect is represented by the dose which elicits a 50% response (IM-ED_{50} value). The IM-ED_{50} comparison between BOTOX® and Dysport® demonstrated a ratio of 1–3.7 (Table 3) while the ratio comparing the systemic effect of the intramuscular dose (IM-ED_{50} value) was 1 to ~2. The safety margins of the two products are demonstrably different, demonstrating differing capacities of the products to remain within the injected murine muscles. However, interspecies differences in response make cross-species extrapolation of safety and efficacy data impossible, and leave the clinical relevance of these observations unknown.

Botulinum toxin type A is also produced in Japan (Nagamine et al., 1991) and China (Zhuang and Yin-chin, 1992) for clinical research, but we have no additional information about the pharmacology of these formulations nor whether they are produced under Good Manufacturing Practice (GMP) standards.

9.2. Specific pharmacology: other serotypes

BoNT/B cleaves synaptic vesicle associated membrane protein (VAMP, also known as synaptobrevin), one of the proteins in the SNARE complex responsible for docking and fusion/release of synaptic vesicle with the presynaptic membrane. It is remarkable that synaptobrevin/VAMP knockout mice (Schoch et al., 2001) continue to demonstrate SNARE function, suggesting that synaptobrevin may not be absolutely required for synaptic fusion, though this speculation requires further investigation.

The formulation of BoNT/B is produced by fermentation of the Bean strain of *Clostridium botulinum* type B, and exists in noncovalent association with hemagglutinin and nonhemagglutinin proteins as a neurotoxin complex. The protein is synthesized as a single polypeptide of approximately 150 kDa, and nicked by proteases to form the heavy and light chains. The commercial preparation is an injectable solution that is clear and colorless-to-light yellow with a molecular weight of approximately 700 kDa. Each single-use vial contains 5000 U of the product per milliliter, and includes 0.05% human serum albumin, sodium succinate, sodium chloride, sodium caprylate, sodium acetyltryptophanate, hydrochloric acid and water at a pH of 5.6 (USFDA, 2001; Neurobloc, 2000). Elan Biopharmaceuticals' method of calculating the LD_{50} in mice is proprietary and may differ in details such as the vehicle, dilution scheme and laboratory protocols. The units of biological activity of Elan's product cannot be compared to or converted into units of any other botulinum toxin. The doses showing efficacy in cervical dystonia trials were between 2500 Units and 15,000 Units (Tsui et al., 1995; Lew et al., 1997; 132-Brashear et al., 1999; Brin et al., 1999). The specific activity ranges between 70 and 130 Units/ng.

There is no specific pharmacology information on serotypes C and F that have been used in human clinical research trials. In clinical studies, BoNT/F has a shorter duration of effect (Greene and Fahn, 1993a, 1992; Ludlow et al., 1992; Rhew et al., 1994; Houser et al., 1998), as may type E in preliminary studies (Eleopra et al., 1998), while type C may have properties similar to those of type A (Eleopra et al., 1998, 1997b). Botulinum toxin type F has been used to treat patients who have antibodies or clinical resistance to type A (Greene and Fahn, 1993a; 135-Greene and Fahn, 1992; Ludlow et al., 1992; Rhew et al., 1994; Houser et al., 1998).

9.3. Risks and adverse effects

BoNT/A has been examined as a therapeutic agent since the late 1970s (Schantz and Johnson, 1994) and in long-term use under medical supervision, it has been proven to be remarkably safe. Weakness or routine EMG changes in muscles distal to the site of injection have not been reported. However, one study reported diminished size of type IIB fibers in muscles distant from the injection site in patients treated for cervical dystonia (Ansved et al., 1997).

Small amounts of BoNT may briefly circulate in blood after administration, raising concern about the potential for long-term adverse effects. "Remote effect," i.e., electromyographic evidence that the toxin has spread to, or had an effect at, more distant muscles has been reported in patients injected with BoNT/A with the lower doses for blepharospasm, as well as patients treated with higher doses for cervical dystonia. This typically manifests as increased jitter in limb muscles on single-fiber EMG (Lange et al., 1993, 1991, 1987; Sanders et al., 1986).

The effect is probably universal in patients treated for cervical dystonia. These physiological abnormalities do not appear to have any clinical significance, and it is not known how long they persist. Nevertheless, in over a decade and a half of experience in treating patients with this agent, there have been no reports of objective generalized weakness in patients without other neurological disease at routine doses.

Dysphagia may represent direct muscle-to-muscle diffusion, or may be a systemic effect. Dry mouth may be a systemic effect, possibly reflecting the impact of toxin that escapes into the bloodstream. The highest rates for dry mouth are seen with MYOBLOC®/NeuroBloc®, followed by Dysport®, followed by BOTOX®. To date, no clinical trials of MYOBLOC®/NeuroBloc have been published in spasticity, and it is unknown whether incidences of dysphagia or dry mouth differ between the three products in this indication.

There are a paucity of data regarding use during pregnancy for any commercially available serotype and teratogenicity has not been established (Scott, 1989; Moser et al., 1997). The authors recommend not injecting patients who are pregnant or lactating. Additionally, although clinicians have treated some patients with pre-existing disorders affecting neuromuscular junction function, the authors recommend proceeding with caution in treating patients with conditions such as myasthenia gravis, Eaton-Lambert syndrome, and motoneuron disease, particularly when large doses are required, such as in the treatment of cervical dystonia (Mezaki et al., 1996; Borodic, 1998; Emmerson, 1994; Tuite and Lang, 1996; Erbguth et al., 1993; Bushara, 1997). Rarely, idiosyncratic reactions can occur, including a persistent rash, localized reactions, and ptosis with injections distant from the face (LeWitt and Trosch, 1997).

10. Antibodies and clinical resistance

In 1984 at Columbia University at the time that Brin and colleagues began to investigate the use of Alan Scott's BoNT/A product, Oculinum (later marketed as BOTOX®), some investigators reflected on the possibility of developing an immune response to chronic therapy. Advisors did not appreciate that antibody formation would occur, because the toxin protein exposure was considered low. In addition, the number of units per treatment was very small compared to the exposure in food-borne botulism, and in the latter case, patients had not been reported to have developed an immune response to the toxin. At that time, there were no guidelines available as to the treatment interval. Brin et al. (1987) initially treated patients on an "as needed" basis, giving booster injections, and injecting some patients approximately every month, if clinical

benefit could be demonstrated. They reported success using this approach; however, after a number of years of treatment, began to see the emergence of clinical resistance to therapy in some patients (Greene and Fahn, 1993b; Greene et al., 1994). As a result, they adjusted their treatment paradigm to avoid injecting patients more often than every 3 months, avoid "booster" injections, and to titrate treatment to the lowest effective dose.

Resistance is characterized by absence of any beneficial effect and by lack of muscle atrophy following the injection. Antibodies against the toxin are presumed to be responsible for most cases of resistance. As noted above, while early studies reported no detectable antibodies in patients exposed either by intestinal colonization (Paton et al., 1982) or for therapeutic indications (Biglan et al., 1986; Gonnering, 1988), clinical investigators (Greene and Fahn, 1993b; Greene et al., 1994; Zuber et al., 1993; Hanna et al., 1999) have shown that small numbers of patients do develop antibodies with repeated BoNT/A treatment. Although antibodies appear to cause no harm, they can render the patient unresponsive to further treatments.

Immunoresistance to BoNT/A may be tested immunologically with a variation of the mouse lethality assay, the mouse neutralization assay, MNA; or mouse protection assay, MPA, or through ELISA testing likely overestimates clinical immunoresistance (Hanna and Jankovic, 1998). The MNA is considered the gold-standard, clinically relevant assay. However, rather than send patients' serum for either type of assay, we often choose to perform the FTAT (frontalis type A antibody test) when clinical resistance is suspected. Fifteen to 20 Units BOTOX are divided into 2 sites of one side of the corrugator muscle. If the muscle does not move within 2 weeks, and the patient cannot furrow that side of their brow, then they are "not resistant;" if the corrugator moves properly, then they are "resistant." In the case of no resistance, the patient may be injected on the opposite side to maintain expression symmetry.

In reports of the early experience, antibodies had been identified via the mouse assay in 3–10% of cervical dystonia patients treated with BoNT/A (both BOTOX® and Dysport®) (Greene and Fahn, 1993b; Greene et al., 1994; Zuber et al., 1993). The product insert for BOTOX® indicates the figure may be as high as 17%. This figure (i.e., 17%) reflects the incidence in patients who had been treated with the original lot of toxin (79-11), some for many years, and many using a treatment paradigm that included booster injections. Lot 79-11, which is no longer distributed, had a significantly higher protein exposure than current BOTOX®.

The authors concur with Greene et al. (1994) and Jankovic and Schwartz (1991) in the recommendations to minimize immunoresistance: (1) use the smallest possible effective dose, (2) extend the interval between treatments as long as reasonable, at least 3 months between treatments, and (3) avoid using booster injections.

In December 1997, Allergan released "current BOTOX®". Current BOTOX® has a higher specific activity than the original batch (Lot 79-11) that was initiated in 1979 (Allergan, Dear Customer letter, November 1997). Current BOTOX® has approximately 4–5 ng neurotoxin complex per 100 Units. The foundation for a product that results in a lower protein exposure to the patient is reviewed elsewhere (Brin, 2000b); comparative protein exposure is summarized in Table 4. Brin et al. (2000) reported on consecutive cervical dystonia patients treated with current BOTOX®, and found no evidence for immunoresistance. Recently, Jankovic reviewed his series of cervical dystonia patients treated with both the original BOTOX® (lot 79-11) and current BOTOX® and reported no patients had developed immunoresistance when treated exclusively with current BOTOX® (Jankovic et al., 2002). After performing an analysis examining the influence of age and cumulative dose, they concluded that the low risk of antibody formation following current BOTOX® treatment is related to the lower protein exposure.

Patients with resistance to one serotype may benefit from injection with other serotypes. In studies of BoNT/A resistant patients, the benefits of BoNT/F seemed to last approximately 1 month (Greene and Fahn, 1993a, 1992; Ludlow et al., 1992; Rhew et al., 1994; Houser et al., 1998; Sheean and Lees, 1995; Mezaki et al., 1995) and both seropositive and seronegative patients benefited.

The registration clinical trials for BoNT/B show that this product is efficacious in type A-resistant patients with cervical dystonia (Brin et al., 1999). The only published antibody rates for MYOBLOC®/NeuroBloc® are found in the U.S. Product Label and in the Summary Basis of Approval (SBA) (USFDA, 2001) available on the FDA website (http://www.fda.gov/cber/products/botelan120800.htm). An ELISA assay was used to identify those patients who developed an antibody response. The mouse neutralization assay (MNA) was then performed. A positive MNA was seen in 9.6% of patients at 12 months, 18.2% of patients by 18 months, and 22.6% of patients by 20 months. Reports suggests that BoNT/C has a duration of effect similar to that of BoNT/A (Eleopra et al., 1998, 1997b), but it is unknown if BoNT/C is effective in type-A or type-B resistant patients.

11. Botulunum toxin for the treatment of pain

11.1. BoNT for the management of musculoskeletal pain

Multiple studies have looked at the neurotoxin's potential for the treatment of painful musculoskeletal conditions, including chronic myofascial and spinal pain syndromes. Musculoskeletal pain is often attributed to myofascial pain syndrome (MPS). Of patients with pain presenting to various specialists, the prevalence of MPS has been reported to vary from 30 to 90%, depending on the subspecialty practice and setting. MPS is characterized by painful muscles with increased tone and stiffness containing trigger points, which are tender, firm nodules, or taut bands, usually 3–6 mm in diameter. Palpation produces aching pain in localized reference zones (Garvey et al., 1989).

Mechanical stimulation of the taut band by needling or brisk transverse pressure produces a localized muscle twitch. Trigger point palpation often elicits a "jump sign"—involuntary reflex-like recoil or flinching from the pain—that is disproportionate to the pressure applied. Multiple treatments, including trigger point injections, have long been advocated; however, reports conflict as to whether any therapeutic substance injected into a muscle provides more benefit than dry needling alone (Wheeler, 2004a).

The pathogenesis of myofascial trigger points is unknown; however, Simons postulates that abnormally increased motor endplate activity caused by excessive release of acetylcholine at the neuromuscular junction results in spontaneous electrical activity and extrafusal muscle contraction in the immediate vicinity of the extrafusal muscle end plates, thus forming the taut band and trigger point (Simons, 1996).

Numerous studies have examined the role of BoNT for treating chronic cervical-thoracic pain associated with myofascial pain and dysfunction. In a randomized, controlled, crossover study, Cheshire et al. (1994) injected myofascial trigger points in the cervical and shoulder region in 6 patients with either BoNT/A (50 Units spread out over 2–3 areas) or normal saline (NS). Crossover occurred at 8 weeks. Four of the 6 patients reported at least 30% pain reduction, as measured by visual analogue scales (VAS), with BoNT/A, but not saline injections.

In a randomized, double-blind, prospective, placebo-controlled study by Wheeler et al. (1998), 33 patients with a single cervical myofascial trigger point were injected with either 50 Units or 100 Units of BoNT or normal saline. All 3 groups showed significant

Table 4
Protein exposure with currently available botulinum toxin preparations.

	BOTOX® (Naumann et al., 2002)	Dysport® (Hambleton and Pickett, 1994)	NeuroBloc®/MYOBLOC™ (USFDA, 2001)
U/ng in current formulation	20 U/ng	40 U/ng	100 U/ng
Ng protein per CD treatment	10 ng/200 U	18 ng/700 U	100 ng/10,000 U

treatment effects as measured by VAS, psychometric testing, and pressure algometry. Group differences were apparent only when the authors considered the number of patients who were asymptomatic from the injections, but no clear statistically significant benefit of BoNT over placebo was demonstrated over 4 months.

However, a striking difference in treatment response was noted between the participants in the 2 BoNT/A treated groups compared with those in the initial placebo group who elected to receive a second, unblinded BoNT/A 100 Unit injection into the same trigger point. Using the same measurement criteria, this second study arm showed a beneficial effect from BoNT/A, but the small number of participant's precluded meaningful statistical analysis (Wheeler, 1998).

In a follow-up open-label study, Wheeler and Goolkasian (2001) examined a 44 patient-cohort with refractory cervical-thoracic (73%) or lumbosacral (9%) muscular pain (or both) who received BTX-A treatment in a private outpatient clinic combined with physical therapy. BoNT/A treatment was directed at painful muscles with spasm or trigger points. BoNT/A dosages were tailored to meet individual patient needs and varied between 50 and 200 Unit. Eighty percent of all patients reported significantly reduced pain after their initial treatment session. Forty-one percent of patients who only underwent one treatment session and an additional 27% who required a second injection session still reported "adequate pain relief" when contacted 2 years later.

However, in a subsequent double-blind, randomized, controlled study, Wheeler et al. (2001) were unable to detect any statistically significant differences in pain reduction between BoNT/A and placebo-treated patients with painful cervical-thoracic paraspinal and trapezius muscles using higher total doses of BoNT/A (e.g., 200–300 Units/session), similar to treatment doses commonly used for cervical dystonia.

In the studies described so far, physicians have used similar injection methodology by placing the neurotoxin into symptomatic trigger points, a practice consistent with the treatment techniques originally described by Simons and Travell (1999). However, some advocate placement of BoNT into the muscle's motor point (e.g., into standardized sites in the mid-belly of affected muscles) (Lang, 2000). Using a BoNT treatment technique that involved injecting the muscle in its motor point pattern with doses of BoNT/A ranging from 20 to 600 Units, Lang (2000) studied the treatment of 72 patients who received 95 injection session treatments. Sixty percent of patients experienced good-to-excellent results at 22–60 days following injection.

In a 12-week randomized, double-blind, placebo-controlled study, 132 patients with cervicothoracic myofascial pain were treated with BoNT/A or saline by Ferrante et al. (2002). No significant differences in outcome were seen between groups. Patients receiving BoNT/A were treated with a total of 50–250 Units of toxin divided among 5 injection sites (Ferrante et al., 2002).

Porta (2000a), in a single blinded study, evaluated the difference between lidocaine/methylprednisolone injections compared with BoNT/A injections into symptomatic myofascial trigger points in the psoas, piriformis, or scalenus anterior muscles; 80–150 Units of toxin were used. Each group received benefit, but the toxin-treated patients experienced a greater duration of relief.

Langevin et al. (2011) performed a Cochrane Review and reported that current evidence fails to confirm either a clinically important or a statistically significant benefit of BoNT/A injection for chronic neck associated with or without associated cervicogenic headache.

Seventy-one percent of his patients reported significant reductions in headache pain frequency and severity. In 2 separate open-label studies, Taqi et al. (2002a,b) showed that either type of BoNT may be effective in the treatment of myofascial pain. Several case reports using BoNT/B injections in the management of chronic myofascial pain have suggested overall beneficial results (Fishman, 2002; Nalamachu, 2002; Smith et al., 2002).

Several publications, including a case report and an open-label study involving 77 patients; have emphasized the benefit of BoNT in the management of chronic myofascial pain (Lang, 2002; Sheean, 2002; De Andres et al., 2010). One published review of BoNT/A treatment for chronic musculoskeletal pain found no clinical evidence of its beneficial efficacy, despite scientific evidence of BTX analgesic qualities (Saenz et al., 2003).

Reports of the use of BoNT for treatment of piriformis muscle syndrome included a randomized, controlled, crossover study of 9 patients who were treated with 100 Units of either BoNT/A or placebo using EMG and fluoroscopic guidance for injection placement. Childers et al. (2002) reported a trend toward greater pain relief for patients receiving toxin as opposed to placebo. Fanucci et al. (2001) reported that 26 of 30 patients with piriformis syndrome who were injected with BoNT/A under computerized axial tomographic guidance obtained relief of their symptoms within 5–7 days.

Fishman et al. (2002) performed 2 studies looking at BoNT use for patients with piriformis syndrome. In one uncontrolled study, the authors concluded that BoNT/A injections may be a useful adjunctive treatment measure primarily when added to physical therapy in the management of this syndrome. In a follow-up dose-ranging study with BoNT/B for piriformis syndrome using EMG guidance, Fishman et al. (2002) reported that patients experienced notable symptom improvement.

A double-blind, randomized, controlled study of BoNT/A use for chronic myofascial pain compared 30 patients with trigger points in the infraspinatus muscle. Subjects were divided into a treatment group who received 50 Units/0.25 mL saline or a placebo group who just received 0.25 mL of isotonic saline. Outcome measures included localized and referred pain, pain detection and tolerance thresholds to mechanical pressure (electronic algometer), and shoulder movement assessed at 3 and 28 days after injection. EMG interference patterns were evaluated at baseline and at 28 days following BoNT/A injections. BoNT/A significantly reduced motor endplate activity and EMG interference patterns; however, no significant differences were found in any of the outcome measures between groups (Gobel et al., 2006). BoNT/A has antinociceptive and muscle-spasmolytic properties that may be hypothesized to alleviate signs and symptoms of myofascial pain syndromes (MPS). A prospective, double-blind 12-week, multicenter, randomized controlled trial by Gobel et al. (2006) found that patients with upper-back MPS who received injections of 400 Ipsen units of BoNT/A (Dysport) to 10 individual trigger points demonstrated significantly improved pain levels at 4–6 weeks after treatment without adverse side effects.

11.2. BoNT for low back pain

The use of BoNT in the management of chronic low back pain remains controversial but has been investigated. In a randomized, controlled study involving 31 patients with chronic low back pain, Foster et al. (2001) studied the effect of 200 Units of BoNT/A (5 sites in the paravertebral levels L1-L5 or L2-S1, 40 Units per site) compared with placebo injections. Pain and extent of disability were noted at baseline and at 3 and 8 weeks using a VAS and the Oswestry Low Back Pain and Disability Questionnaire. At both 3 and 8 weeks, more patients who had received BoNT injections (73.3% and 60%, respectively) experienced 50% or more pain relief compared with the placebo treated group (25% and 12.5%, respectively). At 8 weeks, less disability was noted in the BoNT/A-treated group compared with the placebo-treated group.

Knusel et al. (1998) treated patients with low back pain associated and painful muscle spasm with different doses of BoNT/A and noted that only those treated with the highest doses (240 Units) experienced significantly greater relief than placebo-treated patients.

Two randomized, prospective studies, one double-blind and one open label, were performed to evaluate the long-term efficacy and safety of BoNT/A in 31 and 75 patients with chronic low back pain, respectively. Both studies used a novel injection technique, with placement of 40–50 Units of BoNT/A by injection into the erector spinae muscles at each of 5 levels from L1-L5 (Jabbari, 2008). Jabbari reported significant ($p < 0.05$) reduction of pain intensity and improvement in performance of activities of daily living in 60% and 53% of the patients, respectively. A second study also demonstrated safety with repeated injection sessions over 14 months. The author suggests that BoNT/A should be considered when treatment of low back pain fails other more standard management approaches.

A prospective study by Jabbari et al. (2006) was also performed to study the short-term and long-term effects of BoNT/A on refractory chronic low back pain. Seventy-five patients received BoNT/A injections into paraspinal muscles at 4–5 levels between L1-S1 unilaterally or bilaterally. Dose per site ranged from 40 to 50 Units, whereas the total dose per session ranged from 200 to 500 Units. Reinjection of BoNT/A was performed at 4 months if pain returned over the study duration of 14 months. Outcome data collection included VAS scores, pain frequency (e.g., number of pain days), and self-perceived functional status using the Oswestry Disability Questionnaire (ODQ).

Participants were assessed at baseline, 3 weeks, and then at 2, 4, 6, 8, 10, 12, and 14 months. Forty patients at 3 weeks (53%) and 39 patients at 2 months (52%) reported significant pain relief. Changes in VAS, ODQ scores, and pain frequency were consistent with patient improvements and were statistically significant when the 2-month data were compared with baseline at 2 months and then after each injection period ($p < 0.005$). Among initial responders, 91% continued to show improvement over the length of the study. Three patients (4%) experienced a probable adverse event consisting of a mild flulike reaction that lasted 2–5 days following an initial injection session. The number of responders and durations of chronicity is unclear. Any associated therapies were not noted. Therefore, agreeing with the authors when they suggest that a favorable initial response to BoNT/A predicts subsequent responsiveness is difficult.

The same authors performed an open label prospective study on 60 patients with chronic low back pain (Ney et al., 2006). Patients received 40–50 Units per paraspinal level with multiple levels injected. A maximum dose was 500 Units of BoNT/A per an injection session. Study participants with a beneficial clinical response received a second treatment at 4 months. Pain and clinical status were assessed by VAS, modified OLBPQ, and a CLBPQ at baseline, 3 weeks, 2 months, 4 months, and 6 months after the first treatment. Participants included 18 women and 42 men, aged 21–79 years (mean, 46.6 y); with chronic low back pain over a mean duration of 9.1 years.

Significant improvement in back and radicular pain occurred at 3 weeks in 60% and at 2 months in 58% of the cohort. Again, the authors found that a beneficial clinical response to the first injection predicted the benefit of a second reinjection response, which was indeed measured and determined to be 94%. A significant minority of patients had a sustained beneficial effect from the first injection at 4 months (16.6%) and 6 months (8.3%). Two patients experienced an adverse event described as a transient flulike reaction after the initial treatment. Ney et al. (2006) concluded that BoNT/A is a reasonable therapy for seeking improvement in patients with refractory chronic low back pain.

A beneficial clinical response can be predicted within the first 2 months following treatment. An early positive response from BoNT/A treatment also predicts the high likelihood that the benefit will be sustained with a second treatment. Furthermore, BoNT/A demonstrates a low incidence of mild, but usually transient side effects.

11.3. BoNT in the management of neuropathic pain

BoNT may be effective in the management of neuropathic pain, which by definition is caused by damage or dysfunction within the central or peripheral nervous system. Conditions such as postherpetic neuralgia (PHN), spinal radiculopathy, complex regional pain syndrome (CRPS), spinal cord injury, and brachial plexus injury are examples of neuropathic pain syndromes (Wheeler, 2004b). BoNT/A has demonstrated relief of pain in conditions associated with muscular overactivity, but may also be effective in the treatment of neuropathic pain. Multiple neurochemical and neurophysiological mechanisms have been cited that could explain potential actions of BoNT/A as a therapeutic agent for neuropathic pain.

Freund and Schwartz (2001) reported reduced pain in 7 patients with trigeminal, thoracic, or lumbar PHN of more than 6 months who were treated with subdermal BoNT/A injections at a concentration of 5 Units per 0.1 cm^3 preservative-free normal saline into every 9 cm^3. In another report, 2 patients with cervical spinal cord lesions experienced hyperesthesia, allodynia and burning pain in a segmental dermatomal distribution (Jabbari et al., 2003). Patients were treated with multiple point subcutaneous injections of BoNT/A in the involved dermatome. Definite analgesia was obtained and then maintained by repeating injection treatments every 4–6 months. Injections in one patient were discontinued at 3 years and in the second patient after 2 years, when neuropathic pain symptoms subsided.

Xiao et al. (2010) investigated the therapeutic benefits of BoNT/A in subjects with PHN in a randomized, double-blind, placebo-controlled study. After randomization, one of the following solutions was injected subcutaneously in the affected dermatome: 5 u/mL BoNT/A, 0.5% lidocaine, or 0.9% saline (placebo with 20 patients in each of the three groups. Visual analog scale (VAS) pain and sleeping time (hours) were evaluated at the time of pretreatment, day 1, day 7, and 3 months posttreatment. Opioid usage was calculated at day 7 and 3 months posttreatment (Xiao et al., 2010). Compared with pretreatment, VAS pain scores decreased at day 7 and 3 months posttreatment in all three groups ($P < 0.01$). However, the VAS pain scores of the BoNT/A group decreased more significantly compared with lidocaine and placebo groups at day 7 and 3 months posttreatment ($P < 0.01$). Sleep time (hours) had improved at day 7 and at 3 months compared with pretreatment in all three groups, but the BoNT/A group improved more significantly compared with lidocaine and placebo groups ($P < 0.01$). The percent of subjects using opioids posttreatment in the BoNT/A group was the

lowest (21.1%) compared with the lidocaine (52.6%) and placebo (66.7%) groups ($P < 0.01$) (Xiao et al., 2010).

Long-lasting analgesia of neuropathic pain was demonstrated in 2 experimental studies using rats with either alloxan or streptozotocin-induced diabetic peripheral neuropathy. A single subcutaneous injection of BoNT/A produced a prolonged antinociceptive effect as measured by mechanical sensitivity (Lackovic et al., 2006). Furthermore, more investigation has confirmed that BoNT/A is effective at treating painful conditions that are not primarily caused by excessive or aberrant muscle contraction. In addition to inhibiting vesicular release and membrane fusion with acetylcholine, BoNT/A appears to exert similar influences on pain-mediating neuropeptides that include Substance P and calcitonin gene-related peptide (CGRP) from sensory neurons. BoNT/A also inhibits release of the excitatory neurotransmitter glutamate, and also, suppresses the vanilloid receptor TRPV1 on the surface of peripheral nociceptors (Apfel, 2009; Jeynes and Gauci, 2008; Aoki, 2008).

An abstract by Relja and Militec (2006) presents a prospective study of BoNT/A treatment of painful diabetic neuropathy, whereby 41 symptomatic patients were randomized into a BoNT/A 100 Units treatment or placebo group. Injection methodology was not discussed in the published abstract. All patients completed the study. Significant improvements were noted in all outcome measures; including the endpoint mean pain score, VAS, global assessments, and the SF–36 Questionnaire (Relja and Militec, 2006).

A double-blind, placebo-controlled crossover study of BoNT/A for the treatment of painful diabetic peripheral neuropathy was performed by Yuan et al. (2009) using doses of about 4 u per injection site in 18 subjects. Benefit of reduced pain in the treatment group was noted as early as 1 week followed by statistically significant improvement over through completion of the study at 12 weeks. Responders were defined by >3 cm improvement on a 10 cm "pain right now" VAS. Yuan et al. (2009) reported a 44% responder rate for the active group vs. 0% for the control group, as well as transciently improved sleep quality.

A 2008 study by Ranoux et al. (2008) also, a double-blind RCT, evaluated 29 patients with focal neuropathic pain (e.g., post-traumatic postoperative pain or PHN) treated with standardized BoNT/A dosing of 5 u per injection site and showed statistically significant pain relief at 1 week, and progressing through study's end at 14 weeks. Responders reported >50% pain reduction on weekly pain scores. Responders were 40% in the treatment group compared to 7% in the control group. Ranoux et al. (2008) concluded that BoNT/A may induce direct analgesic effects in patients with chronic neuropathic pain independent of its effects on muscle tone. Ranoux et al. provided the first significant support from human studies in favor of utilizing botulinum toxins for neuropathic pain (Smith, 2009; Murinson, 2008). This study (Ranoux et al., 2008), along with the Xiao (Xiao et al., 2010) and Yuan (Yuan et al., 2009) clinical investigations show promise for the treatment of neuropathic pain with few, if any, adverse events. As will be noted in all BoNT studies they fail suitable design and statistical power to satisfy any FDA or evidence-based analysis.

11.4. BoNT for osteoarticular pain

The rationale for the use of BoNT injections into painful joints followed research findings that implicated intra-articular injections of substance P and calcitonin gene-related peptide (CGRP) as causative of joint pain and inflammation. BoNT was found to produce significant pain relief when injected into painful joints due to either inflammatory or noninflammatory disorders. This rationale presumes that the neurotoxin is capable of binding to nociceptor C-fibers, undergoing endocytosis, and blocking the vesicular release of substance P, CGRP, and glutamate, which are all pain mediators capable of producing neural transmission of noxious stimuli with subsequent nociceptor sensitization.

Singh and Fitzgerald (2010) performed a double-blind, randomized controlled trial to determine the safety and efficacy of intra-articular BoNT/A injections in 43 patients with chronic refractory, moderate-to-severe shoulder joint pain presumed to be due to arthritis. Patients were randomized to receive either 100 U of BoNT/A with lidocaine or saline and lidocaine. Primary outcomes were reduced pain severity on VAS at 1 month (0–10 cm). Secondary outcomes were improvements as measured by the Shoulder Pain and Disability Index (SPADI) disability subscale, quality of life on short-form (SF)-36 subscales, percent of patients who achieved at least a 30% decrease or a 2-point reduction in VAS pain (clinically meaningful pain relief), and safety (Singh and Fitzgerald, 2010). Both BoNT/A ($n = 21$) and placebo ($n = 22$) groups were comparable at baseline. At one month postinjection, pain reduction by VAS, SF-36 subscale scores, and the SPADI disability subscale improvement were significantly greater in the BoNT/A group than in the placebo group. Clinically meaningful pain relief occurred in 61% of the BTX-A treatment group versus 36% of placebo patients ($P = 0.22$). The total number of adverse events was similar, which included 50 events in the BoNT/A group versus 46 events in the placebo group. Therefore, a single injection of BoNT/A produced statistically significant and clinically meaningful pain relief, as well as, producing improvement in quality of life for patients with chronic refractory moderate/severe shoulder arthritis pain at 1 month. These data provided evidence to support the need for a larger multicenter, randomized trial (Singh and Fitzgerald, 2010).

11.5. BoNT for shoulder pain following stroke

BoNT has been studied for use in shoulder pain following stroke. Castiglione et al. (2011) performed a Pilot study with assessments before and after BoNT/A intra-articular injection for 75 patients with refractory hemiplegic shoulder pain. Baseline VAS score was 8.7 ± 1 at rest and 9.8 ± 0.4 during passive arm abduction. It clearly decreased at 2 (1.5 ± 1.1 at rest, $P = .001$; 3 ± 1.2 during 90° arm abduction, $P < .001$) and 8 weeks (1.5 ± 1.2 at rest, $P = .001$; 2.3 ± 1.1 during arm abduction, $P < .001$) after BoNT/A intra-articular injection (Castiglione et al., 2011).

To assess the effects of BoTN/A on hemiplegic shoulder pain associated with spasticity, a larger double-blind, randomized controlled trial looked at a one-time injection of BoTN/A (500 Speywood Units) into the pectoralis major and biceps brachii on the hemiplegic side (Kong et al., 2007). VAS of shoulder pain, shoulder adductor and elbow flexor tone using the Ashworth scale, and passive range of shoulder abduction were assessed as outcomes. However, only 17 patients were enrolled, 8 in the BoTN/A group and 9 in the placebo group. Negative findings in this study include the small sample size and the presence of causes of shoulder pain not related to spasticity, which could have confounded outcome.

A double-blind, randomized controlled trial was performed to determine the efficacy of BTX-A for treatment of shoulder pain in patients with spasticity after stroke (Marco et al., 2007). Two cases dropped out (6.5%) of 31 patients enrolled from an acute-care hospital in Spain. Fourteen subjects were treated with infiltration of 500 U of BoTN/A compared with 15 who received placebo in the pectoralis major muscle of the paretic side. Patients were assessed using a VAS for pain. A significant reduction in pain was considered when the VAS score was below 33.3 mm or less than half the initial score. At 6 months, patients treated with BoTN/A showed significantly greater improvement in pain than placebo from the first week postinfiltration. Patients with shoulder pain from spasticity treated with BoTN/A infiltration into the pectoralis major muscle on the paretic side had a higher likelihood of pain relief, ranging between 2.43 and 3.11-fold.

11.6. BoTN/A for neck pain after radiotherapy/dissection surgery for cancer

Neck dissection surgery and radiation therapy for the treatment of carcinoma of the head and neck often results in chronic pain. Four of 6 volunteers with muscular neck pain and spasm after radiotherapy for treatment of carcinoma of the head and neck who received BoTN/A injections into affected sternocleidomastoid muscle in 1 or 2 locations achieved pain relief (Van Daele et al., 2002).

A prospective, open-label study of 16 patients with chronic neck pain after dissection received 80–320 Units of BoTN/A (Dysport) injected into muscular trigger points. Outcome measures included chronic and shooting pain using VAS and quality of life improvement measures before and 4 weeks after treatment. All patients showed a significant reduction in chronic pain (4.5 before to 3.3 after treatment, $p = 005$) and shooting pain (6.1 before to 4.7 after treatment, $p = 005$), including a trend toward improvement in measures of global quality of life and function (Vasan et al., 2004).

In another study, 23 patients with chronic neuropathic pain after neck dissection were selected for an open, prospective phase II trial (Wittekindt et al., 2006). One group of patients received BoTN/A SC in a low-dose concentration of 10 Units/0.1 mL saline ($n = 13$) compared with a high-dose-group ($n = 10$) of 20 Units/0.1 mL saline. Pain and quality of life measures were assessed at day 0 and day 28. The low-dose BoTN/A group showed significant pain reduction by VAS of 4.3 at day 0–3 at day 28 ($P < .05$); however, the mean pain VAS values did not significantly improve in the high-dose group. Trends toward improvement in quality of life were only in the low-dose BoTN/A group (Wittekindt et al., 2006)].

11.7. BoTN/A for urological pain

Zermann et al. (2000) reported pain relief in 11 patients with chronic prostatic pain who were treated with BoTN/A (200 Units) by transurethral perisphinteric injection.

Giannantoni et al. (2010) looked prospectively at the 2-year efficacy and tolerability of intravesical BoTN/A injections in patients with painful bladder syndrome (PBS) associated with increased urinary frequency refractory to conventional treatments. Preliminary assessment of the 13 participants, who were all women, included voiding diary, urodynamics, urinary tract ultrasonography, and VAS assessment of pain intensity. All patients received multiple injections of 200 U of commercially available BoTN/A diluted in 20 mL NS under cystoscopic guidance.

Clinical evaluation, urodynamic studies, urinary tract ultrasonography, and VAS assessment were repeated at least twice per year during follow-up. A total of 58 injections were administered, with a mean of 4.8 ± 0.8 injections per patient. The mean interval between 2 consecutive injections was 5.25 ± 0.75 months. At 1-month and 4-month follow-ups, 10 patients reported subjective improvement. Three nonresponders to initial BoTN/A intravesical treatment underwent a repeat session 3 months later with satisfactory response.

At the 1 year and 2 year follow-ups, the beneficial effect from intravesical BoTN/A persisted in all patients. The authors did not observe any adverse systemic side effects during the study period. They concluded that intravesical injections of BoTN/A are effective and safe, at least for medium-term management of patients with PBS. As the beneficial BTX effect gradually decreased over several months after previous treatment sessions, repeat injections of the neurotoxin were provided when needed over time.

Liu and Kuo (2007) reported their observations regarding the level of nerve growth factor (NGF) mRNA in bladder tissue and the effect of BoTN/A treatment in patients with interstitial cystitis (IC). Nineteen patients with IC underwent intravesical injections of 100 Units or 200 Units of BoTN/A, followed by cystoscopic hydrodistension 2 weeks later. The bladder mucosa was biopsied before BoTN/A injections and immediately after hydrodistension in study participants and in 12 control subjects. The NGF mRNA and protein levels in bladder tissues were assessed by real-time polymerase chain reaction and immunohistochemistry studies to determine differences in NGF expression between patients with IC before and after BoTN/A treatment and compared with controls.

At 3 months, 14 patients had symptomatic improvement (responders) and 5 did not (nonresponders). At baseline, the NGF mRNA levels in the overall IC patient group were significantly greater than controls. At 2 weeks after BoTN/A treatment, the NGF mRNA levels were found to be decreased and were not significantly different from the NGF mRNA levels in controls. The NGF mRNA levels decreased significantly in responders and were significantly decreased after BoTN/A in 11 patients who reported a reduction in pain of 2 or more as measured by VAS.

Immunoreactivity studies of bladder tissue from patients with IC showed greater NGF density at baseline compared with controls, but the difference was no longer significant after successful BoTN/A treatment. The authors suggest that intravesical BoTN/A injections plus hydrodistension reduce bladder pain in patients with IC. The NGF levels in bladder tissues were significantly increased in patients with IC and dropped to normal levels after treatment in responders.

The use of ureteral stents for ureteral obstruction and after ureteroscopy can result in substantially reduced patient quality of life due to pain, frequency and urgency. Gupta et al. (2010) tested their theory that numerous stent-related symptoms may be caused by detrusor muscle spasm in and around the intramural ureter by evaluating the effect of BoTN/A in patients with indwelling stents after ureteroscopy. Fifty-one patients were enrolled in a prospective, randomized, single-blind study comparing BoTN/A injections into 3 locations around the ureteral orifice (30 patients) compared with no injection after unilateral ureteral stent insertion (21 patients).

Pain and urinary symptoms after stent placement were evaluated using the Ureteral Stent Symptom Questionnaire, which was completed on postoperative day 7. In addition, patients were required to maintain a log of opioid analgesic use between stent placement and its removal. No complications or adverse events occurred during this study. A significant reduction was reported in the postoperative pain score between the patients treated with BoTN/A and the control group (e.g., 3.4 vs. 6 [$p = 0.02$]). Postoperative opioid use was less in the BoTN/A treatment group, who averaged 7.7 pills over 2.7 days compared with 24.7 pills averaged over 7 days in control patients ($p = 0.03$).

With respect to postoperative lower urinary tract symptoms, no significant difference was noted between cohorts using the individual index scores within the Ureteral Stent Symptom Questionnaire. Periureteral BoTN/A injections appear to improve ureteral stent tolerability as referenced by patient report of reduced postoperative pain intensity and decreased opioid intake over a shorter period of time following stent placement.

11.8. BoTN/A for pain associated with rectal disorders

A RCT showed that BoTN/A treatment can be effective in reducing pain after hemorrhoidectomy (Davies et al., 2003). Also, another published review suggested that BotN/A may be effective for the management of severe anorectal pain (Hawley, 2002).

The maximum resting pressure in the anal canal is markedly increased after hemorrhoidectomy, most likely due to postoperative pain, which is the most difficult early management problem after hemorrhoidectomy. Patti et al. (2007) compared the effects of intrasphincter BoTN/A injections with application of glyceryl trinitrate ointment after hemorrhoidectomy for improving wound

healing and reducing postoperative pain at rest or during defecation. Thirty patients with hemorrhoids were randomized into 2 groups. One group received an injection containing 20 Units of BoTN/A, whereas the other group received application of 300 mg of 0.2% glyceryl trinitrate ointment 3 times daily for 30 days. Anorectal manometry was performed preoperatively and then at 5 days and 40 days following hemorrhoidectomy.

Five days after hemorrhoidectomy, maximum resting pressure was significantly reduced compared with baseline values in both groups; however, postoperative pain at rest showed a significant reduction in the BoTN/A group compared with the glyceryl trinitrate group; pain during defecation and time of healing were similar. Adverse effects, such as headaches, were observed only in the glyceryl trinitrate group. At 40 days posthemorrhoidectomy, the maximum resting pressure values in the glyceryl trinitrate group were similar to those obtained preoperatively. However, the maximum resting pressure values remained decreased in the BoTN/A group. These findings support the application of a single intrasphincter injection of BoTN/A for more effective reduction of early postoperative pain at rest, although not necessarily during defecation. BoTN/A is safer and has less side effects than repeated applications of glyceryl trinitrate.

However, Singh et al. (2009) looked at 32 patients undergoing hemorrhoidectomy in a prospective randomized controlled trial. Routine postoperative care included metronidazole and bupivacaine. Patients were also randomized and given an intrasphincteric injection of either placebo or BoTN/A (150 U). A linear analogue score (VAS) was used to assess postoperative pain. The primary endpoint was reduction in postoperative pain. No significant effect on overall or maximal pain scores was noted. Median time for return to normal activities did not differ significantly between groups. BoTN/A reduced anal spasm but failed to demonstrate any significant effect on postoperative pain.

Thrombosed external hemorrhoids are a frequent anorectal emergency. They are associated with swelling and intense pain. Patti et al. (2008) randomized 30 patients with thrombosed external hemorrhoids who refused surgical operation into 2 groups. Patients received an intrasphincteric injection of either 0.6 mL saline or 0.6 mL of a solution containing 30 Units of BoTN/A. Anorectal manometry was performed before treatment and 5 days afterwards. After 5 days of treatment, the maximum resting pressure fell in both groups but was significantly lower in the BoTN/A group ($P = 0.004$). Pain intensity was significantly reduced within 24 h of BoTN/A treatment (PY<Y0.001) but only after 1 week in the placebo group ($P = 0.019$). A single injection of BTX into the anal sphincter seems to be effective in rapidly controlling the pain associated with thrombosed external hemorrhoids and could represent an effective conservative treatment for this condition.

Hollingshead et al. (2011) assessed the effectiveness of botulinum toxin A injections (20–200 Units) into the anal sphincter of 14 patients with functional anal pain (i.e., in the absence of demonstrable anal pathology). Half of these patients reported significant improvement at 3 months post-injection and 4 of 7 were asymptomatic at 3 years post-injection.

11.9. BoTN/A for pelvic pain

Chronic pelvic pain occurs in about 15% of women and has various causes that require accurate diagnosis and appropriate treatment if pain reduction is to be effected. Superficial conditions such as provoked vestibulodynia and deeper pelvic issues such as pelvic floor myalgia were traditionally difficult to diagnose and adequately treat (Abbott, 2009).

To determine whether BoTN/A is more effective than placebo for reducing pain and pelvic floor pressure in women with chronic pelvic pain and pelvic floor muscle spasm, Abbott et al. enrolled 60 women with chronic pelvic pain lasting 2 years or longer who demonstrated evidence of pelvic floor muscle spasm (Abbott et al., 2006). The methodology was a double-blind, randomized controlled trial, wherein 30 women received 80 Units of BoTN/A by injection into the pelvic floor muscles, and 30 women received injections of NS. The severity of dysmenorrhea, dyspareunia, dyschezia, and nonmenstrual pelvic pain were assessed by VAS at baseline and then monthly for 6 months. Pelvic floor pressures were measured by vaginal manometry.

A significant change from baseline in the BoTN/A group was noted for dyspareunia and nonmenstrual pelvic pain. In the placebo group, only dyspareunia was significantly reduced from baseline. A significant reduction in pelvic floor pressure (cm of H_2O) was noted in the BoTN/A group from baseline; the placebo group also had lower pelvic floor muscle pressures. The authors found an objective reduction of pelvic floor muscle spasm, which reduces some types of pelvic pain. BoTN/A reduced pressure in the pelvic floor muscles more than placebo; therefore, BoTN/A may be a useful agent in women with pelvic floor muscle spasm and chronic pelvic pain who do not respond to conservative treatment, including physical therapy.

Rao and Abbott (2009) reviewed the gynecological use of BoTN/A for the treatment of chronic pelvic pain in women. BoTN/A was advocated for use in inflammatory conditions and in areas where muscle spasm was thought to contribute to pain. They acknowledged the limited data that support or specify the use of BoTN/A for gynecological indications. Support for use in the vulva consists of case reports and small series, which indicate that BoTN/A, when used in the vulva, may provide benefit for 3–6 months after injection of 20–40 Units for women with provoked vestibulodynia. Retreatment is reportedly successful, and side effects are limited. Controlled studies are essential to further explore this indication.

For pelvic floor muscle spasm, a greater number of women have been studied and a double-blind, randomized controlled study reported a significant reduction in pelvic floor pressures, with significant pain reduction for some types of pelvic pain compared with baseline. No differences in pain were noted when compared with the control group who had physical therapy as an intervention. Physical therapy can be used as a first line treatment or adjunctively with BoTN/A injections in cases of refractory pain and muscle spasm.

In a review by Rao and Abbott (2009), they cited pain symptoms caused by pelvic floor muscle spasm, daily pelvic pain, and dyspareunia are the most likely to be improved by BoTN/A. Limited data supporting the use of BoTN for provoked vestibulodynia indicate an improvement in pain scores. In the lower GI tract, BoTN/A injection into puborectalis has demonstrated objective improvement in intravaginal pressures, although no randomized controlled trials (class I studies) have validated its use in this setting. Class I studies demonstrate a role for BoTN/A in the management of idiopathic detrusor overactivity, although long-term follow-up data are lacking.

Potential problems with BoTN/A use include reactions to the toxin and urinary and fecal incontinence. A single class I study supports the use of BoTN/A for refractory pelvic floor spasm; however, further adequately powered class I studies for this indication and for provoked vestibulodynia are warranted.

For pelvic floor myalgia, 1 class-I study and 3 class-II to -III studies have indicated efficacy of BoTN/A. In the only double-blind, randomized controlled trial, significant reduction in pelvic floor pressures with significant pain reduction for some types of pelvic pain were reported compared with baseline. No differences in pain occurred compared with the control group who had physical therapy as an intervention. Physical therapy should be used as first-line

treatment and then adjunctively with BoTN/A injections for those who remain refractory to treatment (Rao and Abbott, 2009).

12. Botulinum toxin for the treatment of headache

12.1. Introduction

In 1988 the International Headache Society (IHS) established the first *Classification and Diagnostic Criteria for Headache Disorders, Cranial Neuralgias and Facial Pain* (Headache Classification Committee, 1988). The IHS classification system allowed headache researchers and clinicians to define headache disorders using concordant criteria and terminology (Evans and Olesen, 2003)

In 2004, after years of scrutiny, the Classification Committee of the IHS published the second edition of *International Classification of Headache Disorders* (ICHD-II) which divides headaches into primary and secondary disorders (Headache Classification Committee, 2004). Primary headache disorders are defined as those which occur as the result of a primary neurological process, whereas secondary headaches are attributed to an identifiable underlying cause or condition of the nervous system. Primary headache disorders include migraine, tension-type headaches and cluster headaches, among others. The 2004 ICHD II also cites craniocervical dystonia (CD) as an accepted cause of headache (Headache Classification Committee, 2004).

Migraine is the most common headache disorder that requires physician treatment. Migraine affects an estimated 28 million people in the United States (US) (Lipton et al., 2001). Approximately, 18% of women and 6% of men experience migraine in the US (4). It is more prevalent in primary care settings than diabetes, hypertension and asthma (Lipton et al., 2001). The economic impact of migraine is enormous, with over 112 million missed worked days per year, an estimated cost to American employers of 8 billion dollars per year, and with over one billion healthcare dollars spent annually (Unger et al., 2003; Hu et al., 1999). When migraine-related work absence is combined with reduced productivity, the cost to US employers is estimated at more than 13 billion dollars annually (Hu et al., 1999).

Migraine is defined as a headache with at least moderate intensity that is frequently unilateral, throbbing or exacerbated by physical activity and usually accompanied by nausea, photophobia and phonophobia. Tension-type headache (TTH) is characterized as a band-like tightness without migraine-associated symptoms. In 1994, a revision of the established IHS criteria was proposed using the eponym "chronic daily headache" (CDH) to describe a condition that occurs more than 15 days/month with an average untreated duration of >4 h/day (Silberstein et al., 1994).

12.2. BTX for primary headache disorders

Two BoNT/A formulations (BOTOX® and Dysport®) and one BoNT/B formulation (Myobloc®) are approved for clinical use. Anecdotal reports suggest that Myobloc® can be effective therapy in cases of craniofacial pain; however, no clinical trials have been published.

The potential for BoNT as a treatment for tension-type and migraine-type headaches was discovered by Binder somewhat serendipitously in the early 1970s when he was conducting clinical trials to determine the efficacy of BoNT/A as a treatment for hyperfunctional lines of the face. A retrospective review of headache patients who were receiving BoNT/A injections for neurology, otolaryngology, or cosmetic indications also reported reduced headache frequency (Binder et al., 1998b). A prospective, non-randomized, open label study of 106 patients was designed by Binder et al. (2000) to determine a relationship between BOTOX® treatment and the reduction of headache. The patient cohort was drawn from patients seeking treatment for hyperfunctional facial lines or dystonia who were experiencing concomitant headache. Seventy-seven patients satisfied IHS criteria for migraine. Following treatment with BOTOX®, 51% of 77 patients with migraine had complete resolution of symptoms for a mean duration of 4.1 months, and 38% reported partial improvement for a mean duration of 2.7 months (Binder et al., 2000). A retrospective review published in 2000 by Mauskop and Basedo (2000) suggested that BTX-A was potentially efficacious therapy for migbraine prophylaxis with reduced headache frequency and severity following injections placed in pericranial regions. Eross and Dodick (2002) also demonstrated reduced disability caused by migraine, as measured by the MIDAS questionnaire. Subjects with EM appeared to experience the greatest benefit when compared to subjects with CM (Eross and Dodick, 2002). Troost (2002) used a standardized or "fixed-site" protocol and multiple treatments in a prospective, open-label study of 134 intractable headache patients, who received 30–100 Units of BOTOX®, and were observed over 8 months. Improvements in clinical and patient reports, including overall decreased headache pain, were demonstrated without disclosure of any serious AEs (Troost, 2002).

Other retrospective reviews found support for a beneficial role using BOTOX® as a prophylactic treatment for EM and CM (Blumenfeld, 2002; Mauskop, 2002a,b; Mathew et al., 2002; Miller and Denny, 2002). However, these studies were limited by faulty methodology, low numbers of study patients, poorly defined end points, and heterogeneous patient populations, often including other IHS described types of CDH (Dodick et al., 2004).

12.2.1. Migraine headaches

A double-blind, placebo (PBO)-controlled clinical study of 123 patients with a history of 2–8 moderate to severe EM headaches per month were randomly divided into groups that received a single injection of either 25 Units or 75 Units of BOTOX® or the vehicle-PBO (Silberstein et al., 2000). Injection sites were standardized and located in the bilateral frontalis, glabellar and temporalis muscles. Patients kept daily diaries recording migraine frequency, severity and occurrence of migraine-associated symptoms. In the second study month, the 25 Unit BOTOX® group showed significant reductions in EM frequency and severity, acute medication-use and associated vomiting symptoms when compared to the PBO group. Additional improvements were observed in EM frequency in both treatment groups when these parameters were measured at 3 months. Although the 25 Unit group seemed to derive a greater degree of benefit, the 75 Unit BOTOX® group showed significant improvement in Global Assessment scores at month 2 when compared to PBO. Treatment-related AEs included blepharoptosis, diplopia and injection site weakness. These AEs were all transient and may have been initially and primarily related to injector inexperience and technique, which reportedly improved (Silberstein et al., 2000).

A randomized, PBO-controlled trial published in 2002 evaluated the efficacy and tolerability of BOTOX® as prophylactic treatment for EM (Barrientos and Chana, 2002). Thirty patients who reported 2–8 migraine-type headaches per month were randomly assigned to receive PBO or 50 Units of BOTOX® into 6 regions containing 15 fixed muscle sites: the temporalis, frontalis, trapezius and splenius regions each received 10 Units, the glabellar—8 Units; and procerus—2 Units. No additional prophylactic medications were permitted during the study. Patients were assessed by general neurologic examinations and a migraine survey at baseline, 30, 60 and 90 days after treatment (Barrientos and Chana, 2002) Overall, BOTOX®-treated patients experienced significant reductions in the frequency and duration of EM attacks associated with nausea at all assessment points and reduced duration of migraine attacks

without nausea at 90 days. The use of acute migraine medication therapies (e.g., NSAIDs, triptans) was also reduced throughout the study. Global Assessments by both patients and investigators showed markedly concordant improvements in the majority of the BOTOX®-treated patients. Injections were well tolerated with only one reported minor AE due to a cosmetic asymmetry (Barrientos and Chana, 2002).

However, evidence of benefit was not as clearly demonstrated in three recently reported double blind, randomized, PBO-controlled studies. Relja et al. (2005) randomized patients with EM occurring between 3 and 15 days per month after a single-blind 30-day PBO run-in. Patients were randomized within PBO responder or nonresponder strata into PBO and 3 BOTOX® treatment groups of 225 Units, 150 Units or 75 Units. A fixed-site, fixed-dose injection methodology was employed for 3 treatment sessions at 90-day intervals. The primary efficacy measure was a mean change from baseline and EM frequency between day 150 and day 180 in the PBO nonresponder strata. Four hundred and ninety-five patients (322 PBO nonresponders) were randomized into 1 of the 3 BOTOX® treatment groups or PBO. All PBO and BOTOX® treated groups showed substantial reductions in EM frequency; however, no statistically significant differences were seen among treatment groups. Results may have been confounded by study participants' over usage of acute rescue and abortive medications.

Saper et al. (2005) performed a randomized, double-blind, PBO-controlled study of 232 patients who reported 4–8 moderate to severe EM headaches per month. Patients were randomized into PBO or 1 of 4 BoNT/A treatment groups. Three BoNT/A groups consisted of patients receiving a single injection in the frontal, temporal or glabellar regions; a fourth group received therapeutic injections into all 3 areas. For 3 months following a single injection session, patients recorded pertinent evaluation variables and periodically completed quality of life questionnaires. All BoNT/A groups and the PBO group produced a comparable reduction in frequency of EM from baseline, but there was no significant between-group differences observed for any of the multiple efficacy measures for EM treatment. Again, study results may have been confounded by excessive patient-use of concomitant prophylactic and acute headache medications (Saper et al., 2005).

In a third randomized, PBO-controlled study by Elkind et al. (2005), patients with 4–8 moderate to severe EMs per month were randomized into PBO or into one of 3 BOTOX® treatment groups receiving 7.5 Units, 25 Units, or 50 Units. Injections were placed into predetermined fixed sites in the frontal, glabellar and temporal muscles. Patients were subjected to 3 injection cycles, 4 months apart. Patients receiving PBO or 7.5 Units were then randomized into a second study to receive a masked higher dose of 25 Units or 50 Units. Patients in the initial 25 Units and 50 Units groups received 2 masked treatment sessions of their previously assigned dose. Patients completing the second study arm were next randomized into another 3 treatment groups: BOTOX® 25 Units, BOTOX® 50 Units, or PBO. In the first randomized study-arm, all groups showed a comparable reduction in frequency of EM at all time points; however, the BOTOX® groups demonstrated statistically significant improvement over the PBO group for Global Assessment at 4 months. Improvements in all groups observed in the first study were sustained through both the second and third arms of the study. Therefore, this study failed to show a significant reduction in frequency of migraine compared to PBO, which the investigators determined was the primary reason for prescribing a prophylactic migraine therapy. Again, results may have been affected by excessive patient-use of prophylactic and acute headache medications (Elkind et al., 2005).

12.2.2. Technical considerations and injection strategies

BoNT is an appropriate consideration for treatment of refractory headache, especially CDH, and when standard pharmacology is ineffective or fraught by adverse side effects. The transformation of EM and TTH to CDH may result from peripheral and central sensitization involving vascular and muscular tissues innervated by trigeminal and pericranial (including upper cervical) nerves. Also, BoNT prophylaxis is an enticing alternative to many standard preventive medications which interfere with alertness or cognitive efficiency in people who provide complex intellectual services or operate industrial equipment, including aircraft or other vehicular machinery. Therapeutic benefits may occur due to elimination of localized myalgia, muscular triggers, or painful muscle tension that accompany a headache, however, theorized BoNT CNS antinociceptive effects may prove to be more salient (Wheeler, 2000).

Clinical practice and research have led to two basic BoNT injection paradigms for headache. The "fixed-site" method targets standard craniofacial and cervical sites with a range of predetermined BoNT doses (Blumenfeld et al., 2003). This method is frequently implemented for treatment of EM, and bilateral application is apropos, even when migraine is exclusively unilateral. Symmetrical placement of neurotoxin improves the likelihood of a favorable cosmetic outcome. The "follow the pain" approach is often utilized for treatment of CTTH, but can be applied for EM and CM by distributing injections into areas that demonstrate tenderness or describe the headache location (Blumenfeld et al., 2003) Frequently, craniofacial, pericranial and cervical musculotendinous sites that act as migraine triggers or as pain generators during the headache are targeted by the authors. Palpation of these actively involved muscles may reveal spasm and tenderness. Some clinicians advocate subdermal injections or toxin placement adjacent to emerging branches of the trigeminal nerve, e.g., supraorbital and supratrochlear nerves.

After written informed consent, injection sites and dosing are pre-planned so that the procedure is performed efficiently. Sometimes, a patient may blame BoNT therapy for a preexisting cosmetic flaw; therefore, preinjection photographs are advisable. By avoiding needle contact with the periosteum, using small volumes of concentrated injectate (1 cm^3 preservative-free normal saline per 100 Units of BOTOX®) and using a 30-gauge 1/2 to 1″-inch needle coupled with deliberate rapid injection techniques, topical anesthesia is usually unnecessary. In heavily muscled or obese patients a longer needle, up to 1 1/4-inch, may be required to reach symptomatic muscles in the cervical-thoracic paraspinal and trapezius regions (Wheeler, 2000). Some injectors advocate a higher dilution of BOTOX® 100 u into 2–4 cm^3 of vehicle (Blumenfeld et al., 2003).

Most advocate precise placement of injections into pertinent sites with minimal unwanted diffusion. Areas to avoid include the inferior-lateral frontalis where weakness may cause brow ptosis. Injections into the middle and lower face must be carefully placed and dosed to avoid asymmetry of the mouth or dysphagia. Unwanted diffusion of the neurotoxin behind the orbit causing diplopia or eyelid ptosis is best avoided by performing periorbital injections with the patient sitting, so that the head and neck are vertical.

Following some craniofacial and periorbital injections, patients are less likely to experience ocular side effects if instructed to remain in a vertical posture and to avoid touching or manipulating the injected areas for as long as 3 h. Increased headache pain with muscle spasm at the injection sites can occur in as many as 20% of patients and is maximal during the 2–10 days following treatment. This adverse side effect is often alleviated by manual physiotherapy and/or safely-placed injections of a local anesthetic agent.

The desired degree of paresis induced by chemodenervation is determined by the muscle's role in headache production and

its function. Most people can compensate for dense paresis of the emotive glabellar and frontalis muscles by the facility of the eyes to express feelings. Conversely, the temporalis and masseter muscles work synergistically to perform mouth closure necessary for mastication. The degree of weakness desired in these muscles is determined by the extent that they influence facial pain or headache. Therefore, BoNT dosages must be calculated to maintain masticatory function with sufficient therapeutic dosing to reduce pain (Wheeler, 2000).

Furthermore, dosing for pain relief should produce or preserve a balance of strength between the temporalis muscle and its synergistic partner, the masseter (Wheeler, 2000). EMG needle guidance to assure correct needle placement into the masseter is useful, but usually unnecessary. The needle is guided into the body of the muscle, specifically into symptomatic spasm or trigger points, by grasping the painful muscle between the thumb externally to the needle insertion site on the skin and then placing the second and third fingers intraorally.

Needle depth and placement into the target area is monitored, and any penetration of the needle intraorally should be readily discovered. BoNT should never be injected until the needle has reached the intended target site and the operator is confident that placement is correct. If the needle enters the oral cavity BTX injection should be aborted. The needle must be withdrawn and placed through a new site, with EMG guidance if necessary, into a site that will not allow BTX diffusion through a prior mucosal puncture site. A safer approach would entail delaying the procedure for 6–8 weeks, if the patient did not experience any adverse effects, such as hoarseness or dysphagia. BoNT injection into the oral cavity, especially if swallowed, may cause a serious, potentially lethal, paresis of pharyngeal musculature (Wheeler, 2000).

The upper cervical and occipital muscles, especially the occipitalis, splenius capitis and cervical paraspinal muscles, may cause headache and trigger migraine. Often, these muscles contribute to pain and headache by irritation of the adjacent greater occipital nerve, causing concomitant neuropathic pain or symptoms of neuralgia. Frequently, the trapezius or symptomatic paratho-racic muscles can trigger headache. Injections into this region may induce unwanted weakness of the supraspinatus and infraspinatus muscles, which form part of the rotator cuff, causing the humeral head to rise. Injection of trapezius and levator scapulae muscles may cause the acromion to shift anteriorly and sag inferiorly. This can result in a painful shoulder impingement syndrome, which usually manifests 7–10 days following BTX treatment.

Onset of a BoNT/A clinical effect usually occurs at 7–10 days and plateaus at 3 weeks. The neuromuscular blocking action of BoNT/A lasts 3–4 months; however, the duration of reduced pain can be substantially longer, and a mitigation effect more specific for migraine may continue to develop beyond 2–3 months after the injection session. Headache improvement can be identified through the use of a diary and other self-reporting measures. Other salient measures include reduction of oral prophylactic medications, improved response from abortive therapies, as well as reduced frequency, intensity and severity of headache symptoms.

However, to conduct a large multicenter study, rules that governed injection technique and dosing were necessary to adopt to give the study a reasonable opportunity of using successful injectors, thereby providing the best chance for a successful outcome. Patients were recruited if they satisfied the definition of chronic migraine.

After written consent, injection sites and dosing are preplanned so that the procedure is performed efficiently. Sometimes, a patient may blame BoNT therapy for a preexisting cosmetic flaw; therefore, preinjection photographs are advisable. By avoiding needle contact with the periosteum, using small volumes of concentrated injectate (1–2 cm^3 of preservative-free normal saline per 100 Units) and using a 30-gauge, 0.5–1 inch needle coupled with deliberate rapid injection techniques, topical anesthesia is usually unnecessary.

In heavily muscled or obese patients, a longer needle, as long as 1.5 inch, may be required to reach symptomatic muscles in the cervical-thoracic paraspinal and trapezius regions (Wheeler, 2000). Some injectors advocate a higher dilution of BOTOX® 100 Units into 2–4 cm^3 of vehicle (Blumenfeld et al., 2003).

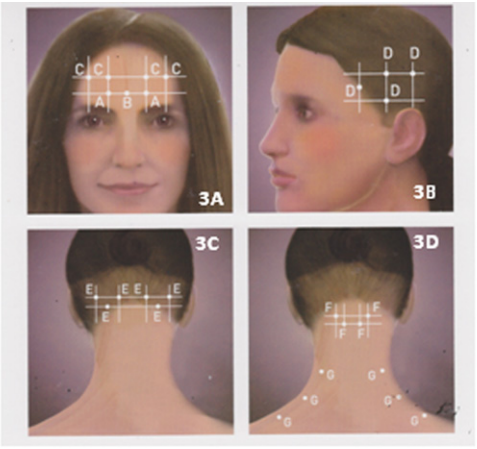
Therapeutic BoNT dosages and injection techniques vary between individuals and between clinician disorders that affect the same muscle groups, as exemplified by hemifacial spasm, dystonia, and cosmetically undersireable hyperkinetic facial lines. The number of injection sites and total BoNT dosages vary among clinicians, but should be individualized for each patient. Factors that may affect dosing include injection methodology, headache type of severity, treatment of adjacent or regional areas of involvement and the subject body's habitus. Many characteristic injection sites contact small, nodular, tender TrPs in distribution patterns similar to those described by Simons and Travell (1983). Standardized criteria for BOTOX® treatment of all primary and secondary headache disorders have been published, but are not yet established (Wheeler, 2000; Blumenfeld et al., 2003). Typical injection sites are demonstrated in Fig. 3, and suggested dosing ranges for BOTOX® are outlined in Table 5. These dosing ranges are based on the extensive medical literature that has been noted in this specific treatise, and is suggested, not proven nor advocated by the FDA. However, not all health insurers will approve nor can all patients afford the FDA recommended dosing of 155 Units.

12.3. FDA-approved treatment paradigm for chronic migraine

Allergan needed to adapt a uniform injection paradigm for chronic migraine treatment among >125 centers to recruit a satisfactory number of participants and achieve safety and efficacy measures that were statistically significant for FDA-approval (Aurora et al., 2010). Allergan mandates this same protocol for clinical use by physicians now, regardless of headache location or despite involvement of regional craniofacial or cervical tissues. Workshops and extensive educational materials are used to enforce precise, standardized methods for reconstitution of BOTOX® and structured injection techniques. Allergan advocates 100 Units of BOTOX® diluted in 2 cm^3 of preservative-free normal saline, which results in a concentration of 5 Units/per 0.1 mL [207].

Reconstitution is performed using a vial of BOTOX®, which must remain upright. A 21-gauge, 2-inch needle is attached to a 5 mm syringe. Dilution of 100 unit vial of BOTOX® is accomplished using 2 mL of preservative-free 0.9% sodium chloride (saline); therefore, a 200-Unit vial is diluted with 4 mL of preservative-free saline. Each vial contains a vacuum, which should pull the saline into the vial. Do not use a BOTOX® vial if the vacuum is absent or reduced and does not pull all the saline freely from the syringe. Leave the reconstitution needle and syringe in the bottle while gently rotating the vial to mix the BOTOX® and saline (© Allergan Inc.)

Next, hold the hub of the needle and remove the reconstitution syringe and replace it with a 1-mL injection syringe. Do not invert the BOTOX® vial while withdrawing the solution into the 1-mL injection syringe. Withdraw 1 mL of the reconstituted solution into the 1-mL injection syringe. Disconnect the first 1-mL injection syringe from the hub of the needle and then attach a sterile 30-gauge 0.5-inch needle to the syringe for injection. Repeat the BOTOX® withdrawal procedure with 3 additional 1-mL injection syringes and then attach 30-gauge, 0.5-inch needles per the Allergan, FDA-approved protocol. An off-label suggestion by the author is to consider attachment of 30-gauge, 1-inch needles to 2 of the syringes for use with the cervical and trapezius injections (© Allergan Inc.).



Order	Muscle	Total Recommended Dosage, Number of Sites**
A	Corrugator*	10 Units divided in 2 sites
B	Procerus*	5 Units in 1 site
C	Frontalis*	20 Units in 4 sites
D	Temporalis*	40 Units in 8 sites
E	Occipitalis*	30 Units divided in 6 sites
F	Cervical paraspinsal*	20 Units divided in 4 sites
G	Trapezius*	30 Units divided in 6 sites
Total Dose		155 Units divided in 31 sites
*Dose distributed bilaterally		**Each IM injection site = 0.1 mL = 5 units BOTOX®

Fig. 3. BOTOX® injection protocol for chronic migraine patients: dosing by muscle(S).

This BOTOX® protocol uses a fixed-site, fixed-dose injection paradigm. Prior to injection the skin over the intended and targeted injection site should be cleaned with an alcohol swab. Each injection consists of 0.1 mL of reconstituted BOTOX® (5 Units). This injection protocol is characterized by fixed injection doses into specific muscle sites (e.g., a fixed number and location of all injection sites with a fixed total dose of BOTOX®) (Table 5). Hold the needle hub with one hand so that it can be angled appropriately away from any danger and to avoid the periosteum. The bevel of the needle and the numbers on the syringe that delineate measurements on the syringe contents should face upward. The second hand controls the plunger.

The first recommended injection sites are the corrugators (A in Fig. 3a). The corrugator muscle injection site (MIS) is located approximately 1 fingerbreadth (approximately 1.5 cm) above the superior edge of the medial orbital ridge. Inject with the beveled

Table 5
Guidelines for headache treatment: botox dosing of specific muscles.

Common injection sites		Botox dose (U)	Botox dose (U)	Number of injection
Muscle	Abbreviation	Per site	Per muscle (each side)	Sites per side
Fixed site method. Characteristic injection sites.				
Procerus	P	2.5	2.5–*10*	1–2 (midline vertical or horizontal relationship)
Corrugator supercilii				
Medial	Mcs	2.5	2.5	1
Lateral	Lcs	2.5	2.5	1
Frontalis	f_1, f_2	2	20	2–6
Temporalis	t_1, t_2	2.5	30	4–6
Occipitalis	O	10	20	1–2
Follow the pain method. Common injection sites.				
Splenius capitis	c_2	5–7.5	5–15	1–2
Cervical paraspinal	c_1	5–7.5	5–15	1–2
Masseter	*M*	10–50	10–50	*1–2*
Trapezius	z_1, z_2	5–10	15–50	3–4

Note: Regular text denotes characteristic "fixed-site" method dosages and injection sites. Italic text denotes "follow-the-pain" location choices, doses, and number of sites.

needle pointing upward at a 45° angle away from the medial aspect of the muscle to avoid ptosis of the eyelid. The Allergan protocol also suggests starting on the left and moving to the right. First the left then the right corrugator MIS are each dosed with 0.1 mL or 5 Units of BOTOX® (A in Fig. 3a).

Corrugator, procerus, frontalis, and temporalis muscles are all injected (by order of protocol) with the patient supine. Each designated muscle site is injected with 0.1 mL of BOTOX® solution. The procerus is located midline on the forehead approximately 1 fingerbreadth above and midline to the medial superior aspect of the orbital ridge of each eye. The injection site for this muscle should appear approximately midway between both corrugator injection sites. A single straight line should connect all 3 of the injection sites. The injection technique is repeated with a beveled needle upward and the needle pointing approximately 45° upward and away from the pain-sensitive periosteum. The procerus is injected with 1 mL (B in Fig. 3a) (© Allergan Inc.).

Next, with the patient remaining supine, a line is drawn upward on the forehead from the medial edge of the orbital ridge about 1 fingerbreadth above each corrugator MIS. These are marked and additional sites are parallel, approximately 1 fingerbreadth lateral to the first 2 frontalis MIS. Each site is injected, first on the left and then on the right. Each of the 4 MIS receives 0.1 mL or 5 Units of BOTOX® solution (C in Fig. 3a) (© Allergan Inc.).

Next the temporalis muscles are dosed with 4 injections of 0.1 mL each bilaterally. The patient is asked to clinch his or her teeth, whereby the examiner is able to palpate the anterior aspect of the temporalis muscle. The first injection is performed about 2 fingerbreadths beyond this point and beneath the hairline. The second injection is performed 0.5 cm superior and approximately 1 fingerbreadth posterior to the first injection into the medial aspect of the muscle. Injection 3 should be parallel and 1.5 cm posterior to the second injection. Injection 4 should be approximately 1.5 cm below and perpendicular to the second injection into the medial aspect of the muscle (D in Fig. 3b) (© Allergan Inc.).

Next, the patient is moved to a sitting posture. Occipital injection sites are identified by palpating the external occipital protuberance. The examiner continues palpation superior to the supranuchal ridge on either side of the occipital protuberance. Beginning with the left occipitalis muscle, the first injection is placed just above the occipital protuberance along the supranuchal ridge, and 1 cm leftward of the external occipital protuberance. The second injection is 1 cm lateral and 1 cm above the first injection site. The third injection is 1 cm medial and superior to the first injection site (E in Fig. 3c).

Next, the cervical paraspinal muscle injections are performed. The first injection into the cervical paraspinal muscles is placed about 1 cm to the left of the midline and 3–5 cm inferior to the occipital protuberance. The second injection site is 1 cm superior diagonally toward the ear from the first injection. The same injection sites are measured as mirror images on the right, leading to a total of 4, each receiving 0.1 mL or 5 Units (F in Fig. 3d) (© Allergan Inc.).

The trapezius muscle is a triangular shaped superficial muscle that spans from the neck to the shoulder. Visualize a proportion of the muscle from the neck to the shoulder into 3 sections per side. Injections are placed into the middle of each of these sections. The protocol recommends that the injector begin by treating the left trapezius muscle. Each of the 3 sites on the left and then on the right (totaling 6) receives 0.1 mL or 5 U of BOTOX® solution (G in Fig. 3d).

Following the procedure the patient is asked to remain vertical for 2–3 h and not to rub the injected areas in any vigorous manner. The patient may return to their normal activities on the following day (© Allergan Inc.).

12.4. Summary

Considerations for the Clinical Use of Botulinum Toxins

- Currently, only BOTOX® is FDA-approved for the treatment of chronic migraine.
- Significant side effects are uncommon. Pain, muscle weakness, and flu-like symptoms have been reported (Baizabal-Carvallo et al., 2011). Spread of toxin has been noted with weakness, sometimes involving muscles that were not directly injected. Autonomic side effects appear to be more commonly seen with type B toxin.
- Contraindications to treatment with BoNT include pregnancy (category C), the concurrent use of aminoglycoside antibiotics, myasthenia gravis, Eaton-Lambert syndrome, or known sensitivity to the toxins.
- Treating more frequently than the recommended interval of 12 weeks may lead to the development of antibodies to the toxin, which may also be associated with the development of clinical resistance.
- There is no valid way to reliably and consistently convert doses of type A toxin to doses of type B toxin are available at present.
- The use of BoNT for pain management is part of a comprehensive treatment program that has been developed based on an accurate diagnosis.
- Be aware of current storage and handling recommendations for each of the toxins.
- Whenever possible, use an injection technique, including needle size, that is the least likely to cause additional pain.
- Guidance techniques such as EMG, CT, or fluoroscopy should be used at the discretion of the injector.
- Prolonged observation following the injections is generally not warranted.
- Follow-up should be arranged for 4–6 weeks following the injections.
- More than one series of injections may be required to achieve maximal analgesic response.

13. Conclusion

Botulinum toxin therapy has had a dramatic impact on clinical practice as it has provided a new and powerful tool for therapeutic and medical management. It has raised both scientific and public awareness about dystonia, other skeletal and smooth muscle contraction disorders, or painful conditions and hypersecretory glandular disorders. This therapy has brought together multiple disciplines to address and rethink human neurophysiology, and has stretched our basic thinking about the pathophysiology of disease.

Botulinum toxin therapy provides a key example of the value of translational research, which relies on the iterative interplay between clinical observation and laboratory discovery. There are clinical conditions effectively treated with botulinum toxin therapy type A, in which we do not fully understand the mechanism of therapeutic action on disease manifestations. Basic scientific investigation has the potential to illuminate these mysteries, which will allow us to address therapeutic issues more precisely.

Botulinum toxin therapy has joined the ranks of innovative drugs that have changed the nature of human suffering across multiple disciplines (Barrientos and Chana, 2002). When used responsibly, botulinum toxin therapy can provide physicians with a therapeutic tool that provides patients with symptomatic relief for long periods, and has a positive impact on their quality of life.

Conflict of interest

The authors declare that there are no conflicts of interest.

References

Abbott, J., 2009. Gynecological indications for the use of botulinum toxin in women with chronic pelvic pain. Toxicon 54, 647–653.

Abbott, J.A., Jarvis, S.K., Lyons, S.D., Thomson, A., Vancaille, T.G., 2006. Botulinum toxin type A for chronic pain and pelvic floor spasm in women: a randomized controlled trial. Obstet. Gynecol. 108, 915–923.

Acquadro, M.A., Borodic, G.E., 1994. Treatment of myofascial pain with botulinum A toxin [letter]. Anesthesiology 80, 705–706.

Alderson, K., Holds, J.B., Anderson, R.L., 1991. Botulinum-induced alteration of nerve-muscle interactions in the human orbicularis oculi following treatment for blepharospasm. Neurology 41, 1800–1805.

Ansved, T., Odergren, T., Borg, K., 1997. Muscle fiber atrophy in leg muscles after botulinum toxin type A treatment of cervical dystonia. Neurology 48, 1440–1442.

Aoki, K.R., 2001a. Pharmacology and immunology of botulinum toxin serotypes. J. Neurol. 248 (3), 10.

Aoki, K.R., 2001b. A comparison of the safety margins of botulinum neurotoxin serotypes A, B, and F in mice. Toxicon 39, 1815–1820.

Aoki, K.R., 2008. Future aspects of botulinum neurotoxins. J. Neural. Transm. 115, 567–576.

Apfel, S.C., 2009. Botulinum toxin for neuropathic pain? Neurology 72, 1456–1457.

Aurora, S.K., Dodick, D.W., Turkel, C.C., DeGryse, R.E., Silberstein, S.D., Lipton, R.B., Diener, H.C., Brin, M.F., PREEMPT 1 Chronic Migraine Study Group. Onabotulinum, 2010. A for treatment of chronic migraine: results from the double-blind, randomized, placebo-controlled phase of the PREEMPT 1 trial. Cephalgia 30, 793–803.

Bai, J., Wang, C.T., Richards, D.A., Jackson, M.B., Chapman, E.R., 2004. Fusion pore dynamics are regulated by synaptotagmin*t-SNARE interactions. Neuron 41, 929–942.

Baizabal-Carvallo, J.F., Jankovic, J., Pappert, E., 2011. Flu-like symptoms following botulinum toxin therapy. Toxicon 58, 1–7.

Barinaga, M., 1993. Secrets of secretion revealed. Science 260, 487–489.

Barrientos, N., Chana, P., 2002. Efficacy and safety of botulinum toxin type A (Botox) in the prophylactic treatment of migraine [abstract S106]. In: Presented at the American Headache Society's 44th Annual Scientific Meeting, Seattle, WA, June 21–23.

Barwood, S., Baillieu, C., Boyd, R., Brereton, K., Low, J., Nattrass, G., Graham, H.K., 2000. Analgesic effects of botulinum toxin A: a randomized, placebo-controlled clinical trial. Dev. Med. Child Neurol. 42, 116–121.

Becher, A., Drenckhahn, A., Pahner, I., Margittai, M., Jahn, R., Ahnert-Hilger, G., 1999. The synaptophysin-synaptobrevin complex: a hallmark of synaptic vesicle maturation. J. Neurosci. 19, 1922–1931.

Bhalla, A., Chicka, M.C., Tucker, W.C., Chapman, E.R., 2006. Ca2+-synaptotagmin directly regulates t-SNARE function during reconstituted membrane fusion. Nat. Struct. Mol. Biol 13, 323–330.

Bigalke, H., Wohlfarth, K., Irmer, A., Dengler, R., 2001. Botulinum A toxin: dysport improvement of biological availability. Exp. Neurol. 168, 162–170.

Biglan, A.W., Gonnering, R., Lockhart, L.B., Rabin, B., Fuerste, F.H., 1986. Absence of antibody production in patients treated with botulinum A toxin. Am. J. Ophthalmol. 101, 232–235.

Binder, W.J., Blitzer, A., Brin, M.F., 1998a. Treatment of hyperfunctional lines of the face with botulinum toxin A [In Process Citation]. Dermatol. Surg. 24, 1198–1205.

Binder, W.J., Brin, M.F., Blitzer, A., Schenrock, L., Diamond, B., 1998b. Botulinum toxin type A (BTX-A) for migraine: an open label assessment. Mov. Disord. 13, 241.

Binder, W.J., Brin, M.F., Blitzer, A., Schoenrock, L.D., Pogoda, J.M., 2000. Botulinum toxin type A (BOTOX) for treatment of migraine headaches: an open-label study. Otolaryngol. Head Neck Surg. 123, 669–676.

Black, J.D., Dolly, J.O., 1986. Interaction of 1251-labeled botulinum neurotoxins with nerve terminals. II. Autoradiographic evidence for its uptake into motor nerves by acceptor-mediated endocytosis. J. Cell. Biol. 103, 535–544.

Blackie, J.D., Lees, A.J., 1990. Botulinum toxin treatment in spasmodic torticollis. J. Neurol. Neurosurg. Psychiatry 53, 640–643.

Blitzer, A., Brin, M.F., Keen, M.S., Aviv, J.E., 1993. Botulinum toxin for the treatment of hyperfunctional lines of the face. Arch. Otolaryngol. Head Neck Surg. 119, 1018–1022.

Blumenfeld, A., 2002. Botulinum toxin type A (BOTOX®) as an effective prophylactic treatment in headache [abstract 81]. In: Presented at the 6th headache Congress, European Headache Federation, Istanbul, Turkey, June 26–30.

Blumenfeld, A., Binder, W., Silberstein, S.D., Blitzer, A., 2003. Procedures for administrating botulinum toxin A for migraine and tension headaches. Headache 43, 884–891.

Borg-Stein, J., Pine, Z.M., Miller, J.R., Brin, M.F., 1993. Botulinum toxin for the treatment of spasticity in multiple sclerosis. New observations. Am. J. Phys. Med. Rehabil. 72, 364–368.

Borodic, G., 1998. Myasthenic crisis after botulinum-toxin [letter]. Lancet 352, 1832.

Borodic, G.E., Ferrante, R.J., Pearce, L.B., Alderson, K., 1994. Pharmacology and histology of the therapeutic application of botulinum toxin. In: Jankovic, J., Hallett, M. (Eds.), Therapy with Botulinum Toxin. Marcus Dekker, New York, pp. 119–157.

Brashear, A., Lew, M.F., Dykstra, D.D., Comella, C.L., Factor, S.A., Rodnitzky, R.L., Trosch, R., Singer, C., Brin, M.F., Murray, J.J., Wallace, J.D., Willmer-Hulme, A., Koller, M., 1999. Safety and efficacy of NeuroBloc (botulinum toxin type B) in type A-responsive cervical dystonia. Neurology 53, 1439–1446.

Brin, M.F., 2000. Botulinum toxin therapy: basic science and overview of other therapeutic applications. In: Blitzer, A., Binder, W.J., Boyd, J.B., Carruthers, A. (Eds.), Management of Facial Lines and Wrinkles. Lippincott Williams & Wilkins, New York, pp. 279–302.

Brin, M.F., 1997. Botulinum toxin: new and expanded indications. Eur. I. Neurol. 4, 5, 9–66.

Brin, M.F., 1998. Treatment of dystonia. In: Lankovic, J., Tolosa, E. (Eds.), Parkinson's Disease and Movement Disorders. Williams & Wilkins, New York, pp. 553–578.

Brin, M.F., Binder, W.J., Blitzer, A., Schenrock, L., Pogoda, J.M., 2002. Botulinum toxin type A for pain and headache. In: Brin, M.F., Hallett, M., Jankovic, J. (Eds.), Scientific and Therapeutic Aspects of Botulinum Toxin. Lippincott Williams & Wilkins, New York, pp. 233–250.

Brin, M.F., Blitzer, A., Stewart, C., Fahn, S., 1992. Treatment of spasmodic dysphonia (laryngeal dystonia} with local injections of botulinum toxin: review and technical aspects. In: Blitzer, A., Brin, M.F., Sasaki, C.T., Fahn, S., Harris, K.S. (Eds.), Neurological Disorders of the Larynx. Thieme, New York, pp. 214–228.

Brin, M.F., Comella, C., O'Brin, C., Jankovic, J., Benabou, R., Jaglin, J., Johnston, T., Judd, D., Hunter, C., 2000. An interim analaysis of the clinical status of patients receiving Current Botox® (lot 2024 or subsequent lots) for the treatment of cervical dystonia (CD). Mov. Disord. 15, 28–29 (Abstract).

Brin, M.F., Fahn, S., Moskowitz, C., Friedman, A., Shale, H.M., Greene, P.E., Blitzer, A., List, T., Lange, D., Lovelace, R.E., 1987. Localized injections of botulinum toxin for the treatment of focal dystonia and hemifacial spasm. Mov. Disord. 2, 237–325.

Brin, M.F., Lew, M.F., Adler, C.H., Comella, C.L., Factor, S.A., Jankovic, J., O'Brien, C., Murray, J.J., Wallace, J.D., Willmer-Hulme, A., Koller, M., 1999. Safety and efficacy of NeuroBloc (botulinum toxin type B) in type A-resistant cervical dystonia. Neurology 53, 1431–1438.

Brin, M.F., Swope, O.M., Abassi, S., O'Brien, C., Pogoda, J.M., 2000a. BOTOX for migraine: double-blind, placebo-controlled. Region-specific evaluation. Cephalalgia 20, 421–422.

Bushara, K.O., 1997. Sialorrhea in amyotrophic lateral sclerosis: a hypothesis of a new treatment-Botulinum toxin A injections of the parotid glands. Med. Hypoth. 48, 337–339.

Carruthers, J., Carruthers, A., 2001a. Botulinum toxin (botox) chemodenervation for facial rejuvenation. Facial Plast. Surg. Clin. North Am. 9, 197–204.

Carruthers, J., Carruthers, A., 2001b. Botox use in the mid and lower face and neck. Semin. Cutan. Med. Surg. 20, 85–92.

Carruthers, A., Langtry, J.A., Carruthers, J., Robinson, G., 1999. Improvement of tension-type headache when treating wrinkles with botulinum toxin A injections. Headache 39, 662–665.

Castiglione, A., Bagnato, S., Boccagni, C., Romano, M.C., Galardi, G., 2011. Efficacy of intra-articular injection of botulinum toxin type A in refractory hemiplegic shoulder pain. Arch. Phys. Med. Rehabil. 92, 1034–1037.

Chen, R., Karp, B.I., Goldstein, S.R., Bara-Jimenez, W., Yaseen, Z., Hallett, M., 1999. Effect of muscle activity immediately after botulinum toxin injection for writer's cramp. Mov. Disord. 14, 307–312.

Cheshire, W.P., Abashian, S.W., Mann, J.D., 1994. Botulinum toxin in the treatment of myofascial pain syndrome. Pain 59, 65–69.

Chheda, M.G., Ashery, U., Thakur, P., Rettig, J., Sheng, Z.H., 2001. Phosphorylation of snapin by PKA modulates its interaction with the SNARE complex. Nat. Cell Biol. 3, 331–338.

Chicka, M.C., Hui, E., Liu, H., Chapman, E.R., 2008. Synaptotagmin arrests the SNARE complex before triggering fast, efficient membrane fusion in response to Ca2+. Nat. Struct. Mol. Biol. 15, 827–835.

Chieregatti, E., Witkin, J.W., Baldini, G., 2002. SNAP-25 and synaptotagmin 1 function in Ca2+-dependent reversible docking of granules to the plasma membrane. Traffic 3, 496–511.

Childers, M.K., Wilson, D.J., Gnatz, S.M., Conway, R.R., Sherman, A.K., 2002. Botulinum toxin type A use in piriformis muscle syndrome: a pilot study. Am. J. Phys. Med. Rehabil. 81, 751–759.

Coffield, J.A., Bakry, N., Zhang, R.D., Carlson, J., Gomella, L.G., Simpson, L.L., 1997. In vitro characterization of botulinum toxin types a, c and d action on human tissues: combined electrophysiologic, pharmacologic and molecular biologic approaches. J. Pharmacol. Exp. Ther. 280, 1489–1498.

Cui, M., Aoki, K.R., 2000. Botulinum toxin type A (BTX-A) reduces inflammatory pain in the rat formalin model. Cephalalgia 20, 414.

DasGupta, B.R., 1994. Structures of botulinum neurotoxin, its functional domains, and perspectives on the crystalline type A toxin. In: Jankovic, J., Hallett, M. (Eds.), Therapy with Botulinum Toxin. Marcel Dekker, New York, pp. 15–39.

DasGupta, B.R., Foley, J.J., 1989. C. botulinum neurotoxin types A and E: isolated light chain breaks down into two fragments. Comparison of their amino acid sequences with tetanus neurotoxin. Biochimoe 71, 1193–1200.

DasGupta, B.R., Sathyamoorthy, V., 1984. Purification and amino acid composition of type A botulinum neurotoxin. Toxicon 22, 415–424.

Davies, J., Duffy, D., Boyt, N., Aghahoseini, A., Alexander, D., Leveson, S., 2003. Botulinum toxin (botox) reduces pain after hemorrhoidectomy: results of a double-blind, randomized study. Dis. Colon Rectum 46, 1097–10102.

Davis, D., Jabbari, B., 1993. Significant improvement of stiff-person syndrome after paraspinal injection of botulinum toxin-A. Mov. Disord. 8, 371–373.

De Andres, J., Adsuara, V.M., Palmisani, S., Villanueva, V., Lopez-Alarcon, M.D., 2010. A double-blind, controlled, randomized trial to evaluate the efficacy of botulinum

toxin for the treatment of lumbar myofascial pain in humans. Reg. Anesth. Pain Med. 35, 255–260.
Dengler, R., Neyer, U., Wohlfarth, K., Bettig, U., Janzik, H., 1992. Local botulinum toxin in the treatment of spastic foot drop. J. Neurol. 239, 375–378.
de Paiva, A., Meunier, F.A., Molgó, J., Aoki, K.R., Dolly, J.O., 1999. Functional repair of motor endplates after botulinum neurotoxin type A poisoning: biphasic switch of synaptic activity between nerve sprouts and their parent terminals. Proc. Natl. Acad. Sci. U.S.A. 96, 3200–3205.
Diaz, J.H., Gould, H.J., 1999. Management of post-thoracotomy pseudoangina and myofascial pain with botulinum toxin. Anesthesiology 91, 877–879.
Dodick, D., Blumenfeld, A., Silberstein, S.D., 2004. Botulinum neurotoxin for the treatment of migraine and other primary headache disorders. Clin. Dermatol. 22, 76–81.
Dolly, J.O., Ashton, A.C., McInnes, C., Wadsworth, J.D., Poulain, B., Tauc, L., Shone, C.C., Melling, J., 1990. Clues to the multi-phasic inhibitory action of botulinum neurotoxins on release of transmitters. J. Physiol. 84, 237–246.
Dolly, J.O., Wang, J., Zurawski, T.H., Meng, J., 2011. Novel therapeutics based on recombinant botulinum neurotoxins to normalize the release of transmitters and pain mediators. FEBS J. 278, 4454–4466.
Dunne, J.W., Heye, N., Dunne, S.L., 1995. Treatment of chronic limb spasticity with botulinum toxin A. J. Neurol. Neurosurg. Psychiatry 58, 232–235.
Emmerson, J., 1994. Botulinum toxin for spasmodic torticollis in a patient with myasthenia gravis. Mov. Disord. 9, 367.
Eleopra, R., Tugnoli, V., De Grandis, D., 1997a. The variability in the clinical effect induced by botulinum toxin type A: the role of muscle activity in humans. Mov. Disord. 12, 89–94.
Eleopra, R., Tugnoli, V., Rossetto, O., De Grandis, D., Montecucco, C., 1998. Different time courses of recovery after poisoning with botulinum neurotoxin serotypes A and E in humans. Neurosci. Lett. 256, 135–138.
Eleopra, R., Tugnoli, V., Rossetto, O., Montecucco, C., De Grandis, D., 1997b. Botulinum neurotoxin serotype C: a novel effective botulinum toxin therapy in human. Neurosci. Lett. 224, 91–94.
Elkind, A.H., O'Carroll, C.P., Blumenfeld, A., DeGryse, R., Dimitrova, R., 2005. A randomized, controlled, 3 study series of multiple treatments with low doses of botulinum toxin type A for the prophylaxis of episodic migraine. J. Neurol. 252, ii51–ii95.
Erbguth, F., Claus, D., Engelhardt, A., Dressler, D., 1993. Systemic effect of local botulinum toxin injections unmasks subclinical lambert-eaton myasthenic syndrome. J. Neurol. Neurosurg. Psychiatry 56, 1235–1236.
Eross, E.G., Dodick, D.W., 2002. The effects of botulinum toxin type A on disability in episodic and chronic migraine. Neurology 58, A497.
Evans, G.M., Williams, R.S., Shone, C.C., Hambleton, P., Melling, J., Dolly, J.O., 1986. Botulinum type B. Its purification, radioiodination and interaction with rat-brain synaptosomal membranes. Eur. J. Biochem. 154, 409–416.
Evans, R.W., Olesen, J., 2003. Migraine classification, diagnostic criteria, and testing. Neurology 60, S24–S30.
Fanucci, E., Masala, S., Sodani, G., Varrucciu, V., Romagnoli, A., Squillaci, E., Simonetti, G., 2001. CT-guided injection of botulinic toxin for percutaneous therapy of piriformis muscle syndrome with preliminary MRI results about denervative process. Eur. Radiol. 11, 2543–2548.
Fernández-Chacón, R., Königstorfer, A., Gerber, S.H., García, J., Matos, M.F., Stevens, C.F., Brose, N., Rizo, J., Rosenmund, C., Südhof, T.C., 2001. Synaptotagmin I functions as a calcium regulator of release probability. Nature 410, 41–49.
Ferrante, M., Bearn, L., Rothrock, R., King, L., 2002. Botulinum toxin type A in the treatment of myofascial pain. In: Presented at the Annual Meeting of the American Society of Anesthesiologists.
Filippi, G.M., Errico, P., Santarelli, R., Bagolini, B., Manni, E., 1993. Botulinum-A toxin effects on rat jaw muscle spindles. Acta Oto-Laryngol. 113, 400–404.
Fishman, L.M., 2002. Myobloc in the treatment of piriformis syndrome: a dose finding study. In: Presented at the American Academy of Pain Medicine's Annual Meeting, San Francisco, CA.
Fishman, L.M., Anderson, C., Rosner, B., 2002. BOTOX and physical therapy in the treatment of piriformis syndrome. Am. J. Phys. Med. Rehabil. 81, 936–942.
Fishman, V.M., Parkman, H.P., Schiano, T.D., Hills, C., Dabezies, M.A., Cohen, S., Fisher, R.S., Miller, L.S., 1996. Symptomatic improvement in achalasia after botulinum toxin injection ofthe lower esophageal sphincter. Am. J. Gastroenterol. 91, 1724–1730.
Foster, L., Clapp, L., Erickson, M., Jabbari, B., 2001. Botulinum toxin A and chronic low back pain: a randomized, double-blind study. Neurology 56, 1290–1293.
Freund, B., Schwartz, M., 2001. Subcutaneous BTX-A in the treatment of neuropathic pain: a pilot study. In: Presented at the 38th Interagency Botulism Research Coordinating Committee Meeting, Easton, (MD) October 17–19.
Freund, B., Schwartz, M., 2000a. Treatment of chronic cervical-associated headache with botulinum toxin A: a pilot study. Headache 40, 231–236.
Freund, B.J., Schwartz, M., 2000b. The use of botulinum toxin A in the treatment of refractory cluster headache: case reports. Cephalalgia 20, 329–330.
Freund, B., Schwartz, M., Symington, J.M., 2000. Botulinum toxin: new treatment for temporomandibular disorders. Br. J. Oral Maxillofac. Surg. 38, 466–471.
Garvey, T.A., Marks, M.R., Wiesel, S.W., 1989. A prospective, randomized, double-blind evaluation of trigger-point injection therapy for low-back pain. Spine 14, 962–964.
Gerona, R.L., Larsen, E.C., Kowalchyk, J.A., Martin, F.J., 2000. The C terminus of SNAP25 is essential for Ca2+-dependent binding of synaptotagmin to SNARE complexes. J. Biol. Chem. 275, 6328–6336.
Giannantoni, A., Mearini, E., Del Zingaro, M., Proietti, S., Porena, M., 2010. Two-year efficacy and safety of botulinum a toxin intravesical injections in patients affected by refractory painful bladder syndrome. Curr. Drug Deliv. 7, 1–4.
Ginies, P.R., Fraimount, J.L., Siou, D.K., Chevallier, J., Mann, C., Colson, P., 1996. Treatment of cluster headache by subcutaneous injection of botulinum toxin (abst). In: Jensen, T.S. (Ed.), Progress in Pain Research and Management. , 8th ed. IASP Press, Seattle, p. 501.
Giraudo, C.G., Eng, W.S., Melia, T.J., Rothman, J.E., 2006. A clamping mechanism involved in SNARE-dependent exocytosis. Science 313, 676–680.
Girdler, N.M., 1994. Use of botulinum toxin to alleviate facial pain. Br. J. Hosp. Med. 52, 363.
Glocker, F.X., Guschlbauer, B., Lucking, C.H., Deuschl, G., 1995. Effects of local injections of botulinum toxin on electrophysiological parameters in patients with hemifacial spasm: role of synaptic activity and size of motor units. Neurosci. Lett. 187, 161–164.
Gobel, H., Heinze, A., Reichel, G., Hefter, H., Benecke, R., 2006. Efficacy and safety of a single botulinum type A toxin complex treatment (Dysport) for the relief of upper back myofascial pain syndrome: results from a randomized double-blind placebo-controlled multicentre study. Pain 125, 82–88.
Granata, A., Watson, R., Collinson, L.M., Schiavo, G., Warner, T.T., 2008. The dystonia-associated protein torsinA modulates synaptic vesicle recycling. J. Biol. Chem. 283, 7568–7579.
Grazko, M.A., Polo, K.B., Jabbari, B., 1995. Botulinum toxin A for spasticity, muscle spasms, and rigidity. Neurology 45, 712–717.
Greene, P., Fahn, S., 1993a. Use of botulinum toxin type-F injections to treat torticollis in patients with immunity to botulinum toxin type-A. Mov. Disord. 8, 479–483.
Greene, P., Fahn, S., 1993b. Development of antibodies to botulinum toxin type A in patients with torticollis treated with injections of botulinum toxin type A. In: DasGupta, B.R. (Ed.), Botulinum and Tetanus Neurotoxins: Neurotransmission and Biomedical Aspects. Plenum Press, New York, pp. 651–654.
Greene, P., Fahn, S., 1992. Treatment of torticollis with injections of botulinum toxin type F in patients with antibodies to botulinum toxin type A. Mov. Disord. 7, 134.
Greene, P., Fahn, S., Diamond, B., 1994. Development of resistance to botulinum toxin type A in patients with torticollis. Mov. Disord. 9, 213–217.
Greene, P., Kang, U., Fahn, S., Brin, M., Moskowitz, C., Flaster, E., 1990. Double-blind, placebo-controlled trial of botulinum toxin injections for the treatment of spasmodic torticollis. Neurology 40, 1213–1218.
Gonnering, R.S., 1988. Negative antibody response to long-term treatment of facial spasm with botulinum toxin. Am. J. Ophthalmol. 105, 313–315.
Gupta, M., Patel, T., Xavier, K., Maruffo, F., Lehman, D., Walsh, R., 2010. Prospective randomized evaluation of periureteral botulinum toxin type A injection for ureteral stent pain reduction. J. Urol. 183, 598–602.
Hallett, M., 2002. Effects of botulinum toxin at the neuromuscular junction. In: Brin, M.F., Hallett, M., Jankovic, J. (Eds.), Scientific and Therapeutic Aspects of Botulinum Toxin. Lippincott Williams & Wilkins, Philadelphia, PA, pp. 167–170.
Hallett, M., 2000. How does botulinum toxin work? Ann. Neurol. 48, 7–8.
Hallett, M., 1995. Is dystonia a sensory disorder? Ann. Neurol. 38, 139–140 (editorial).
Hallett, M., Glocker, F.X., Deuschl, G., 1994. Mechanism of action of botulinum toxin. Ann. Neurol. 36, 449–450 (letter).
Hambleton, P., Pickett, A.M., 1994. Potency equivalence of botulinum toxin preparations. J. R. Soc. Med. 87, 719.
Hanna, P.A., Jankovic, J., 1998. Mouse bioassay versus Western blot assay for botulinum toxin antibodies: correlation with clinical response. Neurology 50, 1624–1629.
Hanna, P.A., Jankovic, J., Vincent, A., 1999. Comparison of mouse bioassay and immunoprecipitation assay for botulinum toxin antibodies. J. Neurol. Neurosurg. Psychiatry 66, 612–616.
Hatheway, C.L., Dang, C., 1994. Immunogenicity of the neurotoxins of *Clostridium botulinum*. In: Jankovic, J., Halltt, M. (Eds.), Therapy with Botulinum Toxin. Marcel Dekker, New York, pp. 93–107.
Hawley, P.H., 2002. Botulinum toxin for severe anorectal pain. J. Pain Symptom Manage. 24, 11–13.
Headache Classification Committee of the International Headache Society, 2004. The International Classification of Headache Disorders, 2nd ed. Cephalalgia 24, 1–160.
Headache Classification Committee of the International Headache Society, 1988. Classification and Diagnostic Criteria for Headache Disorders, Cranial Neuralgias and Facial Pain. Cephalalgia 8, 1–96.
Heckmann, M., Ceballos-Baumann, A.O., Plewig, G., 2001. Botulinum toxin A for axillary hyperhidrosis (excessive sweating). N. Engl. J. Med. 344, 488–493.
Hesse, S., Reiter, F., Konrad, M., Jahnke, M.T., 1998. 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 [In Process Citation]. Clin. Rehabil. 12, 381–388.
Hinz, B., Becher, A., Mitter, D., Schulze, K., Heinemann, U., Draguhn, A., Ahnert-Hilger, G., 2001. Activity-dependent changes of the presynaptic synaptophysin-synaptobrevin complex in adult rat brain. Eur. J. Cell Biol. 80, 615–619.
Hollingshead, J.R., Maeda, Y., Brown, T.J., Warusavitarne, J., Vaizey, C.J., 2011. Long-term outcome of the use of botulinum toxin injection for functional anal pain. Colorectal Dis. 13, e293–e296.
Houser, M.K., Sheean, G.L., Lees, A.J., 1998. Further studies using higher doses of botulinum toxin type F for torticollis resistant to botulinum toxin type A. J. Neurol. Neurosurg. Psychiatry 64, 577–580.

Hu, X.H., Markson, L.E., Lipton, R.B., Stewart, W.F., Berger, M.L., 1999. Burden of migraine in the United States: disability and economic costs. Arch. Intern. Med. 159, 813–818.
Hughes, R., Whaler, B.C., 1962. Influence of nerve ending activity and of drugs on the rate of paralysis of rat diaphragm preparations by clostridium botulinum type A toxin. J. Physiol. (Lond.) 160, 221–233.
Huttner, W.B., 1993. Cell biology. Snappy exocytoxins. Nature 365, 104–105.
Hyman, N., Barnes, M.D., Bhakta, B., Cozens, A., Bakheit, M., Kreczy-Kleedorfer, B., Poewe, W., Wissel, J., Bain, P., Glickman, S., Sayer, A., Richardson, A., Dott, C., 2000. Botulinum toxin (Dysport) treatment of hip adductor spasticity in multiple sclerosis: a prospective, randomized, double-blind, placebo-controlled, dose ranging study. J. Neurol. Neurosurg. Psychiatry 68, 707–712.
Ilardi, J.M., Mochida, S., Sheng, Z.H., 1999. Snapin: a SNARE-associated protein implicated in synaptic transmission. Nat. Neurosci. 2, 119–124.
Ishikawa, H., Mitsui, Y., Yoshitomi, T., Mashimo, K., Aoki, S., Mukuno, K., Shimizu, K., 2000. Presynaptic effects of botulinum toxin type Aon the neuronally evoked response of albino and pigmented rabbit iris sphincter and dilator muscles. Jpn. J. Ophthalmol. 44, 106–109.
Ivanhoe, C.B., Lai, J.M., Francisco, G.E., 1997. Bruxism after brain injury: successful treatment with botulinum toxin-A. Arch. Phys. Med. Rehabil. 78, 1272–1273.
Jabbari, B., 2008. Botulinum neurotoxins in the treatment of refractory pain. Nat. Clin. Pract. Neurol. 4, 676–685.
Jabbari, B., Maher, N., Difazio, M.P., 2003. Botulinum toxin A improved burning pain and allodynia in two patients with spinal cord pathology. Pain Med. 4, 206–210.
Jabbari, B., Ney, J., Sichani, A., Monacci, W., Foster, L., Difazio, M., 2006. Treatment of refractory, chronic low back pain with botulinum neurotoxin A: an open-label, pilot study. Pain Med. 7, 260–264.
Jankovic, J., Ahsan, J., Vuong, K.D.P., 2002. Comparison of immunogenicity of old versus current BOTOX in cervical dystonia. Naunyn Schmied. Ardl. Pharmacol. (Abstract).
Jankovic, J., Brin, M.F., 1997. Botulinum toxin: historical perspective and potential new indications. Muscle Nerve 20, S129–S145.
Jankovic, J., Hallett, M., 1994. Therapy with Botulinum Toxin. Marcel Dekker, New York.
Jankovic, J., Schwartz, K.S., 1991. Clinical correlates of response to botulinum toxin injections. Arch. Neurol. 48, 1253–1256.
Jankovic, J., Schwartz, K., 1990. Botulinum toxin injections for cervical dystonia. Neurology 4, 277–280.
Jeynes, L.C., Gauci, C.A., 2008. Evidence for the use of botulinum toxin in the chronic pain setting: a review of the literature. Pain Practice 8, 269–276.
Johnstone, S.J., Adler, C.H., 1998. Headache and facial pain responsive to botulinum toxin: an unusual presentation of blepharospasm. Headache 38, 366–368.
Jost, W.H., 1997. One hundred cases of anal fissure treated with botulin toxin: early and long-term results. Dis. Colon Rectum 40, 1029–1032.
Kaji, R., Mezaki, T., Kubori, T., Murase, N., Kimura, J., 1996. Treatment of spasticity with botulinum toxin and muscle afferent block. Rinsho Shinkeigaku 36, 1334–1335.
Kaji, R., Kohara, N., Katayama, M., Kubori, T., Mezaki, T., Shibasaki, H., Kimura, J., 1995a. Muscle afferent block by intramuscular injection of lidocaine for the treatment of writer's cramp. Muscle Nerve 18, 234–235.
Kaji, R., Rothwell, J.C., Katayama, M., Ikeda, T., Kubori, T., Kohara, N., Mezaki, T., Shibasaki, H., Kimura, J., 1995b. Tonic vibration reflex and muscle afferent block in writer's cramp. Ann. Neurol. 38, 155–162.
Kaji, R., Shibasaki, H., Kimura, J., 1995c. Writer's cramp: a disorder of motor subroutine? Ann. Neurol. 38, 837–838.
Kong, K.H., Neo, J.J., Chua, K.S., 2007. A randomized controlled study of botulinum toxin A in the treatment of hemiplegic shoulder pain associated with spasticity. Clin. Rehabil. 21, 28–35.
Kozaki, S., Sakaguchi, G., 1982. Binding to mouse brain synaptosomes of Clostridium botulinum type E derivative toxin before and after tryptic activation. Toxicon 20, 841–846.
Knusel, B., DeGryse, R., Grant, M., Loeser, J., Ripp, C., Stanton-Hicks, M., 1998. Intramuscular injection of botulinum toxin type A (BOTOX) in chronic low back pain associated with muscle spasm. 100:110. Presented at the American Pain Society Annual Scientific Meeting. San Diego, CA.
Krack, P., Deuschl, G., Benecke, R., Ceballos-Baumann, A.O., Marion, M.H., Oertel, W.H., Poewe, W., 1998. Dose standardization of botulinum toxin. Mov. Disord. 13, 749–751 (letter).
Lackovic, Z., Bach-Rojecky, L., Salcovic-Petrisic, M., 2006. Antinociceptive effect of botulinum toxin type A in experimental diabetic neuropathy [abstract P06.188]. Presented at the American Academy of Neurology's 58th Annual Meeting, San Diego, CA, April 1–8.
Lang, A., 2000. A pilot study of botulinum toxin type A (BOTOX), administered using a novel injection technique, for the treatment of myofascial pain. Am. J. Pain Manage. 10, 108–112.
Lang, A.M., 2002. Botulinum toxin therapy for myofascial pain disorders. Curr. Pain Headache Rep. 6, 355–360.
Lange, D.J., Brin, M.F., Greene, P., Lovelace, R.E., Fahn, S., 1993. Distant effects of locally injected botulinum toxin—a double-blind study of single fiber EMG changes—a reply). Muscle Nerve 16, 677.
Lange, D.J., Brin, M.F., Warner, C.L., Fahn, S., Lovelace, R.E., 1987. Distant effects of local injection of botulinum toxin. Muscle Nerve 10, 552–555.
Lange, D.J., Rubin, M., Greene, P.E., Kang, U.J., Moskowitz, C.B., Brin, M.F., Lovelace, R.E., Fahn, S., 1991. Distant effects of locally injected botulinum toxin: a double-blind study of single fiber EMG changes. Muscle Nerve 14, 672–675.
Langevin, P., Peloso, P.M., Lowcock, J., Nolan, M., Weber, J., Gross, A., Roberts, J., Goldsmith, C.H., Graham, N., Burnie, S.J., Haines, T., 2011. Botulinum toxin for subacute/chronic neck pain. Cochrane Database Syst. Rev. 7, CD008626.
Lew, M.F., Adornato, B.T., Duane, D.D., Dykstra, D.D., Factor, S.A., Massey, J.M., Brin, M.F., Jankovic, J., Rodnitzky, R.L., Singer, C., Swenson, M.R., Tarsy, D., Murray, J.J., Koller, M., Wallace, J.D., 1997. Botulinum toxin type B (BotB): a double-blind, placebo-controlled, safety and efficacy study in cervical dystonia. Neurology 49, 701–707.
LeWitt, P.A., Trosch, R.M., 1997. Idiosyncratic adverse reactions to intramuscular botulinum toxin type A injection. Mov. Disord. 12, 1064–1067.
Lipton, R.B., Diamond, S., Reed, M., Diamond, M.L., Stewart, W.F., 2001. Migraine diagnosis and treatment: results from the American Migraine Study II. Headache 41, 638–645.
Liu, H.T., Kuo, H.C., 2007. Intravesical botulinum toxin A injections plus hydrodistension can reduce nerve growth factor production and control bladder pain in interstitial cystitis. Urology 70, 463–468.
Lorentz, I.T., Subramaniam, S.S., Yiannikas, C., 1991. Treatment of idiopathic spasmodic torticollis with botulinum toxin A: a double-blind study on twenty-three patients. Mov. Disord. 6, 145–150.
Lu, C.S., Chen, R.S., Tsai, C.H., 1995. Double-blind, placebo-controlled study of botulinum toxin injections in the treatment of cervical dystonia. J. Formos. Med. Assoc. 94, 189–192.
Ludlow, C.L., Hallett, M., Rhew, K., Cole, R., Shimizu, T., Sakaguchi, G., Bagley, J.A., Schulz, G.M., Yin, S.G., Koda, J., 1992. Therapeutic use of type F botulinum toxin. N. Engl. J. Med. 326, 349–350 (letter).
Ludlow, C.L., Hallett, M., Sedory, S.E., Fujita, M., Naunton, R.F., 1990. The pathophysiology of spasmodic dys·phonia and its modification by botulinum toxin. In: Berardelli, A., Benecke, R., Manfredi, M., Marsden, C.M. (Eds.), Motor Disturbances II. Academic Press, New York, pp. 273–288.
Marco, E., Duarte, E., Vila, J., Tejero, M., Guillen, A., Boza, R., 2007. Is botulinum toxin type A effective in the treatment of spastic shoulder pain in patients after stroke? A double-blind randomized clinical trial. J. Rehabil. Med. 39, 440–447.
Martin, T.F.J., 1997. Stages of regulated exocytosis. Trends Cell Biol. 7, 271–276.
Mathew, N.T., Kallasam, J., Kaupp, A., Meadors, L., 2002. Disease modification in chronic migraine with botulinum toxin type A: long-term experience [abstract S107]. Presented at the American Headache Society's 44th Annual Scientific Meeting, Seattle, WA, June 21–23.
Mauskop, A., Basedo, R., 2000. Botulinum toxin A is an effect prophylactic therapy of migraines. Cephalalgia 20, 422.
Mauskop, A., 2002a. The use of botulinum toxin in the treatment of headaches. Curr. Pain Headache Rep. 6, 320–323.
Mauskop, A., 2002. Long-term use of botulinum toxin Type A (BOTOX®) in the treatment of episodic and chronic migraine headaches [abstract S105]. Presented at the American Headache Society's 44th Annual Scientific Meeting, Seattle, WA, June 21–23.
McLellan, K., Das, R.E., Ekong, T.A., Sesardic, D., 1996. Therapeutic botulinum type A toxin: factors affecting potency. Toxicon 301, 975–985.
Melling, J., Hambleton, P., Shone, C.C., 1988. Clostridium botulinum toxins: nature and preparation for clinical use. Eye 2, 16–23.
Memin, B., Pollack, P., Hommel, M., Perret, J., 1992. Effects of botulinum toxin on spasticity. Rev. Neurol. (Paris) 148, 212–214.
Mezaki, T., Kaji, R., Kohara, N., Fujii, H., Katayama, M., Shimizu, T., Kimura, J., Brin, M.F., 1995. Comparison of therapeutic efficacies of type A and F botulinum toxins for blepharospasm: a double-blind, controlled study. Neurology 45, 506–508.
Mezaki, T., Kaji, R., Hirota, N., Kohara, N., Kimura, J., 1999. Treatment of spasticity with muscle afferent block. Neurology 53, 1156–1157.
Mezaki, T., Kaji, R., Kohara, N., Kimura, J., 1996. Development of general weakness in a patient with amyotrophic lateral sclerosis after focal botulinum toxin injection. Neurology 46, 845–846.
Miller, T., Denny, L., 2002. Retrospective cohort analysis of 48 chronic headache patients treated with botulinum toxin type A (Botox) in a combination fixed-injection-site and follow the pain protocol [abstract S138]. Presented at the American Headache Society's 44th Annual Scientific Meeting, Seattle, WA, June 21–23.
Misura, K.M., Scheller, R.H., Weis, W.I., 2000. Three-dimensional structure of the neuronal-Sec1-syntaxin 1a complex. Nature 404, 355–362.
Molteni, F., 1995. Botulinum toxin and rehabilitation programs in lower limb spasticity. Eur. J. Neurol. 2, 61–67.
Moser, E., Ligon, K.M., Singer, C., Sethi, K.D., 1997. Botulinum toxin A (Botox) therapy during pregnancy. Neurology 48, 399.
Murinson, B., 2008. Botulinum toxin type a treatment of painful focal neuropathies: new evidence for efference of afferents. Ann. Neurol. 64, 236–238.
Nagamine, T., Kaji, R., Hamano, T., Kimura, J., 1991. Treatment of focal dystonia with botulinum toxin. Clin. Neurol. 31, 31–37.
Nalamachu, S., 2002. Treatment with botulinum toxin type B (Myobloc) injections in three patients with myofascial pain. Presented at the American Academy of Pain Medicine's Annual Scientific Meeting. San Francisco, CA.
Naumann, M., Lowe, N.J., 2001. Botulinum toxin type A in treatment of bilateral primary axillary hyperhidrosis: randomized, parallel group, double-blind, placebo controlled trial. BMJ 323, 596–599.
Naumann, M., Yakovleff, A., Durif, F., Botox® Cervical Dystonia Prospective Study Group, 2002. A randomized, double-masked, crossover comparison of the efficacy and safety of botulinum toxin type A produced from the original bulk toxin source and current bulk toxin source for the treatment of cervical dystonia. J. Neurol. 249, 57–63.

Neurobloc European Public Assessment Report, 2000. European Agency for the Evaluation of Medicinal Product. http://www.eudra.org/humandocs/humans/epar/neurobloc/neuroblocM.htm

Ney, J.P., Difazio, M., Sichani, A., Monacci, W., Foster, L., Jabbari, B., 2006. Treatment of chronic low back pain with successive injections of botulinum toxin a over 6 months: a prospective trial of 60 patients. Clin. J. Pain 22, 363–369.

Nix, W.A., Butler, I.J., Roontga, S., Gutmann, L., Hopf, H.C., 1992. Persistent unilateral tibialis anterior muscle hypertrophy with complex repetitive discharges and myalgia: report of two unique cases and response to botulinum toxin. Neurology 42, 602–606.

Pan, P.Y., Tian, J.H., Sheng, Z.H., 2009. Snapin facilitates the synchronization of synaptic vesicle fusion. Neuron 61, 412–424.

Pang, Z.P., Shin, O.H., Meyer, A.C., Rosenmund, C., Südhof, T.C., 2006. A gain-of-function mutation in synaptotagmin-1 reveals a critical role of Ca2+-dependent soluble N-ethylmaleimide-sensitive factor attachment protein receptor complex binding in synaptic exocytosis. J. Neurosci. 26, 12556–12565.

Paton, J.C., Lawrence, A.J., Manson, J.I., 1982. Quantitation of Clostridium botulinum organisms and toxin in the feces of an infant with botulism. J. Clin. Microbiol. 15, 1–4.

Patti, R., Almasio, P.L., Arcara, M., et al., 2007. Long-term manometric study of anal sphincter function after hemorrhoidectomy. Int. J. Colorectal Dis. 22, 253–257.

Patti, R., Arcara, M., Bonventre, S., Sammartano, S., Sparacello, M., Vitello, G., 2008. Randomized clinical trial of botulinum toxin injection for pain relief in patients with thrombosed external haemorrhoids. Br. J. Surg. 95, 1339–1343.

Pearce, L.B., Borodic, G.E., First, E.R., Maccallum, R.D., 1994. Measurement of botulinum toxin activity: evaluation of the lethality assay. Toxicol. Appl. Pharmacol. 128, 69–77.

Pearce, L.B., Borodic, G.E., Johnson, E.A., First, E.R., MacCallum, R., 1995. The median paralysis unit: a more pharmacologically relevant unit of biologic activity for botulinum toxin. Toxicon 33, 217–227.

Peng, K., Merlino, G., Addeo, J., Fosler, S., Spanoyannis, A., Aoki, K.R., 1998. BOTOX® is six fold more potent than Dysport in the mouse digit abduction scoring assay. Mov. Disord. 13, 110 (Abstract).

Poewe, W., Schelosky, L., Kleedorfer, B., Heinen, F., Wagner, M., Deuschl, G., 1992. Treatment of spasmodic torticollis with local injections of botulinum toxin. One-year follow-up in 37 patients. J. Neurol. 239, 21–25.

Polo, K.D., Jabbari, D., 1994. Effectiveness of botulinum toxin type A against painful limb myoclonus of spinal cord origin. Mov. Disord. 9, 233–235.

Porta, M., 2000a. A comparative trial of botulinum toxin type A and methylprednisolone for the treatment of myofascial pain syndrome and pain from chronic muscle spasm. Pain 85, 101–105.

Porta, M., 2000b. A comparative trial of botulinum toxin type A and methylprednisolone for the treatment of tension-type headache. Curr. Rev. Pain 4, 31–35.

Ranoux, D., Attal, N., Morain, F., Bouhassira, D., 2008. Botulinum toxin type A induces direct analgesic effects in chronic neuropathic pain. Ann. Neurol. 64, 274–284.

Rao, A., Abbott, J., 2009. Using botulinum toxin for pelvic indications in women. Aust. N. Z. J. Obstet. Gynaecol. 49 (August (4)), 352–357.

Reim, K., Mansour, M., Varoqueaux, F., McMahon, H.T., Südhof, T.C., Brose, N., Rosenmund, C., 2001. Complexins regulate a late step in Ca2+-dependent neurotransmitter release. Cell 12, 71–81.

Relja, M.A., 2000. Treatment of tension-type headache by local injection of botulinum toxin: 1-year follow-up. Cephalalgia 20, 336.

Relja, M., 1997. Treatment of tension-type headache by local injection of botulinum toxin. Eur. J. Neurol. 4, S71–S74.

Relja, M., Militec, Z., 2006. Botulinum toxin type-A in the treatment of painful diabetic neuropathy: a prospective study [abstract S07.006]. Presented at the American Academy of Neurology's 58th Annual Meeting, San Diego, CA, April 1–8.

Relja, M., Poole, A.C., Schoenen, J., Pascual, J., Saulay, M., Kumar, C., 2005. A multicenter, double-blind, randomized, placebo-controlled, parallel group study of multiple treatments of botulinum toxin type A (BoNTA) for the prophylaxis of migraine headaches [abstract]. Presented at the European Neurological Society, Vienna, Austria, June 20.

Rhew, K., Ludlow, C.L., Karp, B.I., Hallet, M., 1994. Clinical experience with botulinum toxin F. In: Jankovic, J., Hallet, M. (Eds.), Therapy with Botulinum Toxin. Marcus Dekker, New York, pp. 323–328.

Rijsdijk, B.A., van ES, R.J., Zonneveld, F.W., Steenks, M.H., Koole, R., 1998. [Botulinum toxin type A treatment of cosmetically disturbing masseteric hypertrophyl] De toepassing van botuline A toxine bij cosmetisch storende M.-masseterhypertrofie. Ned. Tifdschr. Geneeskd. 142, 529–532.

Rollnik, J.D., Matzke, M., Wohlfarth, K., Dengler, R., Bigalke, H., 2000. Low-dose treatment of cervical dystonia, blepharospasm and facial hemispasm with albumin-diluted botulinum toxin type A under EMG guidance. An open label study. Eur. Neurol. 43, 9–12.

Rosales, R.L., Arimura, K., Takenaga, S., Osame, M., 1996. Extrafusal and intrafusal muscle effects in experimental botulinum toxin-A injection. Muscle Nerve 19, 488–496.

Ruder, C., Reimer, T., Delgado-Martinez, I., Hermosilla, R., Engelsberg, A., Nehring, R., Dorken, B., Rehm, A., 2005. EBAG9 adds a new layer of control on large dense-core vesicle exocytosis via interaction with Snapin. Mol. Biol. Cell 16, 1245–1257.

Saenz, A., Avellanet, M., Garreta, R., 2003. Use of botulinum toxin type A on orthopedics: a case report. Arch. Phys. Med. Rehabil. 84, 1085–1086.

Sakaguchi, G., Kozaki, S., Ohishi, I., 1984. Structure and function of botulinum toxins. In: Alouf, J.E.F. (Ed.), Bacterial Protein Toxins. Academic Press, London, pp. 433–443.

Salazar, G., Craige, B., Wainer, B.H., Guo, J., De Camilli, P., Faundez, V., 2005. Phosphatidylinositol-4-kinase type II alpha is a component of adaptor protein-3-derived vesicles. Mol. Biol. Cell 16, 3692–3704.

Sanders, D.B., Massey, E.W., Buckley, E.G., 1986. Botulinum toxin for blepharospasm: single-fiber EMG studies. Neurology 36, 545–547.

Saper, J.R., Mathew, N.T., Loder, E.W., DeGryse, R., VanDenburgh, A.M., 2005. A double-blind, randomized, placebo-controlled exploratory study comparing injection sites with low doses of botulinum toxin type A in the prevention of episodic migraine [abstract]. Presented at the European Neurological Society, Vienna, Austria, June 20.

Schantz, E.J., 1964. Purification and characterization of C. botulinum toxins. In: Lewis, K.H., Cassel Jr., K. (Eds.), Botulism. Public Health Service. U.S. Dept. of HEW, Washington D.C., pp. 91–104.

Schantz, E.J., Johnson, E.A., 1994. Preparation and characterization of botulinum toxin type A-for human treatment. In: Jankovic, J., Hallett, M. (Eds.), Therapy with Botulinum Toxin. Marcus Dekker, New York, pp. 41–49.

Schantz, E.J., Kautter, D.A., 1977. Microbiological methods: standardized assay for clostridium botulinum toxins. J. Assoc. Off. Anal. Chem. 61, 96–99.

Schiavo, G., Rossetto, O., Montecco, C., 1994. Clostridial neurotoxins as tools to investigate the molecular events of neurotransmitter release. Semin. Cell Biol. 5, 221–229.

Schiavo, G., Rossetto, O., Santucci, A., DasGupta, B.R., Montecucco, C., 1992. Botulinum neurotoxins are zinc proteins. J. Biol. Chem. 267, 23479–23483.

Schoch, S., Deak, F., Konigstorfer, A., Mozhayeva, M., Sara, Y., Sudhof, T.C., Kavalali, E.T., 2001. SNARE function analyzed in synaptobrevin/VAMP knockout mice. Science 294, 1117–1122.

Schulte-Mattler, W.J., Wieser, T., Zierz, S., 1999. Treatment of tension-type headache with botulinum toxin: a pilot study. Eur. J. Med. Res. 4, 183–186.

Scott, A.B., 1989. Clostridial toxins as therapeutic agent. In: Simpson, L.L. (Ed.), Botulinum Neurotoxin and Tetanus Toxin. Academic Press, New York, pp. 399–412.

Sellin, L.C., Thesleff, S., 1981. Pre- and post-synaptic actions of botulinum toxin at the rat neuromuscular junction. J. Physiol. 317, 487–495.

Settler, P.E., 2002. Therapeutic use of botulinum toxins: background and history. Clin. J. Pain 18, S19–24.

Sheean, G., 2002. Botulinum toxin for the treatment of musculoskeletal pain and spasm. Curr. Pain Headache Rep. 6, 460–469.

Sheean, G.L., Lees, A.J., 1995. Botulinum toxin F in the treatment of torticollis clinically resistant to botulinum toxin A. J. Neurol. Neurosurg. Psych. 59, 601–607.

Silberstein, S., Mathew, N., Saper, J., Jenkins, S., 2000. Botulinum toxin type A as a migraine preventive treatment. Headache 40, 445–450.

Silberstein, S.D., Lipton, R.B., Solomon, S., Mathew, N., 1994. Classification of daily and near-daily headaches: proposed to the revisions to the IHS criteria. Headache 34, 1–7.

Simons, D.G., 1996. Clinical and etiological update of myofascial pain from trigger points. In: Russell, I.J., (Ed.), Clinical Overview and Pathogenesis of Fibromyalgia Syndrome, Myofascial Pain and Other Pain Syndromes. Haworth Medical Press, Binghamton, NY, pp. 93–122.

Simons, D.G., Travell, J.G., 1999. Travell and Simons' Myofascial Pain and Dysfunction: The Trigger Point Manual, 2nd ed. Williams & Wilkins, Baltimore, MD.

Simons, D.G., Travell, J.G., 1983. Myofascial origins of low back pain. 1. Principles of diagnosis and treatment. Postgrad. Med. 73, 68–70.

Simpson, L.L., 1989. Peripheral actions of the botulinum toxins. In: Simpson, L.L. (Ed.), Botulinum Neurotoxin and Tetanus Toxin. , 1st ed. Academic Press, New York, pp. 153–178.

Simpson, L.L., 1984. The binding fragment from tetanus toxin antagonizes the neuromuscular blocking actions of botulinum toxin. J. Pharmacol. Exp. Ther. 229, 182–187.

Simpson, L.L., 1981. The origin, structure, and pharmacological activity of botulinum toxin. Pharmacol. Rev. 33, 155–188.

Simpson, L.L., DasGupta, B.R., 1983. Botulinum neurotoxin type E: studies on mechanism of action and on structure-activity relationships. J. Pharmacol. Exp. Ther. 224, 135–140.

Singh, B., Box, B., Lindsey, I., George, B., Mortensen, N., Cunningham, C., 2009. Botulinum toxin reduces anal spasm but has no effect on pain after haemorrhoidectomy. Colorectal Dis. 11, 203–207.

Singh, J.A., Fitzgerald, P.M., 2010. Botulinum toxin for shoulder pain. Cochrane Database Syst. Rev. 8 (September), CD008271.

Smith, H.S., 2009. Botulinum toxins for analgesia. Pain Phys. 12, 479–481 (Editorial).

Smith, H., Audette, J., Dey, R., Khan, S., Bajwa, Z., 2002. Botulinum toxin type B for a patient with myofascial pain. Presented at the American Academy of Pain Medicine's Annual Scientific Meeting. San Francisco, CA.

Smuts, J.A., Barnard, P.W.A., 2000. Botulinum toxin type A in the treatment of headache syndromes: a clinical report of 79 patients. Cephalalgia 20, 332.

Südhof, T.C., Rothman, J.E., 2009. Membrane fusion: grappling with SNARE and SM proteins. Science 323, 474–477.

Talbot, K., Cho, D.S., Ong, W.Y., Benson, M.A., Han, L.Y., Kazi, H.A., Kamins, J., Hahn, C.G., Blake, D.J., Arnold, S.E., 2006. Dysbindin-1 is a synaptic and microtubular protein that binds brain snapin. Hum. Mol. Genet. 15, 3041–3054.

Tan, E.K., Jankovic, J., 2000. Treating severe bruxism with botulinum toxin. J. Am. Dent. Assoc. 131, 211–216.

Tang, J., Maximov, A., Shin, O.H., Dai, H., Rizo, J., Südhof, T.C., 2006. A complexin/synaptotagmin 1 switch controls fast synaptic vesicle exocytosis. Cell 126, 1175–1187.
Taqi, D., Gunyea, I., Bhakta, B., Movva, V., Ward, S., Jenson, M., Royal, M., 2002a. Botulinum toxin type A (BOTOX) in the treatment of refractory cervicothoracic myofascial pain. A Prospective trial. Pain 3, 16.
Taqi, D., Gunyea, I., Bhakta, B., Movva, V., Ward, S., Jenson, M., Royal, M., 2002b. Botulinum toxin type B (Myobloc) in the treatment of refractory myofascial pain. Pain 3, 16.
Tian, J.H., Wu, Z.X., Unzicker, M., Lu, L., Cai, Q., Li, C., Schirra, C., Matti, U., Stevens, D., Deng, C., Rettig, J., Sheng, Z.H., 2005. The role of Snapin in neurosecretion: snapin knock-out mice exhibit impaired calcium-dependent exocytosis of large dense-core vesicles in chromaffin cells. J. Neurosci. 25, 10546–10555.
Troost, B., 2002. Botulinum toxin type A (BOTOX®) therapy for intractable headache. Headache 42, 435.
Tsui, J.K., Eisen, A., Mak, E., Carruthers, M.J., Scott, A., Calne, D.B., 1985. A pilot study on the use of botulinum toxin in spasmodic torticollis. Can. J. Neurol. Sci. 12, 314–316.
Tsui, J.K., Eisen, A., Stoessl, A.J., Calne, S., Calne, D.B., 1986. Double-blind study of botulinum toxin in spasmodic torticollis. Lancet 2, 245–247.
Tsui, J.K., Fross, R.D., Calne, S., Calne, D.B., 1987. Local treatment of spasmodic torticollis with botulinum toxin. Can. J. Neuro. Sci. 14, 533–535.
Tsui, J.K.C., Hayward, M., Mak, E.K.M., Schulzer, M., 1995. Botulinum toxin type B in the treatment of cervical dystonia. A pilot study. Neurology 45, 2109–2110.
Tuite, P.J., Lang, A.E., 1996. Severe and prolonged dysphagia complicating botulinum toxin A injections for dystonia in Machado-Joseph disease. Neurology 46, 846.
Unger, J., Cady, R.K., Farmer-Cady, K., 2003. Understanding migraine: pathophysiology and presentation. Emerg. Med. 43, 19–26.
United States Food and Drug Administration (USFDA), 2001. Myobloc: Summary Basis of Approval Cervical Dystonia. Available from US Government through Freedom of Information.
Van Daele, D.J., Finnegan, E.M., Rodnitzky, R.L., Zhen, W., McCulloch, T.M., Hoffman, H.T., 2002. Head and neck muscle spasm after radiotherapy: management with botulinum toxin A injection. Arch. Otolaryngol. Head Neck Surg. 128, 956–959.
Van den Bergh, P.Y., Lison, D.F., 1998. Dose standardization of botulinum toxin. Adv. Neurol. 78, 231–235.
Van Zandijcke, M., Marchau, M.M., 1990. Treatment of bruxism with botulinum toxin injections. J. Neurol. Neurosurg. Psychiatry 53, 530 (letter).
Vasan, C.W., Liu, C., Klussmann, J.P., Guntinas-Lichius, O., 2004. Botulinum toxin type A for the treatment of chronic neck pain after neck dissection. Head Neck 26, 39–45.
Viriyavejakul, A., Vachalathiti, R., Poungvarin, N., 1998. Botulinum treatment for post-stroke spasticity: low dose regime. J. Med. Assoc. Thai. 81, 413–422.
Wheeler, A.H., 2004a. Myofascial pain disorders: theory to therapy. Drugs 64, 45–62.
Wheeler, A.H., 2004b. Botulinum toxin A for the treatment of neuropathic pain. AJPM 14, 151–156.
Wheeler, A.H., 2000. Botulinum toxin injection technique for treatment of headaches. Aesthetic Surg. J. 22, 656–658.
Wheeler, A.H., 1997. Therapeutic uses of botulinum toxin. Am. Fam. Physician 55, 541–545, 548.
Wheeler, A.H., 1998. Botulinum toxin A, adjunctive therapy for refractory headaches associated with pericranial muscle tension. Headache 38, 468–471.
Wheeler, A.H., Goolkasian, P., 2001. Open label assessment of botulinum toxin in a private outpatient setting. J. Musculoskeletal Pain 9, 67–82.
Wheeler, A.H., Goolkasion, P., Gretz, S.S., 2001. High-dose botulinum toxin for the treatment of chronic neck pain. Pain 94, 255–260.
Wheeler, A.H., Goolkasian, P., Gretz, S.S., 1998. A randomized, double-blind, prospective pilot study of botulinum toxin injection for refractory, unilateral, cervicothoracic, paraspinal, myofascial pain syndrome. Spine 23, 1662–1666.
Wittekindt, C., Liu, W.C., Preuss, S.F., Guntinas-Lichius, O., 2006. Botulinum toxin A for neuropathic pain after neck dissection: a dose-finding study. Laryngoscope 116, 1168–1171.
Wohlfarth, K., Goschel, H., Frevert, J., Dengler, R., Bigalke, H., 1997. Botulinum A toxins: units versus units. Naunyn Schmiedebergs Arch. Phannacol. 355, 335–340.
Xiao, L., Mackey, S., Hui, H., Xong, D., Zhang, Q., Zhang, D., 2010. Subcutaneous injection of botulinum toxin A is beneficial in postherpetic neuralgia. Pain Med. 11, 1827–1833.
Yoshida, K., Kaji, R., Kubori, T., Kohara, N., Iizuka, T., Kimura, J., 1998. Muscle afferent block for the treatment of oromandibular dystonia. Mov. Disord. 13, 699–705.
Yuan, R-Y., Sheu, J-J., Yu, J-M., Chen, W.T., Tseng, I.J., Chang, H.H., Hu, C.J., 2009. Botulinum toxin for diabetic neuropathic pain: a randomized double-blind crossover trial. Neurology 72, 1473–1478.
Zermann, D., lshigooka, M., Schubert, J., Schmidt, R.A., 2000. Perisphincteric injection of botulinum toxin type A. a treatment option for patients with chronic prostatic pain? Eur. Urol. 38, 393–399.
Zhang, X., Kim-Miller, M.J., Fukuda, M., Kowalchyk, J.A., Martin, T.F., 2002. Ca2+-dependent synaptotagmin binding to SNAP-25 is essential for Ca2+-triggered exocytosis. Neuron 34, 599–611.
Zhuang, D., Yin-chin, W., 1992. Treatment of blepharospasm, hemifacial spasm and strabismus with botulinum toxin. Chin. Med. J. 105, 476–480.
Zuber, M., Sebald, M., Bathien, N., Derecondo, J., Rondot, P., 1993. Botulinum antibodies in dystonic patients treated with type-A botulinum toxin—frequency and significance. Neurology 43, 1715–1718.
Zwart, J.A., Bovim, G., Sand, T., Sjaastad, O., 1994. Tension headache: botulinum toxin paralysis of temporal muscles. Headache 34, 458–462.
Zwirner, P., Murry, T., Swenson, M., Wooodson, G., 1992. Effects of botulinum toxin therapy in patients with adductor spasmodic dysphonia: acoustic, aerodynamic, and videoendoscopic findings. Laryngoscope 102, 400–406.