Botulinum Toxin: Pharmacology and Clinical Developments: A Literature Review

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Abstract: Botulinum toxin is used as first line therapy for some muscular disorders, and is efficacious in treating hypersecretory and some pain syndromes. When used appropriately it has a good safety profile. It has been evaluated in treating a number of conditions that as yet do not have obvious effective or beneficial treatment. With the greater acceptance and use of botulinum toxin therapy for cosmetic purposes, botulinum toxin use will increase. An understanding of the pharmacology, and potential adverse effects is essential for the physician when managing patients having or who would benefit from botulinum toxin therapy.

Key Words: Botulinum toxin, botulism, neurotoxin, neuromuscular transmission.

INTRODUCTION

Botulinium toxins are increasingly used in the treatment of a number of medical conditions as a result of the purification of the botulinum neurotoxin (BoNT). BoNT was once considered to be the most deadly toxin known to humans. The aim of this article is to review the pharmacology and clinical uses of BoNTs as an understanding of the pharmacology, and potential adverse effects is essential for medical practitioners when treating patients who would benefit from BoNT therapy.

HISTORY

The presence of botulinum neurotoxin has been known about since the 18th century. Botulism (i.e. poisoning by BoNT) was first identified by Justinus Kerner in 1822. He linked deaths from food intoxication with a poison found in smoked sausages (*Botulus* means sausage in Latin) [1]. Botulism is characterized by descending flaccid paralysis with blurred vision, mydriasis, diplopia, ptosis, dysphagia, and dysarthria. Mental function remains intact. The progressive flaccid paralysis leads to respiratory failure and death in the absence of life support measures [2]. Kerner concluded that the poison interfered with the motor and autonomic nervous system excitability. He suggested that "the capacity of nerve conduction is interrupted by the toxin in the same way an electrical conductor is by rust" [3]. He proposed a number of medical uses for the toxin including the treatment of hypersecretion of bodily fluids, ulcers from malignant diseases, and skin alterations from burns, rabies and tuberculosis. It is now known that BoNT inhibits acetylcholine release at the neuromuscular junction [4].

Emile Pierre Marie van Ermengem suggested that *Clostridium botulinum* caused botulism in 1895 following the isolation of the bacillus from the remnants of a meal that killed three musicians in Ellezelle, Belgium [2,5].

In the 1920s there were several outbreaks of food borne serotype A botulism in California [6]. This spurred on the first attempts to purify the BoNT. It is believed that at this time Dr. Hermann Sommer at the University of California isolated a crude form of BoNT type A (BoNT/A) as a stable acid precipitate [7]. In 1946 Dr. Edward Schantz prepared crystalline forms of BoNT/A, and further studies on BoNT type A were facilitated. Several other serotypes have been characterized subsequently [2]. In the 1950s Dr Vernon Brooks discovered that BoNT/A, when injected into a hyperactive muscle, blocked the release of acetylcholine from presynaptic motor nerve endings and this induced a reversible paralysis of the injected muscle [7]. In the 1970s the perception of BoNT as a toxin began to change when it was used as a research tool to study spinal cord physiology [8]. In 1973 Dr Alan B. Scott, an ophthalmologist, published a study on the effect of BoNT injection into the lateral rectus muscle of the monkey [7]. In 1981 he reported the therapeutic efficacy of BoNT in the treatment of strabismus in humans after injecting BoNT into the extra ocular muscles in 67 patients [9]. After 4 to 5 days the toxin caused weakness in the extraocular muscles and the treatment corrected strabismus without any systemic complications [10]. Successful correction of strabismus with BoNT/A led the way for the use of BoNTs in a wide array of potential therapeutic applications. In the late 1980s BoNT/A was used for blepharospasm and other focal dystonias [1].

In 1989 the United States Food and Drug Administration (FDA) approved the use of Botox (the first therapeutic BoNT based product) for the treatment of strabismus, benign essential blepharospasm and disorders of the VIIth cranial nerve. Since then world wide experience has shown that this therapeutic agent is safe and effective for numerous indications. Subsequently, in 1991, another formulation of BoNT type A complex (Dysport) was approved in the United Kingdom. In December 2000, BoNT/A and two botulinum neurotoxin B complex preparations (Myobloc in USA; Neurobloc

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1573-4064/07 $50.00+.00

in Europe) were both approved by the FDA for use in cervical dystonia.

The use of BoNT/A for cosmetic purposes was first described by Carruthers and Carruthers in 1992 when 18 patients were injected with BoNT/A for the treatment of glabellar frown lines. In April 2002, BoNT/A was approved by the FDA for the treatment of brow furrow wrinkles [11]. The number of disorders currently treated with botulinum toxin has expanded [2]. Despite its deadly potential, BoNT is useful a therapeutic agent in a number of neurological and muscle disorders.

CHEMISTRY

Botulinum toxin, with lethal doses as low as 10^{-9}g/kg body weight, is one of the most potent neurotoxins known [12,13]. It is produced by *Clostridium botulinum*, a gram positive anaerobic sporulating rod. Other species such as *Clostridium barati* and *Clostridium butyricum* are also capable of producing the toxin. *Clostridium botulinum* is commonly found in soil and water but is relatively innocuous unless ingested in large amounts [3] There are approximately 24 cases of food-borne botulism per year in the United States [14].

Different strains of the *Clostridium botulinum* produce different serotypes of botulinum toxin. Seven serotypes of botulinum neurotoxin (A to G) have been identified. There is approximately 50% homology between these serotypes.(6) They vary in molecular size (from 300-900 kilodaltons, kD), cellular mechanism of action, formulation, and clinical usefulness [10]. Botulism is caused by BoNT serotypes A, B, E, F and potentially G [1]

The seven BoNT serotypes are macromolecular complexes that are antigenically distinct and have a common subunit structure [15]. The large BoNT complexes are most stable in the pH range of 5 to 7. At a pH of >7, the protein subunits dissociate [12] to a neurotoxin protein (150 kD) and non-toxin proteins. The non-toxic proteins account for about 70% of total mass [13]. The non-toxic proteins protect the neurotoxin subunit, especially during transit in the gastrointestinal tract prior to its absorption. The non-toxic proteins also stabilize the labile neurotoxin during its purification and manufacture, and reduce the undesired spread of the toxin to adjacent muscles when injected into the target muscle.

The 150 kD neurotoxin is a single polypeptide chain. (Fig. **1**). It has little pharmacological activity until it is cleaved by protease enzymes to form two polypeptide fragments. The cleavage occurs at a peptide bond approximately one third the length of the polypeptide from the N terminus. The resultant dipeptide consists of a 100 kD heavy chain and a 50 kD light chain that are linked by heat labile disulphide bonds.

The heavy chain has two functional domains, a 50KDa C-terminal fragment (Hc) which binds to acceptor sites, and a 50KDa N-terminal fragment (Hn) which is associated with translocation of the light chain into the cytosol [16,17]. The Hc fragment consists of the most disparate amino acid sequence among the serotypes, and it provides specificity of each serotype (binding sites). It is also the region associated

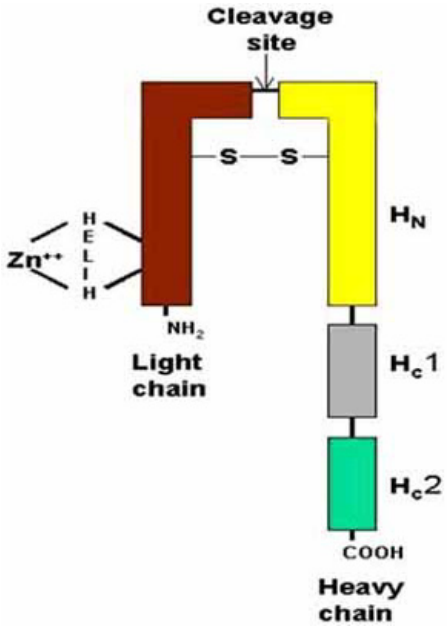

Fig. (1). The dipeptide structure of botulinum toxin.

with the development of antibodies. The light chain possesses a zinc binding site that has intracellular proteolytic activity and causes neurotoxicity [17].

The various serotypes differ in the extent to which they are cleaved and thus activated. More than 95% of BoNT serotype A is cleaved. Type B is cleaved to a similar extent. The amount of cleaved neurotoxin is important because the uncleaved neurotoxin which has no therapeutic activity contributes to overall protein content and immunogenicity [14]. Whilst the various serotypes of BoNT have common structural similarities and mechanisms of action, variations in acceptor binding, enzyme action, and species sensitivity indicate that each serotype is distinct pharmacologically [13].

MECHANISM OF ACTION

BoNT has a high affinity for the neuromuscular junction and causes a reversible cholinergic blockade at the neuromuscular junction by inhibiting vesicle exocytosis. This reduces acetylcholine release into the synapse, and thus paralyzes the innervated muscle. Inhibition of acetylcholine release occurs in 3 steps: (i) target cell binding, (ii) translocation and internalization, and (iii) inhibition of acetylcholine release.

1. Binding to Target Cell

BoNTs bind to cholinergic nerve terminals *via* the Hc binding domain of their heavy chains (Fig. **2**). Each serotype binds to its own unique acceptor site on the presynaptic nerve membrane and therefore does not compete with other serotypes [2,12] The toxin binding domain interacts with two receptors. Initially it binds in a nonspecific, low affinity manner to trisialogangliosides located at the nerve terminal. Then it binds in a high affinity and specific nature to an as yet unidentified protein receptor that appears to be serotype specific [14]. Following this, BoNTs are internalized into the

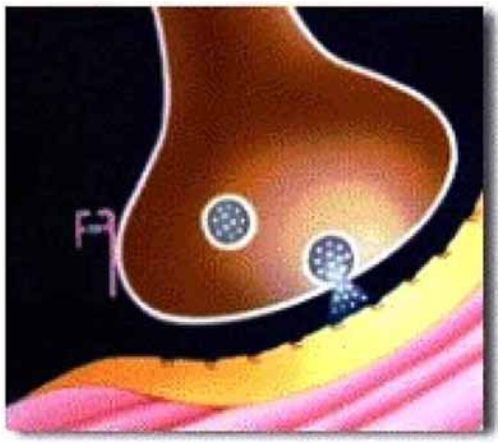

Fig. (2). Binding of BoNT to a nerve terminal *via* its heavy chain.

acidic environment of the endosome *via* endocytosis. This process is both temperature and energy dependent.

2. Internalization and Translocation

The nerve cell plasma membrane invaginates around the toxin receptor complex to form a vesicle (Fig. **3**). The acidic pH within the cell triggers a structural change in BoNT that increases its hydrophobicity and enhances penetration of bilipid membranes [4]. Once BoNT is bound to the acceptor sites on the neuron and the process of translocation into the cytosol has begun, the process is irreversible. Antitoxins administered after the process has commenced only neutralize the unbound protein and are therefore not effective at reversing the effects of the toxin [2].

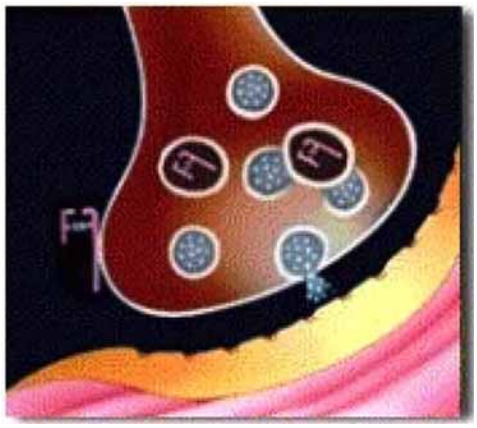

Fig. (3). Invagination and translocation of BoNT at the nerve ending.

3. Inhibition of Neurotransmitter Release

The vesicle then releases the light chain, a zinc dependent endopeptidase that cleaves Soluble N-ethylmaleimade-sensitive factor Attachment Receptor Protein (SNARE). SNARE is a 'docking' protein that is involved in the fusion of acetylcholine containing synaptic vesicles with the plasma membrane (Fig. **4**). Cleavage of SNARE proteins results in a non-functional complex and the coupling between Ca^{++} influx and fusion is disrupted. Through this mechanism BoNT proteins interfere with vesicle docking and exocytosis [18].

Up to 26 different SNARE proteins exist in a typical mammalian cell but only a few of them are involved in the release of neurotransmitter containing vesicles [18]. The 7 BoNT serotypes cleave specific SNARE proteins. Each BoNT serotype cleaves its targeted protein at a unique amino acid sequence. BoNTs A and E act on SNAP-25 (synaptosomal-associated protein of 25kDa), BoNTs B, D, F, and G cleave synaptobrevin, and BoNT C1 act on both SNAP-25 and syntaxin [2,4,19,20]

Injection of BoNT produces several changes at the neuromuscular junction. The most obvious histological features are axonal sprouting, muscle fibre atrophy, and formation of new end plates [21] The effect of BoNT at the motor end plate is only temporary. Two mechanisms mediate the termination of the effects of BoNT: (1). axonal sprouting, and (2) restoration of the SNARE protein complex. Collateral axonal sprouts develop over time at the nerve terminal and these sprouts can release acetylcholine into the synaptic space so that muscle activity returns. The newly formed sprouts degenerate as the parent axonal terminal recovers function with the restoration of the SNARE proteins [6,22]. These phenomena explain the clinical observation that repeated doses of BoNT are required to maintain a therapeutic effect [23].

ELECTROPHYSIOLOGICAL EFFECTS

Electrophysiologically BoNT reduces the amplitude of compound muscle action potentials (CMAPs) induced by single and repetitive supramaximal nerve stimulation. The CMAPs represent the summed response evoked by supramaximal stimulation of the associated nerve and reflect nerve conduction velocity and the initial depolarization of the innervated motor fibres. The peak amplitude is correlated with the number of activated extrafusal motor fibres and their orchestration. In severe botulism, the most consistent electrophysiological feature is a reduction in evoked CMAP in clinically affected muscle [13]. After BoNT injections maximal reduction in CMAP amplitude is achieved with repetitive stimulation at 2 Hz. The extent of muscle paralysis is enhanced by proximity of the injection site to the motor endplate. It can be further accentuated by muscle activation with electrical stimulation [2].

The minimum effective dose (MED) is defined as the dose producing a mean decrement of the CMAP of 70% in the injected muscle. Dose response studies demonstrated that the MED in the monkey abductor pollicis brevis was 0.09 units for BoNT type A and 0.44 units for BoNT/B. The ratio of MED between BoNT types A and B should not be extrapolated directly to humans [13].

When a muscle is stretched, afferent signals from the muscle spindles of the intrafusal muscle fibres traveling in Ia and II nerve fibers excite the alpha motor neurons of the stretched muscle as well as interneurons which inhibit the alpha motor neurons of its antagonist muscle. Acetylcholine is also a neurotransmitter in intrafusal muscle fibers. It is postulated that the mechanism of action of BoNT may be also related to blockade of the intrafusal muscle fibers. Injection of BoNT can attenuate the afferent signal in the Ia and II nerve fibers. This reduces the feedback to the alpha motor neurons and other pathways to reduce muscle activity of

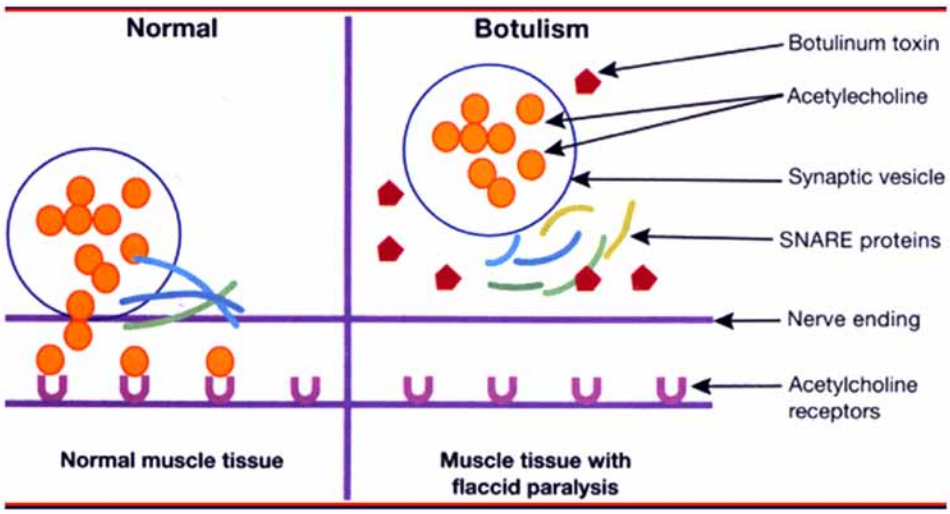

Fig. (4). (a) Normal acetylcholine vesicle docking by SNARE proteins. (b) Inhibition of docking *via* the cleavage of SNARE proteins by the light chain of botulinum neurotoxin.

noninjected muscles. The reduction of overall muscle contraction reduces excess muscle contraction and any associated pain. Animal data supporting this hypothesis are limited, and the evidence for the intrafusal action of BoNT is weak [27].

Acceptors for BoNT are also found on autonomic nerve terminals where they inhibit acetylcholine release at glands and smooth muscle [23]. Whilst BoNT readily inhibits the release of acetylcholine it prevents the release of other neurotransmitters such as noradrenaline, dopamine, serotonin, γ-amino-butyrate, glycine, and peptide methionine-enkephalin when present in large quantities [7]. BoNT does not cause any CNS effects when administered peripherally because it cannot cross the blood brain barrier [7].

ANALGESIA

BoNT therapy can alleviate pain associated with various conditions with or without concomitant excess muscle contractions. Several mechanisms have been proposed for the analgesic effect of BoNT. These include a reduction of muscle contraction, inhibition of release of neurotransmitter , retrograde axonal transport resulting in effects in the ventral roots of the spinal cord and spread into the synapses in the spinal cord.

Pathological muscle contraction can produce pain through compression of small blood vessels which make tissues ischaemic and lead to the release of bradykinin and other mediators which sensitize or excite peripheral nociceptors [6]. BoNT inhibits muscle contraction *via* inhibition of the alpha motoneuron and possibly *via* inhibition of the muscle spindle. This prevents pathological muscle contracture and alleviates the pain.

There is evidence that the antinociceptive effect of botulinum toxin type A is mediated by the inhibition of the release of substance P, calcitonin gene related peptide (CGRP), and other pain mediators [21,30]. SNARE proteins are found in vesicles that contain substance P and CGRP as well as those that contain acetylcholine. BoNT cleaves these SNARE proteins and prevent the release of pain mediators so that peripheral and central sensitization is reduced [3].

In a rat model BoNT/A significantly inhibited formalin induced glutamate release, formalin induced Fos-like immunoreactive cells in the dorsal horn of the spinal cord. The excitation of wide dynamic range neurons of the dorsal horn was also significantly reduced [25]. Therefore BoNT can inhibit both peripheral and central sensitization.

BoNT uptake is enhanced in nerve terminals that are most active, whether the hyperactivity is induced by nerve stimulation or increased voluntary activity. This uptake is responsible for the weakening of those muscle fibers that are pathologically overactive. The clinical significance of this finding is not known [24].

In vivo animal studies indicated that some BoNT enter the CNS after peripheral administration. Histoautoradiography studies in animals suggest that BoNT or its fragments are transferred to the ventral roots and to the adjacent spinal cord by retrograde axonal transport following intramuscular injection and then spread partly to the contralateral spinal cord. Direct stimulation of the injected muscles increase retrograde transport to the ipsilateral spinal cord segments. Significant decreases in central synaptic transmission, reflexes and motor neuronal firing pattern are observed following high dose intramuscular injections of BoNT or in experimental botulism in animals and these may be related to retrograde axonal transport and central spread.

PHARMACODYNAMICS

The biological activity of botulinum neurotoxin is measured in mouse units, mouse LD50 or simply a ‘unit’. This is defined as the amount of toxin required to kill 50% of a group of Swiss-Webster mice (18-22g), after intraperitoneal injection. The LD50 for a 70 kilogram a human is between 2700-3000 mouse units [1]. Differences in serotype, formulation, and the way lethality tests vary between manufacturers results in the large variability in the estimated equivalent potency between the various products [5]. Therefore the dose of one product cannot be easily converted into the dose of

another product [1]. For example, with the two commercially available BoNT/A preparations, doses of Dysport can be 3 to 6 times higher than the doses of Botox typically used to treat the same condition. The major determinants of clinical response to BoNT treatment are the toxin preparation, length of toxin storage (after reconstitution), mass of targeted muscle, dose and response relationships, and immunogenicity [1,8,26]

Dose Effect Correlation

The relationship between the amount of BoNT applied and the extent of paresis produced is demonstrated by the reduction of maximal amplitude of electromyographic recordings of muscles. Dose effect correlation curves can be used to optimize BoNT doses in muscle tissue. However, lower doses diminish the duration of effect.

Onset and Duration of Effect

The clinical effects of the serotype A and B neurotoxins generally begin within 24-48 hours and peak at 2-3 weeks and then plateau for 1 to 2 months. Patients require reinjection approximately every 3 months. A retrospective review that included 60 cervical dystonia patients treated for at least 1 year showed that the mean clinical response lasted 15.6 weeks (range: 12.2-24.3 weeks). The benefit was longest in patients who experienced moderate symptoms [2]. When relatively low doses were applied there was a correlation between dose and duration of effect. With higher doses the duration of action of BoNT serotypes A and B reached a maximum at about 3 months.

Pharmacokinetics

Studies on the pharmacokinetic profile of botulinum toxins have mainly focused on diffusion from the site of injection as this influences the risk of unwanted effects on adjacent structures and also potential systemic effects. Several factors (dose, volume, site of injection, muscle size, and muscular fascia) influence the extent of diffusion and possible side effects. Several methods (electrophysiological assessment of end plate activity, histological evidence of muscle atrophy, and immunochemical localization of the toxin) have been used to assess the diffusion of BoNT [14].

(a) Spread to Adjacent Muscles

BoNT is directly injected into the muscles involved in the wide variety of clinical conditions. Animal models demonstrate that BoNT can penetrate muscle fascia but this spread is limited (approximately 20-25%). In humans, some weakness and end-plate dysfunction can be demonstrated in muscles adjacent to the injection site.

(b) Spread to Remote Muscles

Electromyography studies on muscle fibres show subtle abnormalities in the end plate function in muscles remote from injection sites although their maximal compound muscle action potentials are unchanged [24]. The potential spread of BoNT action varies between different serotypes. Sudies performed on a monkey hand model suggested that there was less spread of BoNT/B to nearby and relatively distant non-injected muscles compared with BoNT/A when doses are adjusted to produce an equivalent direct effect [13]. This may be because the BoNT/B formulation is buffered in a slightly acidic solution (pH 5.6) to maintain its integrity when it is injected.

(c) Systemic Spread

The tissue specificity of BoNT results in minimal uptake into the systemic circulation and therefore causing minimal systemic side effects. BoNT cannot penetrate the blood brain barrier because of its large molecular size. However there is potential for it to enter the CNS *via* retrograde axonal spread. This process is slow, and BoNT is likely to be inactivated before it can enter the [8].

PREPARATIONS

BoNT is produced from an anaerobic culture of a specific *Clostridium botulinum* strain. The toxin is recovered and purified *via* a series of salt precipitation and chromatography processes [21]. Either sodium chloride (Botox), or lactose (Dysport) is added to protect the steric structure of the neurotoxin. Human serum albumin is then added to inhibit loss by surface adsorption. Finally the toxin is then dried either with freezing (Dysport) or without freezing (Botox) [27].

There are differences in neurotoxin complex protein size, amount of neurotoxin in the activated or cleaved form, intracellular protein target, and potency among the Botulinum neurotoxin serotypes. These properties vary between preparations that contain the same botulinum toxin serotype as a result of differences in formulation. As a result each product has unique properties in terms of efficacy, duration of action, stability, toxicity and antigenic potential [16,21,28] and also interspecies variation [12].

Of the eight serotypes of BoNT identified, only BoNT/A and BoNT/B are commercially available. The vast majority of commercial developments of botulinum toxin for clinical use are based on botulinum toxin type A. Other serotypes studied include BoNT/C (profile of action similar to BoNT/A) [19]; BoNT/E (shorter duration of action than BoNT/A) and BoNT/F (shorter duration of effect than BoNT/A) [6].

Botox® (Allergan, Irvine, California, USA)

The first formulation of botulinum toxin type A was marketed in the United States in 1989 by Allergan as Botox. In 2002 the same formulation was marketed for cosmetic use as Botox Cosmetic. It is supplied in vials containing 100U BoNT/A, 0.5mg human albumin, and 0.9mg of sodium chloride in a sterile, vacuum-crystalline form without a preservative. It is a vacuum dried preparation that has a pH of approximately 7 [9]. The manufacturers recommend that the vial should be stored below -5^{o}C and reconstituted with sterile saline solution. BoNT/A should be used within 4 hours of dilution. The reconstituted product can be stored refrigerated for a week without any loss of efficacy [10]. Drying processes in the drug manufacture can cause inactivation (up to 40%), aggregation, and denaturation of BoNT/A preparations (12,13,29,30). It is recommended that the maximum dose in a single treatment should not exceed 400 units [29].

Dysport® (Speywood Pharmaceuticals, UK)

In 1991, a different formulation of botulinum toxin type A was marketed outside the United States as Dysport. Each vial of Dysport contains 500U of BoNT/A, 125mcg of human albumin and 2.5mg of lactose. It is a lyophilized formulation with a pH of approximately 7. Before reconstitution the manufacturer recommends storage in a refrigerator at 2-8C. The maximum dose in a single treatment should not exceed 1500 units [29].

One unit of Botox is approximately equivalent to 2.5 units of Dysport. Different studies have demonstrated that the ratio (Botox:Dysport) to be 2.33 in hemifacial spasm, and approximately 3 in torticollis, and 4 in blepharospasm and hemifacial spasm [6].

***Myobloc*® (Elan Pharmaceuticals, California, USA) or *Neurobloc*® (Elan Pharmaceutical International, Ireland)**

Botulinum toxin type B was approved by the FDA in 2000 and is marketed by Elan Pharmaceuticals as Myobloc in the United States. It is known as Neurobloc in Europe. Myobloc is presented as a solution with pH of 5.6 in 0.5ml, 1.0ml, and 2.0ml vials. It causes more pain on injection because it is more acidic than BoNT/A preparations. Each vial contains 5000U/ml of BoNT/B, 0.05% human albumin, 0.1mol/L sodium chloride, and 0.01mol/L sodium succinate. Myobloc is not lyophilized and as a result it does not need to be reconstituted before use. The manufacturer recommends the Myobloc/Neurobloc should be stored at 2-8^{o}C. Studies have shown that unopened BoNT/B vials are stable for 30 months when refrigerated and for 9 months at room temperature [11,13]. Unlike BoNT/A, it does not require reconstitution prior to use. The maximum dose for a single treatment should not exceed 10,000 units Neurobloc [29].

Dosage and Administration

Exact doses and injection sites depend on the patient, the pathology treated and the formulation of BoNT used. Injection of a high concentration in small volumes results in a more localized effect, with a dispersion area of about 1cm. In contrast, injection of a low concentration in large volumes results in greater diffusion of the toxin and can cause paralysis of unintended muscles. The use of eletromyographic guidance to locate the appropriate muscles accurately substantially reduces the dose of BoNT [2]. The onset of action of BoNT is 1 to 2 days after injection. This decreases the predictability of effect of BoNT when injected. To overcome this, addition of local anaesthetic agent to BoNT enables the clinician to be confident about the accuracy of the injection of BoNT into the target muscle. The addition of lignocaine and adrenaline to BoNT does not reduce its potency [9].

CLINICAL APPLICATIONS

BoNT therapy is approved by the FDA for the treatment of several disorders such as strabismus, disorders of the VIIth nerve, blepharospasm, and treatment of brow furrow wrinkles. Numerous trials performed and case reports suggest the use of BoNT in the management of other conditions.

(i) Muscle Disorders (Table 1)

BoNT is used in the treatment for a wide variety of muscle movement disorders.

(a) Dystonia

BoNT has become first line treatment for cervical dystonia and provides relief (with improvement in head posture, range of motion, and pain) in about 85% of patients [2,31]. More than 75% of patients with jaw-closing oromandibular dystonias improve significantly [1]. More than 90% of patients of laryngeal dystonia treated with BoNT experience a satisfactory result. The efficacy in upper limb dytonias is variable, improving symptoms in 35-58% of patients. Pain is the most frequent symptom that is improved, regardless of whether or not there is an improvement in motor function [1,2]. In the treatment of leg dystonia the major reason for reduced efficacy is limitation (no more than 400U Botox) of the BoNT dose that can be used safely.

(b) Blepharospasm

In 1989 the FDA approved the use of Botox for the treatment of blepharospasm. A small number of controlled studies have confirmed the results of open trials that showed the efficacy (response rates = 90%) of BoNT in treating blepharospasm. Long-term follow up of patients receiving multiple treatments showed sustained reduction in intensity of blepharospasm [2].

(c) Strabismus

In 1981, the first series of botulinum toxin use in humans was published by Dr. Alan B. Scott who treated 67 patients with strabismus. Further evidence of the benefit of this treatment of this condition led to the approval for the use of Botox for treatment of strabismus by the FDA in 1989.

(d) Hemifacial Spasm

BoNT/A is currently first-line therapy for involuntary facial spasms, with response rates of 95% for hemifacial spasm (tics) [10]. Surgical decompression of the facial nerve is only needed in patients who have a poor response.

(e) Spasticity

BoNT is used in the treatment of spasticity resulting from cerebral palsy, multiple sclerosis, traumatic brain injury, spinal cord injury, and stroke [32]. BoNT injection into spastic muscles reduces resistance to passive movement of the involved muscles. Continued physical management may then improve resting posture and help regain active function. However no improvement in purposeful muscle movement has been demonstrated [1,24,29].

(f) Tremor

BoNT is used to treat a number of forms of tremor such as essential tremor, dystonic tremor, head tremor, voice tremor, and rest tremor. It is most efficacious in the treatment of voice tremor and head tremor. In essential voice tremor BoNT injected into the thyroarytenoid muscles reduced tremor amplitude and laryngeal resistance and eased vocal strain when speaking [2].

Table 1. Botulinum Toxin and Muscle Disorders

Author	Design	No.	Comments	Results	Adverse Effect	Year	Ref
Cervical dystonia							
Myobloc							
Figgitt *et al.*	Review of three RCTs			all 3 RCTs BoNT/B - effectively reduced severity, disability and pain of cervical dystonia	Most common adverse effects: dry mouth and dysphagia. No serious adverse events	2002	[34]
Hemifacial Spasm							
Mahant *et al.*	1 RCT, several OL			Objective improvement in 84% after BoNT/A injection, and none after placebo		2000	[30]
Spasticity							
Speth *et al.*	RT	20	Aim: Determine if BoNT/A increased upper limb function in treatment programme. Control group - no placebo injections	Treatment group - clinically relevant increase in active dorsal flexion, and tone reduction of the wrist. No statistically significant differences in functional outcome measures		2005	[17]
Bhakta *et al.*	DB P RCT	40	Patients randomized to receive BoNT/A or placebo	Significant improvement at 2 weeks, nil demonstrated subsequently. No pain improvement	No serious adverse effects reported	2000	[35]
Simpson *et al.*	DB P RCT	39	BoNT/A or placebo into the biceps, flexor carpi radialis, and flexor carpi ulnaris muscles	300 unit BoNT/A - statistically and clinically significant improvement in spasticity	no serious adverse effects	1996	[36]
Barbaud *et al.*	DB P RCT	23	Patients examined on days 0, 30, 90, and 120 and received one injection at day 0 and day 90	clear subjective improvement in foot spasticity after BoNT/A ($P = 0.0014$) but not after placebo		1996	[37]
Hyman *et al.*	DB P RCT	74	Dysport (500, 1000, or 1500 Units), or placebo to the hip adductor muscles of both legs	Improved efficacy with Dysport compared to placebo	Adverse events in 32/58 (55%) Dysport patients; 10/16 (63%) placebo patients	2000	[38]
Cosgrove and Graham	RCT	20	BoNT injections into the hamstrings and gastrocnemius in cerebral palsy children	increased the length of hamstring muscles		2004	[39]
Oromandibular Dytonia							
Mahant *et al.*	Review: 1 RCT, several OL			Data supportive		2000	[30]
Tics							
	OL	35		BoNT/A injections effective, well tolerated		2000	[33]
Tinnitus							
Scolozzi *et al.*	CS			BoNT injection into masticator muscles -n relieved severe and disabling postpolio tinnitus		2005	[36]

DB = double blind, P = placebo, R = randomized, CT = controlled trial, OL = open label study, CS = case study, M = multicentre, Pr = prospective, Re = retrospective

The efficacy of BoNT is variable in the treatment of numerous other movement disorders (e.g. myoclonic jerks, laryngeal dystonias, vocal tics, stuttering, crico-pharyngeal spasms, bruxism, nystagmus, chronic VI nerve palsy, myokymia, VII nerve disorders, tardive dyskinesias, in Tourettes syndrome [for tonic cervical tics, vocal tics and coprolalia], and in Parkinson's disease to manage prominent tonic components and freezing of gait) [1]. Botulinum toxin therapy has been reported to be useful in the temporary inactivation of abnormal muscle function after orthopaedic or neurosurgical procedures, and in the treatment of tetanus [27].

(ii) Hypersecretory Disorders (Table 2)

(a) Hyperhidrosis

BoNT is useful in the treatment of several conditions associated with increased sweating (hyperhidrosis). BoNT is considered first line treatment for gustatory sweating. The duration of treatment effect in patients with Frey's syndrome (gustatory sweating of the cheek following parotid surgery) is much longer (mean duration 17.3 months) than in patients treated for other indications [15]. BoNT/A is a safe and efficacious alternative to other less effective medical or more invasive surgical options for the treatment of palmar and axillary hyperhidrosis. Quality of life is improved and the associated body odor is reduced [10].

(b) Sialorrhoea

BoNT injection into the parotid gland effectively controls drooling in conditions such as Parkinson's disease, motor neuron disease, and bulbar or pseudobulbar palsy [1]. BoNT/B may be an effective treatment for sialorrhoea caused by autonomic dysfunction [23].

Botulinum toxin has also been used with some success in the treatment of hyperlacrimation, and nasal hypersecretion.

(iii) Pain Syndromes (Table 3)

As more experience is gained, the success of BoNT therapy in treatment of pain syndromes is likely to increase. Numerous trials have reported variable results on the efficacy of BoNT in tension headache. Apart from one study all the studies on tension headache have not shown any reduction in the frequency of headaches with BoNT.

BoNT is effective in the prophylaxis of migraine. The exact mechanism is unknown but it is postulated that BoNT reduces the afferent volley of pain impulses [17].

Botulinum toxin has variable efficacy in the treatment of backache, dystonia-complex regional pain syndrome, myofascial pain, and tennis elbow.

(iv) Urogenital and Gastrointestinal Conditions (Table 4)

Hyperactivity of the urethral sphincter leads to sphincter-detrusor dyssynergia. BoNT can be injected into the urethral sphincter *via* either transperineal or transurethral injections. External urethral pressure, voiding pressure, and post-void residual volume decrease following treatment. These effects last between 2 to 9 months [7].

Injection of BoNT into the detrusor muscle of the bladder under cystoscopic guidance reduces hyperreflexia of the urinary bladder [1]. The efficacy of BoNT is variable in the treatment of neurogenic detrusor overactivity, chronic prostatic pain, and vaginismus.

Botulinum toxin is useful in the treatment gastrointestinal disorders such as anal fissures, anismus, achalasia, Hirschprung disease, and disorders of the sphincter of Oddi.

(v) Dermatological / Cosmetic Conditions (Table 5)

The most common use of botulinum toxin therapy today is in cosmesis. It is efficacious in the treatment of hyperfunctional facial lines, facial asymmetries, and hypertrophic platysma muscle bands.

ADVERSE EFFECTS

Botulinum toxin therapy has been shown to be safe in a variety of conditions when administered appropriately. In the 25 years that it has been used in humans there are no reported deaths from an overdose [5,20]. Adverse effects may be caused by either local or systemic spread of unbound botulinum toxin.

(a) Local Adverse Effects

Untoward sequelae that may occur at any injection site include pain, oedema, erythema, ecchymoses, and short term hyperesthesia. Pain at the injection site is greater with BoNT/B compared with BoNT/A [11].

Reduction of physiological muscle strength is uncommon because of the wide therapeutic window. The severity of effect depends upon the site of injection. Approximately 50% of patients treated for cervical dystonia develop radiological evidence of dysphagia, 33% develop new symptoms of swallowing difficulties especially with coarse solids [2]. In the treatment of abductor spasmodic dysphonia with BoNT injections into the thyroarytenoid muscles severe stridor (caused by paralysis of the posterior cricoarytenoid muscle) can occur. There are anecdotal reports of such patients requiring tracheostomies [5]. In patients with lingual dystonia it is prudent to avoid injecting the tongue because a flaccid tongue can cause life threatening airway obstruction [1].

Although mild muscle atrophy occurs following BoNT injections, repeated injections do not cause irreversible change or fibrosis of the muscle [2]. Muscle biopsies obtained from patients who have received numerous doses of BoNT for more than 7 years did not show any signs of atrophy or permanent muscle degeneration [33].

Dental caries and gum disease caused by an excessively dry mouth have been reported when BoNTs are used to treat hypersalivation [27].

(b) Systemic Effects

There have been case reports of generalized mild muscle weakness as a result of systemic dissemination [1,2]. following localized injections of BoNT. Using electromyographic studies, Olney *et al.* showed that injections into neck muscles for cervical dystonia caused decreased excitability in the biceps brachii muscles. These muscles recovered and returned to normal after 3 to 6 months [2].

Table 2. Botuinum Toxin and Hypersecretory Disorders

Author	Design	No.	Comments	Results	Adverse Effect	Year	Ref
Hyperhidrosis							
Botox							
Naumann *et al.*	M, Initial DB RCT	207	16-month study with initial DB randomization to 50 U of BoNT/A or placebo per axilla	174 (84%) completed the study. Subjects' satisfaction after treatments -consistently high, quality of life improved, and reduction in the impact of the disease on their lives	BoNT/A Safety profile - excellent, no neutralizing antibodies reported	2003	[40]
Laccourreye *et al.*	Pr OL	33	An inception cohort with a minimum of 18 months follow-up	Severity of gustatory sweating reduced, - amenable to further treatment		1999	[41]
Dysport							
Heckmann *et al.*	M RCT	145	BoNT/A injected into one axilla, placebo in other. 2 weeks later placebo injected axilla treated with BoNT/A	Treatment well tolerated, good efficacy		2001	[42]
Sialorrhoea							
Botox							
Suskind *et al.*	Pr OL, dose-finding study	22	Intraglandular BoNT in treat-ment of sialorrhoea in children with cerebral palsy	Difficulty in objectively assessing amount of 'drool', and any reduction. No control group used	no adverse effects on swallowing	2002	[43]
Bhatia *et al.*	Pr OL	4	BoNT injections into parotid gland in four patients with excessive drooling	beneficial response beginning at end of the first week and lasting 6 weeks in one patient and 3 to 4 months in the others	Dysphagia, mild chewing difficulties, dry mouth. No facial weakness	1999	[44]
Pal *et al.*	Pr OL	9	Intraparotid injections of BoNT/A in reducing salivary secretions and drooling in PD	Marked objective reduction in secretion, and 2/3 had subjective improvement in drooling	No side effects observed	2000	[45]
Giess *et al.*	Pr OL	5	BoNT/A) injected into the salivary glands. Patients with bulbar ALS and sialorrhoea	BoNT/A improved sialorrhoea and quality of life	No major adverse effects	2000	[46]
Porta *et al.*	Pr OL	10	BoNT/A injection into parotid and submandibular glands using ultrasound in 10 patients	9 (90%) reported a subjective reduction in salivation post-treatment and one patient (10%) found no improvement	No serious adverse events	2001	[47]
Ellies *et al.*	Re	4	55 to 65U Botox injected under sonographic control into parotid and submandibular glands	All4 patients - distinct improvement of symptoms within 1 week after injection. Salivary flow rate decreased	No side effects	2002	[48]
Dysport							
Lipp *et al.*	Pr DB P C dose-finding study	32	Efficacy of three different doses BoNT/A vs vehicle in patients with sialorrhoea	Primary endpoint was achieved with 75 MU BoNT/A per side. -mean saliva reduction of approximately 50%	No adverse events	2003	[49]
Myobloc							
Ondo *et al.*	DB P CT	16	Patients received either BoNT/B (1,000U into each parotid and 250U into each submandibular) or a pH-matched placebo	BoNT/B- improvement on the Visual Analogue Scale ($p < 0.001$), global impressions of change ($p < 0.005$), Drooling Rating Scale ($p < 0.05$), and Drooling Severity and Frequency Scale ($p < 0.001$)	Adverse events mild, - dry mouth (3); worsened gait (2), diarrhoea (1), and neck pain (1)	2004	[50]

DB = double blind, P = placebo, R = randomized, CT = controlled trial, OL = open label study, CS = case study, M = multicentre, Pr = prospective, Re = retrospective

Table 3. Botulinum Toxin and Pain Syndromes

Author	Design	No.	Comments	Results	Adverse Effect	Year	Ref
Tension Headache							
Freund *et al.*	Pr OL	60	BoNT/A into masticatory muscles, 50U into each masseter and 25U into each temporalis muscle	63% - subjective improvement in facial pain. Number of headache free days improved post injection		2002	[51]
Ondo *et al.*	P RCT	60	Primary efficacy point = no. of headache-free days for 12 weeks after BoNT injection	All improved in the BoNT group compared with placebo	Adverse events mild	2004	[52]
Burch *et al.*	DB P RCT	41	injection of 50U of Botox or sterile saline into the glabella and forehead region	Reduced number of headaches - not statistically significant, Headache intensity reduced significantly		2001	[53]
Padberg *et al.*	DB P RCT	40	BoNT (maximum 100U) or saline in muscles with increased tenderness	Botulinum toxin - not effective in treatment of chronic tension-type headache		2004	[54]
Wolfgang *et al.*	DB P RCT	60	Patients followed up at 4 and 8 weeks	number of pain-free days increased only in BoNT/A group	Side effects – no difference	2001	[55]
Zwart *et al.*	OL	6		Unilateral Temporal Injection not effective		1994	[56]
Wheeler	OL	4		Effective in 4 patients		1998	[56]
Schulte-Mattler *et al.*	OL	9		Effective in 8 out of 9 patients		1999	[56]
Rollnik *et al.*	DB P CT	21		Not effective		2000	[56]
Migraine							
Silberstein *et al.*	DB, vehicle-CT	123		Effective prophylaxis		2000	[56]
Binder *et al.*	OL	77		51% complete response - mean duration = 4 months		2003	[43]
Silberstein *et al.*	PR DB vehicle CT			significant reduction in migraine frequency, severity, quick relief medication use, and vomiting with 25 units BoNT/A		2003	[43]
Cervicogenic Headache							
Freund *et al.*	DB P RCT	26		Effective		2000	[24]
Temperomandibular Disorders							
Freund *et al.*	OL	15		Effective		1999	[24]
Freund *et al.*	OL	46		Effective		2000	[24]
Bruxism							
Ivanhoe *et al.*	CS			bruxism post head injury responding to BoNT/A		1997	[30]

(Table 3. Contd….)

Author	Design	No.	Comments	Results	Adverse Effect	Year	Ref
Backache							
Foster *et al.*	DB P RCT	31	Injection at 5 lumbar paravertebral levels on side of maximum discomfort	BoNT group - >50% pain relief clinically significant $p<0.05$	No side effects	2001	[57]
Myofascial pain							
Wheeler *et al.*	DB RCT	33		No significant difference		1998	[24]
Porta	RCT	40		chronic myofascial pain syndrome, Botox better than methylprednisolone		2000	[24]
Fibromyalgia							
Paulson *et al.*	RCT	5		Not effective		1996	[24]
Chronic Pancreatitis							
Sherman *et al.*	OL	7		Not effective		1995	[24]
Pain relief in cervical dytonia							
T. Sycha *et al.*	Review of 14 RCTs		All except one showed significant pain relief following BoNT			2004	[31]

(DB = double blind, P = placebo, R = randomized, CT = controlled trial, OL = open label study, CS = case study, M = multicentre, Pr = prospective, Re = retrospective

A relatively uncommon adverse effect is an influenza like syndrome with fever, lethargy and generalized myalgia [6]. This may be related to the non-toxin proteins in the BoNT. Preparations which have lower levels of non-toxin proteins may be advantageous [1]

Some studies reported a high incidence of dry mouth in the patients. This is observed more frequently after BoNT/B injections and may be related to the higher systemic absorption and the greater affinity for autonomic nerve endings [1,11]. Other evidence of systemic spread of BoNT is the occurrence of subtle changes in cardiovascular reflexes and blood pressure but these are rarely of clinical importance.

ANTIBODY FORMATION

Exposure to BoNT antigens can trigger an immune response with B cell and T cell activation, memory cell formation, and cytokine production [3]. Antibodies may be produced and can cause resistance to BoNT. The incidence of antibody mediated resistance is between 3 and 10% [1] Preparations containing the lowest amount of neurotoxin protein decrease the formation of neutralizing antibodies [12,28]. The protein content of Botox is 5ng/100 Units, Dysport 2.5ng/100 units, Myobloc 1ng/100 units. However the number of biological units per dose to treat a specific condition is not the same for each formulation. The protein load associated with the use of Botox is lower than that of the other two commercially available products because the number of biological units per dose is smaller. More purified forms of botulinum toxin have been manufactured with lower concentrations of non-toxin protein to prevent the formation of neutralizing antibodies. In a recent study of patients treated for cervical dystonia, blocking antibodies were not detected in any of 119 patients treated exclusively with a more purified form of Botox [1]. Several strategies are suggested to reduce formation of neutralizing antibodies and development of clinical resistance to BoNT. The use of the smallest possible effective dose and longer treatment intervals (at least 3 months) can reduce the likelihood of antibody development [2,3,34]. In patients with suboptimal outcome, supplemental injections should be avoided. Alternating different serotypes to minimize the formation of neutralizing antibodies has been suggested. However, preclinical studies suggest that certain fragments of botulinum toxin type A stimulate the production of antibodies that react with the antigens in the heavy chain proteins on other serotypes (eg BoNT/B) [3].

A lack of response to botulinum toxin therapy is not always caused by neutralizing antibodies. It may be due to misplaced toxin, sub-optimal dosing, or administration of toxin that has been inactivated by improper storage. In a survey of 155 patients receiving BoNT treatment, 21.8% discontinued treatment and the major reason for discontinuation cited was the lack of efficacy [2]. High cost of treatment, and the perception of lack of efficacy were common reasons for treatment cessation and adverse events were not the major reason for treatment cessation. In patients who suffer from "body dysmorphic disorder" there is a potential for the abuse of BoNT ("Botulinophilia") [15].

Table 4. Botulinum Toxin: Use in Urology, Gastroenterology

Urogenital Practice						
Sphincter-detrusor dyssnergia						
Dykstra and Sidi	DB	5		BoNT group urethral and bladder pressure during voiding decreased, post void residual volume of urine decreased	1990	[23]
Petit *et al.*	OL	17		reductions in residual volume, detrusor voiding pressures, and urethral pressures	1998	[23]
de Seze *et al.*	R DB	13		Improvement in post void residual volume, micturition volume, maximal detrusor pressure, satisfaction scores	2002	[23]
Chronic Prostatic Pain						
Zermann *et al.*	OL	11		9/11 reported subjective pain relief, with the average pain level on VAS decreasing from 7.2 to 1.6	2001	[23]
Urinary Retention						
Phelan *et al.*	OL	19		BoNT/A injections into the urethral sphincter = effective treatment for urinary retention	2000	[23]
Gastroenterological Disorders						
Anal fissures						
Mahant *et al.*	Review of 1 RCT, 2 CS			BoNT/A injections into the internal anal sphincter. High efficacy, low incidence of incontinence.	2000	[30]
Achalasia						
Storr *et al.*	Pr OL	40	Injection in LOS. Patients evaluated before treatment, 1 week and 1 month afterwards.	BoNT injection significant reduction in Lower Oesophageal Sphincter tone. Global symptom scores - significantly decreased after 1 week and 1 month.	2002	[58]

DB = double blind, P = placebo, R = randomized, CT = controlled trial, OL = open label study, CS = case study, M = multicentre, Pr = prospective, Re = retrospective

CONTRAINDICATIONS

A known hypersensitivity to any ingredient in the formulation is an absolute contraindication to the use of BoNT. Medications that interfere with neuromuscular transmission such as aminoglycosides, penicillamine, quinine, and calcium channel blockers, can potentiate the effect of BoNT and should be avoided during BoNT treatment Relative contraindications to BoNT therapy include evidence of infection at the proposed injection site, bleeding diathesis, lactating or pregnant mothers, neuromuscular disease and medications that may interact with BoNT [1,7,34].

FUTURE DIRECTIONS AND THERAPY

There are still several unanswered questions concerning the basic and clinical science of BoNT. Although much is known about the intracellular targets of BoNT, the identities of the serotype specific protein receptors present at the neuromuscular junction have yet to be determined. Further research is required in defining the efficacy of BoNT in a number of diseases. Studies in newer applications of BoNT (eg. treatment of rhinorrhoea) are currently being undertaken. The efficacy and potential use of the other BoNT serotypes is under further evaluation. Short acting derivates have been synthesized and may have applications in the treatment of sports injuries. Formulations of BoNT that have a longer duration of action due to blocking reactivation are being developed. The deactivated heavy chain is being studied as a carrier for other active substances to the nerve ending [1,4]. The bacterium *Clostridium botulinum* may also be useful in treating cancer . Apoptosis inducing Clostridia specifically colonize hypoxic areas of tumours, and may be use-

Table 5. Botulinum Toxin and Cosmetic Therapy

Hyperfunctional Facial Lines							
Carruthers *et al.*	Review 2 RCTs			Confirmed efficacy and safety of BoNT/A		2002	[43]

DB = double blind, P = placebo, R = randomized, CT = controlled trial, OL = open label study, CS = case study, M = multicentre, Pr = prospective, Re = retrospective

ful for transporting anti-cancer drugs specifically into tumor cells [4]. In a mouse model, BoNT/A has demonstrated to potentiate the effects of radiotherapy (by vasodilation and alleviating tumour hypoxia which causes resistance to radiotherapy) and chemotherapy (*via* increased delivery of drug) of fibrosarcoma and hepatocarcinoma [59].

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