

## Basic Aspects of Botulinum Toxin

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## Physiology and Pharmacology of Therapeutic Botulinum Neurotoxins

*K. Roger Aoki*

Neurotoxin Research Program, Biological Sciences, Allergan Inc., Irvine, Calif., USA

The therapeutic benefits derived from a local injection of a botulinum neurotoxin preparation are based on the site-specific delivery (i.e. intramuscular, subcutaneous, intrasphincter etc. injection) and its high-affinity uptake by cholinergic neurons. This results in a temporary chemodenervation and the loss or reduction of activity of the target organ (muscle, sweat gland, sphincter etc.) with minimal risk of systemic adverse effects. Worldwide experience since the approval of the first therapeutic product based on botulinum neurotoxin (Botox<sup>®</sup>, Allergan, Irvine, Calif., USA) in 1989 has shown that this is a safe therapeutic agent effective in numerous indications [Brin, 2000; Johnson, 1999; Aoki, 1998]. Subsequently, another preparation containing type A complex was approved in the UK in 1991 (Dysport<sup>®</sup>, Speywood). Although these products are based upon serotype A botulinum neurotoxins, they have sufficient differences in efficacy and safety profiles so that these products and other future botulinum-toxin-based products should not be considered generic equivalents comparable by simple dose ratios.

The mechanism of action of the 7 botulinum neurotoxin serotypes (A, B, C1, D, E, F and G) have been reviewed in other papers. This will provide an overview of the physiology and pharmacology of the therapeutically relevant neurotoxins A, B and F administered by injection to treat blepharospasm or cervical dystonia [Jankovic and Schwartz, 1990; Jankovic and Brin, 1991; Mezaki et al., 1995; Brashear et al., 1999; Brin et al., 1999]. Although serotypes C1 [Eleopra et al., 1997] and E [Eleopra et al., 1998] have been used in a limited number of volunteers and patients, their role as therapeutic agents requires further clinical studies. To date, the commercial development of botulinum toxin for clinical use has been based on botulinum toxin type A (BTX-A). However, studies indicate that botulinum neurotoxin serotypes B (BTX-B) and F (BTX-F) may be

useful for the treatment of cervical dystonia or blepharospasm [Mezaki et al., 1995; Lew et al., 1997; Brashear et al., 1999], especially in patients with BTX-A-nonresponsive cervical dystonia [Chen et al., 1998; Brin et al., 1999]. Clinical studies have found that BTX-A is more potent (total amount of units administered per patient per session) than BTX-B [Sloop et al., 1997; Brashear et al., 1999; Brin et al., 1999] and has a longer duration of action than either BTX-B for electromyography-based efficacy only [Sloop et al., 1997] or BTX-F for clinical responses [Mezaki et al., 1995; Chen et al., 1998; Houser et al., 1998].

Local efficacy/safety and duration of action are due to the dose (total units), inherent properties of the serotype (e.g. BTX-A, BTX-B, BTX-F) or its formulation. To illustrate the physiological effects of local injections of botulinum toxin, clinical and preclinical examples will be presented. The exquisite efficacy of botulinum neurotoxin therapy is primarily due to the injection method of delivery and the high-affinity uptake of the neurotoxin by nerves (cholinergic nerves are more sensitive to BTX-A than other exocytic cells). This combination of delivery method, low dose and neuronal uptake provides a reasonable measure of local efficacy with minimal systemic adverse effects. For example, Botox (BTX-A purified neurotoxin complex) from Allergan is the first approved medical product using botulinum toxin [Jankovic and Brin, 1997; Aoki, 1998; Johnson, 1999; Dressler, 2000]. The clinical use requires precise injections of BTX-A into specific muscles to cause a temporary chemodenervation of the skeletal muscle and the relief of the clinical symptoms. During the clinical experience of benign essential blepharospasm, it was observed that patients in warm climates did not sweat in the injected areas on the face. This observation then led to investigations on the utility of botulinum toxin therapy in hyperhidrosis patients addressed in this book.

Pioneering physicians have utilized the known mechanism of action of botulinum toxin and their knowledge of anatomy, physiology and disease mechanisms to treat other skeletal-muscle-related disorders such as cervical dystonia [Jankovic and Brin, 1991], juvenile cerebral palsy [Koman et al., 1994; Cosgrove et al., 1994; Graham et al., 2000], focal spasticity [Das and Park, 1989; Snow et al., 1990], disorders of the smooth muscle system, achalasia [Pasricha et al., 1993], anal fissure [Maria et al., 1998; Brisinda et al., 1999], disorders of sweat glands [Schnider et al., 1996] and other dermatological conditions [Brin, 2000]. These and other clinical conditions are summarized in other chapters of this book.

### **Recovery of Clinical Response: Effect on Nerve Sprouting**

The prolonged temporary chemodenervation caused by botulinum toxin was originally thought to be due to the axotomy-like changes in the motor

neuron, based on histological evidence of botulinum-toxin-treated patients [Alderson et al., 1991] and cats [Printer et al., 1991]. Once the neuromuscular connection has been disrupted, the motor neuron responds by sending sprouts from the nerve terminal and node of Ranvier. These sprouts eventually reach the muscle fiber. However, since these were histological evaluations, it was not known whether these were functional connections. In 1999, the laboratory of Prof. Oliver Dolly reported that a single intramuscular injection of BTX-A into the sternomastoid muscle of mice formed functional connections with the muscle fiber [dePaiva et al., 1999]. The most interesting aspect of this report was that the primary, BTX-A-intoxicated nerve terminal was incapable of neurotransmitter exocytosis and produced sprouts which eventually demonstrated exocytosis with subsequent upregulation of nicotinic receptors, thus forming a functional synapse. However, this original BTX-A-intoxicated terminal resumed exocytosis and the sprouts regressed. This observation could explain the clinical experience where most patients chronically treated with BTX-A maintained a stable dose regimen over long periods of treatment.

#### **Alpha, Gamma Motor Neuron, Ia Afferents and Indirect Effects on the CNS**

Many reports have suggested that the injection of BTX-A caused a profound reduction of spasticity in areas, which are larger than expected and not related to the zone of diffusion [Shaari and Sanders, 1993; Borodic et al., 1994]. This observation may be related to the effects of BTX-A on the gamma motor neurons reducing the Ia afferent signal from the muscle spindles [Filippi et al., 1993; Rosales et al., 1996] and could therefore reduce spasticity in an area larger than expected from a local injection of BTX-A. Thus, an injection of BTX-A into a muscle will reduce the alpha motor neuron activity on the extrafusal muscle fibers and reduce muscle contraction. Simultaneously, muscle spindles, when present in the area, are also inhibited by BTX-A by the inhibition of the gamma motor neuron control of the spindle intrafusal fibers and subsequent reduction of the Ia afferent signal. This attenuated Ia signal then reduces the feedback to the alpha motor neurons and other pathways to reduce muscle activity of other noninjected muscles.

The overall impact of a long-term reduction of alpha, gamma and Ia neuronal activity has an indirect effect on the CNS. This was demonstrated preclinically by the work of the laboratory of Prof. Delgado-Garcia [1998]. These investigators have demonstrated that a single injection of BTX-A into the lateral rectus muscle of cats caused inhibition of abduction, alerted electromyographic signals of the contralateral ocular muscles and a disruption of abducens motor



neuron discharge patterns lasting longer than 2 months [Moreno-Lopez et al., 1994]. Further investigations have demonstrated an elimination of inhibitory postsynaptic potentials and a reduction of gephyrin-immunoreactive clusters (glycine receptor clustering protein) onto abducens motor neuron somata starting from 5 days and significant at 19 and 35 days after BTX-A (3 ng/kg) administration into the cat lateral rectus muscle [Pastor et al., 1997; Moreno-Lopez et al., 1997, 1998]. The authors concluded that '...our findings indicate that the long-term paralysis of a muscle involved in many complex motor responses, both reflex and spontaneous, may induce the reorganization of central motor programs and the appearance of compensatory movements' [Moreno-Lopez et al., 1997]. The clinical significance of these preclinical observations remains to be elucidated.

### Safety and Antigenicity

Botulinum toxin therapy has been demonstrated to be safe in a variety of conditions when administered appropriately. The most common adverse effects are either excessive weakness of the treated muscle and the diffusion of the neurotoxin from the injection site causing unwanted weakness in adjacent muscles, for example (1) hand weakness when excess BTX-A diffuses into the muscles from the subcutaneous locations used to treat palmar hyperhidrosis, (2) ptosis when the levator muscle is affected during treatment of blepharospasm, brow furrows or headaches, (3) dysphasia in patients treated for cervical dystonia [Van den Berg and Lison, 1998]. All of these muscle weakness adverse effects with BTX-A are generally mild and of limited duration.

The escape of minute quantities of BTX-A from the treated cervical muscles has been reported [Olney et al., 1988; Langer and Birnbaum, 1991]. These effects were measured by a single-fiber electromyographic technique and recorded as an 'electromyographic jitter' in a distal limb. There were no clinically significant weaknesses associated with these observations.

The preclinical efficacy and safety of the two commercial botulinum neurotoxin preparations were compared following a single intramuscular injection in mice [Aoki, 1999]. The mouse digit abduction scoring (DAS) assay was used to assess the local muscle weakening efficacy of the BTX-A preparations (Botox, Dysport). In a single study where both Botox and Dysport were compared for local efficacy (DAS score) and the first dose that caused a significant weight loss in mice (10 per dose group), compared with vehicle, Botox was found to have a larger safety margin than Dysport (table 1). These results suggest that the two preparations of BTX-A possess different dose ratios for local efficacy than ratios at doses where the toxin escapes the injection site to exert a

**Table 1.** Relative safety margin for two commercial preparations of BTX-A

	Efficacy (DAS ED <sub>50</sub> ) U/kg	Safety (weight loss dose) U/kg	Ratio
Botox	3.5	30	8.6
Dysport	15.2	50	3.3

Values determined from a single experiment, 10 mice per dose group (see text for methods). From Aoki [1999].

**Table 2.** Incidence of dry mouth reported with BTX-B (units per patient) treatment of cervical dystonia

	Dose of type B (Elan)			
	0 units	2,500 units	5,000 units	10,000 units
Lew et al. [1997]	1 of 30 (3)	1 of 31 (3)	3 of 31 (10)	10 of 30 (33)
Brashear et al. [1999]	1 of 36 (3)	NT	5 of 36 (14)	9 of 37 (24)
Brin et al. [1999]	1 of 38 (3)	NT	NT	17 of 39 (44)

NT = Not tested. Figures in parentheses indicate percentages.

systemic effect. Thus, simple conversion of units between the two products should be avoided, especially at the higher doses. Any simple unit conversion factor does not address these differences nor considers the antigenic potential of the preparations.

One unusual adverse effect of a dose-related dry mouth was reported for cervical dystonia patients treated with a BTX-B preparation (table 2). The authors did not report the duration of this effect. Dry mouth is rarely observed following treatment with BTX-A [Jankovic and Schwartz, 1990; Tsui et al., 1986]. The dry mouth symptom of these BTX-B-treated patients was unexpected since the target organ (e.g. salivary glands) is further from the injection site than the muscles associated with swallowing and no other lower facial muscles were significantly affected.

The observed adverse effect of dry mouth may be due to escape of the BTX-B from the muscle and systemic distribution to the salivary glands. Since there was no obvious oral or distal weakness reported with the dry mouth, it suggests that BTX-B may have a higher affinity or there is a higher number of BTX-B-specific receptors on the cholinergic neurons innervating the salivary gland than the motor nerve. In support of this differential binding to different

nerve types, it has been reported that BTX-B may have a greater affinity than BTX-A for autonomic nerve terminals [Konig et al., 1975; Hughes, 1991]. Further research will be necessary to elucidate the mechanism by which BTX-B caused dry mouth in some patients.

Botulinum neurotoxin preparations that exhibit low potency and/or short duration of action will require higher doses and/or more frequent injections to achieve the desired therapeutic efficacy levels in chronic conditions. Higher doses may increase the amount of drug that diffuses away from the injection site, leading to more adverse events. Additionally, since high doses and frequent injections have been associated with neutralizing antibody formation [Jankovic and Schwartz, 1995; Atassi and Oshima, 1999], increased antigenicity with lower-potency, shorter-duration serotypes is a concern.

Neutralizing antibody formation is a particular concern with low-potency, short-acting botulinum toxin serotypes. This is because high neurotoxin doses and frequent injections have been associated with the formation of neutralizing antibodies [Jankovic and Schwartz, 1995]. Importantly, neurotoxin preparations containing different serotypes vary in the doses needed for clinical efficacy and therefore may vary in antigenic potential. For example, even though BTX-A and BTX-F have similar potency, doses of type F have been increased in an attempt to mimic the longer duration of action observed with type A [Chen et al., 1998; Houser et al., 1998]. In a study of dystonia patients treated with BTX-F, 4 of 18 patients (22%) became nonresponsive following 12-66 months of treatment [Chen et al., 1998]. Since the incidence of antibody formation with type A for the treatment of cervical dystonia has historically been  $<5\%$  [Greene et al., 1994], the finding of Chen and colleagues is consistent with the hypothesis that increasing doses to achieve adequate duration of muscle weakness will also increase antigenicity. Large clinical studies are needed to determine the overall incidence of neutralizing antibody formation with serotypes other than type A.

Another factor that can contribute to the overall neurotoxin protein load of a preparation is the amount of unnicked (nonactivated) neurotoxin. The single-chain neurotoxin will contribute to the overall neurotoxin protein load of the preparation while contributing little to therapeutic efficacy. The amount of in situ activation is variable and unpredictable. BTX-A and BTX-F are released as the nicked form whereas BTX-B is variable and depends upon the strain and the fermentation conditions. Therefore, botulinum neurotoxin preparations that produce the desired amount of muscle relaxation while exposing patients to the lowest amount of neurotoxin complex protein are likely to reduce the risk of antibody formation [Borodic et al., 1996].

An additional concern with the development of antibodies is that serum cross-reactivity among botulinum neurotoxin serotypes may be possible [Atassi and Oshima, 1999]. Despite the historical separation of botulinum neurotoxin



serotypes, recent evidence suggests that cross-reactivity may occur. Dertzbaugh and West [1996] found that mice treated with BTX-A fragments developed antibodies that cross-reacted with other serotypes. Furthermore, one study found that human spastic patients treated with BTX-A produced measurable titers of antibodies against several other serotypes [Doellgast et al., 1997].

A further problem with increasing doses of lower-potency or shorter-acting botulinum toxins is the increased potential for diffusion as the number of molecules injected is increased. Many of the adverse events observed following botulinum neurotoxin treatment (e.g. dysphagia, ptosis) are due to diffusion away from the injection site [Biglan et al., 1988; Greene et al., 1990]. Thus, botulinum neurotoxin preparations administered at higher doses are likely to exhibit less favorable safety profiles.

Despite all of these local or distal adverse effects described in this section, BTX-A with both commercial products has provided safe and effective therapy for thousands of patients worldwide.

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