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Pharmacology and immunology of botulinum toxin serotypes

Abstract Botulinum toxin preparations can provide patients with a therapeutic modality that may improve both their medical condition and quality of life. The mechanism of action of the various botulinum toxin preparations and serotypes is similar: they all block neurotransmitter release. The majority of clinical conditions treated are based upon the targeted temporary chemodenervation of the selected organ. The antinociceptive effects of botulinum toxin type A (BTX-A), based on preclinical studies and clinical experiences in treating movement disorders and other painful conditions, will also be reviewed to illustrate how this compound may act as it alleviates the discomfort associated with various conditions. Chronic therapies with preparations with the lowest amount of neurotoxin protein provide the best chance for long-term therapy by minimizing the potential of the patient to form neutralizing antibodies. Differences in formulations or serotypes impart unique efficacy and safety profiles and thus does not support a simple dose ratio conversion between products.

Key words Botox® · Dysport® · Myobloc™ · Chemodenervation · Analgesia · Safety margin · Botulinum toxin type A · Botulinum toxin type B

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Introduction

Botulinum toxin preparations can achieve organ-selective temporary chemodenervation when injected near the nerve that controls the target organ. This can provide patients with a therapeutic modality that may improve both their medical condition and quality of life. The pharmacology of the various botulinum toxin preparations will be reviewed in this article to illustrate their common as well as their unique efficacy and safety profiles. The antinociceptive effects of botulinum toxin type A (BTX-A), based on preclinical studies and clinical experiences in treating movement disorders and other painful conditions, will also be reviewed to illustrate how this compound may act as it alleviates the discomfort associated with various conditions.

History and mechanism of action

The therapeutic benefits derived from a local injection of a botulinum toxin preparation are based on site-specific delivery (e. g., intramuscular, subcutaneous) and the fact that these compounds have a high affinity for uptake by cholinergic neurons. This results in a temporary chemodenervation and the loss or reduction of neuronal activity at the target organ (e. g., muscle, glands) with minimal risk of systemic adverse effects, when used at the appropriate dose. Worldwide experience since the 1989 United States approval of BTX-A (Botox®, Allergan), the first therapeutic botulinum-neurotoxin based product for the treatment of strabismus, benign essential blepharospasm and disorders of the VIIth nerve has shown that this therapeutic agent is safe and effective for numerous indications, including movement disorders [1–4] and currently is approved for numerous indications worldwide. Subsequently, another botulinum toxin type A complex (Dysport®, Ipsen) was approved in the United Kingdom in 1991, but is not cur-

rently available in the United States. Recently, the U. S. FDA has approved a BTX-B complex preparation (Myobloc™, Elan; in Europe Neurobloc®) for use in cervical dystonia patients. Although these three products are based on botulinum neurotoxins, they have sufficiently different doses, efficacy and safety profiles that these and other future botulinum-toxin-based products should not be considered generic equivalents comparable by simple dose ratios.

The mechanism of action of the seven botulinum neurotoxin serotypes (A, B, C1, D, E, F, and G) has been reviewed in other publications. This article will provide a brief overview of the physiology and pharmacology of botulinum neurotoxin serotypes A, B, and F [1, 5–8] as well as reports of antinociceptive observations with BTX-A. Although serotypes C1 and E have been used in a limited number of volunteers and patients, their role as therapeutic agents requires further clinical studies [9, 10]. To date, the vast majority of commercial development of botulinum toxin for clinical use has been based on 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 [6, 7, 11], especially for patients with cervical dystonia that no longer respond to BTX-A [8, 12]. Clinical studies have found that BTX-A is more potent (less total number of units administered per patient per session) than BTX-B [7, 8, 13] and has a longer duration of action than either BTX-B, as indicated by electromyography (EMG) results only [13], or BTX-F as indicated by clinical response [6, 12, 14]. Reports of BTX-B duration of action in cervical dystonia patients may be over estimated since a statistical method of duration of effect was utilized. Direct clinical comparisons of duration of BTX-A and BTX-B in cervical dystonia patients have not been studied.

The efficacy of botulinum neurotoxin therapy is primarily due to the injection method of delivery and the high affinity of cholinergic nerves for neurotoxin uptake. 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 [2, 3, 15, 16]. In clinical use, BTX-A is precisely injected into specific muscles to cause a temporary chemodenervation of the skeletal muscle and, thus, relief from clinical symptoms. 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 [5], juvenile cerebral palsy [17, 18], and focal spasticity [19, 20]; disorders of the smooth muscle systems such as achalasia [21] and anal fissure [22, 23, 80, 81], hyperhidrosis [24] and other dermatological conditions [1].

Local efficacy/safety and duration of action of botulinum toxin preparations are due to the dose (total units), inherent properties of the serotype (e. g., BTX-A, BTX-B, and BTX-F), or their formulation. To illustrate the physiological effects of local injections of botulinum toxin, clinical and preclinical examples will be discussed.

Recovery of clinical response: effect on nerve sprouting

Based on histological evidence from botulinum-toxin treated patients [25] and cats [26], the prolonged temporary chemodenervation caused by botulinum toxin was originally thought to be due to the axotomy-like changes in the motor neuron. Researchers also believed that once the neuromuscular connection was disrupted, the motor neuron responded by sending sprouts from the nerve terminal and nodes of Ranvier; these sprouts eventually reached the muscle fiber. However, since these were histological evaluations, it was not known whether these were functional connections.

In 1999, the laboratory of Professor Oliver Dolly (Imperial College, London, UK) reported that a single intramuscular injection of BTX-A into the sternomastoid muscle of mice caused the formation of functional neuronal sprouts that connected with the muscle fiber [27]. The most interesting aspect of this report was that the primary BTX-A-intoxicated nerve terminal was incapable of neurotransmitter exocytosis and produced sprouts that eventually demonstrated exocytosis with subsequent upregulation of adjacent nicotinic receptors on the muscle fiber, thus, forming a functional synapse. However, this original BTX-A-intoxicated terminal resumed exocytosis, and the sprouts regressed to return the neuromuscular junction to its original state. This observation could explain how most patients chronically treated with BTX-A were maintained on a stable dosing regimen over long periods of treatment.

Alpha and gamma motor neurons, Ia afferents, and indirect effects on the central nervous system

The results of many reports suggest that the injection of BTX-A causes a profound reduction of spasticity in areas that are larger than expected and not related to the zone of diffusion (e. g., whole limbs or the face rather than the injection site, which was expected to be approximately 2 to 4 cm for Botox®) [28, 29]. 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 [30, 31] and therefore reducing 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 muscle contraction. Simultaneously, muscles spindles, when present in the area, are also inhibited by BTX-A through 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. This reduction of overall muscle contraction presumably could reduce excess muscle contraction associated with pain.

The overall impact of a long-term reduction of alpha, gamma, and Ia neuronal activity may have an indirect effect on the central nervous system (CNS). This was demonstrated preclinically by the laboratory of Professor Delgado-Garcia. These investigators demonstrated that a single injection of BTX-A into the lateral rectus muscle of cats caused inhibition of abduction, altered EMG signals of the contralateral ocular muscles, and caused a disruption of abducens motor neuron discharge patterns lasting longer than 2 months [32]. Further investigations have demonstrated an elimination of inhibitory postsynaptic potentials and reduction of gephyrin-immunoreactive clusters (glycine-receptor-clustering protein) onto abducens motor neuron somata starting from 5 days and becoming significant at 19 and 35 days after BTX-A (3 ng/kg) administration into the cat lateral rectus muscle [33–35]. The authors [34] 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.*" The clinical significance of these preclinical observations remains to be established. However, it is tempting to speculate that this indirect CNS effect of a peripheral botulinum toxin injection could influence chronic pain through prevention or reversal of the central wind-up or sensitization process.

Safety and antigenicity

Botulinum toxin therapy has been demonstrated to be safe in a variety of conditions (BTX-A only, BTX-B has only been studied in cervical dystonia) when administered appropriately. The most common adverse effects are either excessive weakness of the treated muscle or the local diffusion of the neurotoxin from the injection site causing unwanted weakness in adjacent muscles. For example, the following can occur: hand weakness when excess BTX-A diffuses into the muscles from the subcutaneous locations used to treat palmar hyperhidrosis; ptosis when the levator muscle is affected during treatment of blepharospasm, brow furrows, or headaches; and dysphagia (BTX-A or BTX-B) in patients treated for cervical dystonia [36, 50, 51]. 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 [37, 38]. These events were measured by a single fiber electromyographic technique and recorded as an "EMG jitter" in a distal limb. There was no clinically significant weakness associated with these observations. Similar human EMG jitter studies remain to be conducted with the BTX-B preparation.

The preclinical efficacy and safety of the two BTX-A commercial botulinum neurotoxin preparations (Botox® and Dysport®) were compared following a single intramuscular injection in mice [39]. The mouse digit abduction scoring (DAS) assay was used to assess the local muscle-weakening efficacy of these preparations. The systemic effect was measured as the first dose to cause significant reduction of weight gain in the treated mice. Botox® was observed to have a larger safety margin than Dysport® (Table 1) when compared with Dysport® for the ratio of local efficacy (DAS score) and the first dose that caused a significant weight loss (10 mice per dose group). 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 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 or consider the antigenic potential of the preparations. This concept should apply to other botulinum toxin serotype preparations as well.

One unusual dose-related adverse effect, dry mouth, was reported for patients in cervical dystonia who were treated with BTX-B (Myobloc™ resp. Neurobloc®) (Table 2). The authors did not report the duration of this adverse effect. Dry mouth is rarely observed following treatment with BTX-A [4, 40]. Dry mouth in these BTX-B-treated patients was unexpected because the target organ (e. g., salivary gland) is further from the injection site than the muscles associated with swallowing and other lower facial muscles, including the tongue, were not significantly affected.

The dry mouth symptoms may be caused by the BTX-B which has escaped from the injected muscle and has

Tab. 1 Relative safety margin for two commercial preparations of botulinum toxin type A*

Preparation	Efficacy (DAS ED_{50}, 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) (adapted with permission from Aoki [39]. † ED_{50} indicates median effective dose of an intramuscular injection.

Tab. 2 Incidence of dry mouth reported in patients with cervical dystonia in BTX-B trials

Report	No. (%) of Patients by BTX-B Dose*			
	0 Units	2500 Units	5000 Units	10,000 Units
Lew et al, 1997 [11]	1/30 (3 %)	1/31 (3 %)	3/31 (10 %)	10/30 (33 %)
Brashere et al, 1999 [7]	1/36 (3 %)	NT	5/36 (14 %)	9/37 (24 %)
Brin et al, 1999 [8]	1/38 (3 %)	NT†	NT	17/39 (44 %)

* Units per patient. † NT indicates not tested.

reached the salivary gland through a systemic distribution. The absence of obvious lingual or lower facial weakness after treatment of cervical muscles suggests that either BTX-B may have a higher affinity for the cholinergic neurons innervating the salivary gland compared with the motor nerve. Alternatively, there may be a higher number of BTX-B-specific acceptors on the cholinergic neurons innervating the salivary gland compared with the motor nerve. In support of this differential binding of BTX serotypes to different nerve types, it has been reported that BTX-B may have a greater affinity than BTX-A for autonomic nerve terminals [41, 42]. Further research will be necessary to elucidate the mechanism by which BTX-B causes 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 and leads to more adverse events. In addition, high doses and frequent injections of botulinum toxin have been associated with neutralizing antibody formation [43, 44]. Neutralizing antibody formation is a particular concern with low-potency, short-acting botulinum toxin serotypes [44]. Thus, neurotoxin preparations containing different serotypes vary in the doses needed for clinical efficacy and may vary in antigenic potential.

For example, although 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 [12, 14]. In a study of patients with dystonia who were treated with BTX-F, 4 of 18 patients (22 %) became nonresponsive following 12 to 66 months of treatment [12]. Because the incidence of antibody formation with type A for the treatment of cervical dystonia has historically been less than 5 % [45], the finding of Chen and colleagues [12] is consistent with the hypothesis that increasing doses of botulinum toxin to achieve adequate duration of muscle weakness will also increase antigenicity. Larger prospective clinical studies are needed to determine the overall incidence of neutralizing antibody formation with BTX-A, BTX-B, BTX-F or other serotypes.

Another factor that can contribute to the overall neurotoxin protein load of a preparation is the amount of unnicked (e. g. single chain neurotoxin) or "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 in the nicked form whereas BTX-B is variable and depends on the clostridial 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 [46].

Information about the antigenicity of botulinum toxin type B (Myobloc™, resp. Neurobloc®) and the original preparation of botulinum toxin type A (original Botox® from Allergan) has recently become available [76, 77]. Both products have recently been approved for the treatment of cervical dystonia in the United States. The product inserts contain data on percentages of patients with neutralizing antibodies, as required by the Food and Drug Administration (FDA). A study of patients treated with the *original*, higher neurotoxin protein botulinum toxin type A product (original Botox® from Allergan), found that 17 % of patients had neutralizing antibodies [76]. The results of prospective antigenicity studies with the *current* preparation of botulinum toxin type A (current Botox® from Allergan), which contains 80 % less neurotoxin complex protein, are not yet available. However, preclinical [79] and retrospective clinical data [78] suggests that the antigenic potential of the current Botox® preparation is likely to be substantially lower than the original preparation. The rate of neutralizing antibody formation in response to treatment with BTX-B was reported in the package insert for Myobloc™ [77]. The incidence of neutralizing antibody formation in cervical dystonia patients treated with the type B preparation (Myobloc™) is 10 % for one year and 18 % after 18 months. Further studies are needed to determine the time course and other risk factors associated with development of neutralizing antibody formation in patients treated with botulinum toxin preparations.

An additional concern with the development of antibodies is that serum cross-reactivity among botulinum neurotoxin serotypes may be possible [47, 74]. Despite historic separation of botulinum neurotoxin serotypes, evidence suggests that cross-reactivity and cross neutralization may occur [74]. Dertzbaugh and West [48] found that mice treated with BTX-A fragments developed antibodies that cross-reacted with other serotypes. Halpern et al. [74] demonstrated that sera from mice immunized with synthetic peptides from tetanus toxin cross-reacted with BTX-B, BTX-C but not BTX-A. In a

clinical study, patients with spasticity who received BTX-A produced measurable titers of antibodies (determined in vitro) against several other serotypes [49]. Thus, cross reactive epitopes between botulinum neurotoxin serotypes could prime the immune response to stimulate the production of neutralizing antibodies to the second serotype faster than in a naïve individual devoid of anti-toxin antibodies. This hypothesis remains to be demonstrated in patients.

Botulinum neurotoxin preparations administered at higher doses are likely to exhibit less-favorable safety profiles and increase antigenicity potential. Despite all of the local and distal adverse effects described in this section, BTX-A therapy with both commercial products has provided safe and effective treatment for thousands of patients worldwide. Further experience with the BTX-B preparation remains to be established beyond the limited experience with cervical dystonia patients.

Antinociceptive observations

Botulinum toxin therapy has been reported to alleviate pain associated with various conditions with or without concomitant excess muscle contractions. Early observations in patients with cervical dystonia who were treated with BTX-A suggested that the pain relief exceeded the motor benefit [4, 40, 52–54]. In other areas, the pain associated with myoclonus of spinal cord origin has been treated effectively with BTX-A [55]. Tension-associated headaches have been reported to be alleviated with BTX-A therapy [56–61]. In a double-blind placebo-controlled trial, Professor H. Kerr Graham and coworkers reported profound antinociceptive activity of intramuscular BTX-A (Botox®) when administered prior to adductor-release surgery in children with cerebral palsy [62]. The effect was so dramatic that the trial was terminated early. Children treated with Botox® had a reduced need for narcotic analgesics, were discharged earlier, and had better outcomes than the placebo group. In a recent pilot study [26], patients with chronic whiplash-associated neck pain were successfully treated with BTX-A (Botox®) [63]. Other reports of BTX-A for reduction of primary pain include trigger point injections [64], myofascial pain [60, 65] and migraine headache prophylaxis [66, 67], and back pain [68]. However, not all reports have demonstrated positive results [69]. This variable response to BTX-A therapy is similar to the early experience in the movement disorders. As the physicians became more experienced in selecting the patient, target muscle and dose, the success rate increased. Therefore, the treatment of chronic pain continues to mature with a sufficient number of successes that warrant further investigations.

Theoretical/potential mechanism for antinociceptive effect

Botulinum toxin can affect neurons within the CNS. For example, botulinum toxin serotypes B and F and tetanus toxin are internalized by cultured rat hippocampal astrocytes and cleave the appropriate substrate [70]. Neuropeptide release was reported to be inhibited by botulinum toxin (BTX-A, B, C1, F) treatment in vitro from embryonic rat dorsal root ganglia neurons [71, 72] and from isolated rabbit iris sphincter and dilatory muscles [73]. More importantly, the in vitro release of acetylcholine and substance P (but not norepinephrine) from the rabbit ocular tissue was also inhibited with BTX-A [73]. Therefore, based on these in vitro and limited in vivo data, it can be hypothesized that botulinum toxin treatment may reduce the local release of nociceptive neuropeptides from either cholinergic neurons or from C or A delta fibers in vivo. The reduced neuropeptide release could prevent the local sensitization of nociceptors and thus reduce the perception of pain. A reduction of nociceptive signals from the periphery could then re-

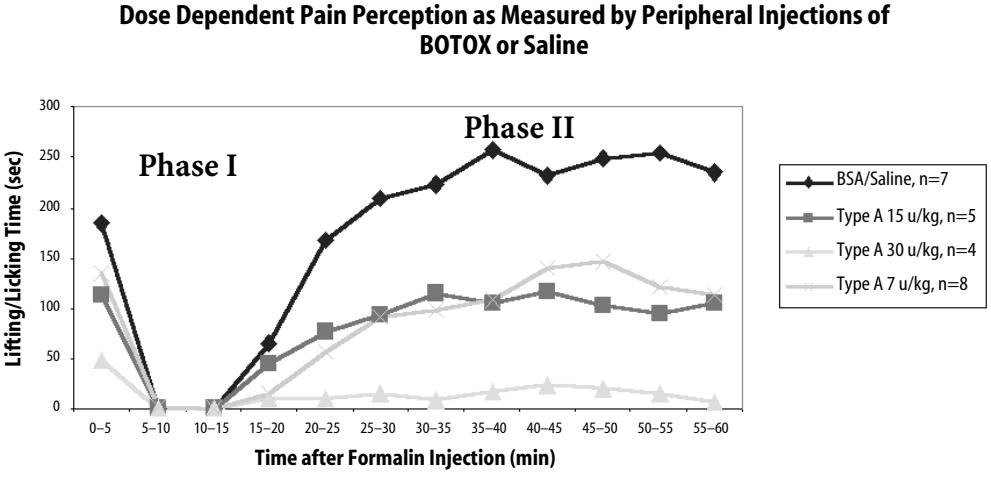

Fig. 1 The time course of the dose-dependent reduction of formalin-induced pain by Botox®. Rats were treated with saline or different doses of Botox® (7, 15 and 30 u/kg). Formalin test was conducted 5 days after the s. c. injection of Botox®. Botox® dose dependently inhibited the formalin-induced pain.

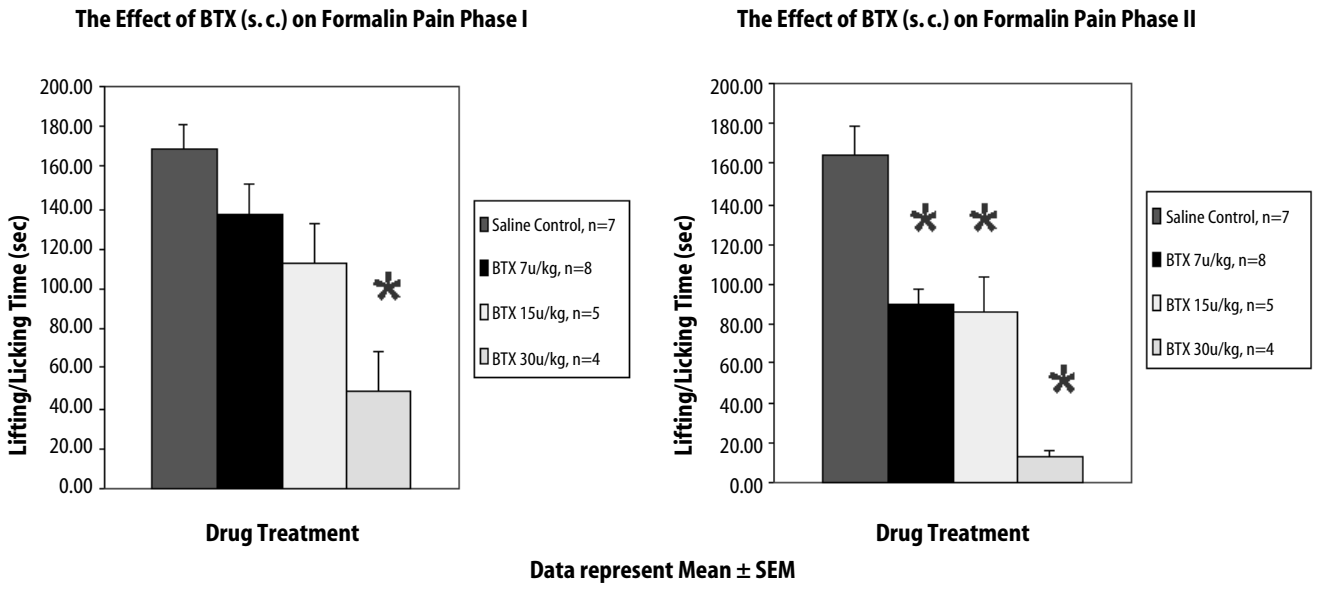

Fig. 2 The summarized antinociceptive effect of Botox® on phase I and phase II of formalin test. Botox® at 7, 15 and 30 u/kg all significantly inhibited the second phase of formalin-induced pain, whereas only rats treated with 30 u/kg showed a significant pain reduction in phase I. Asterisk (*) indicates a p value < 0.05 determined by ANOVA.

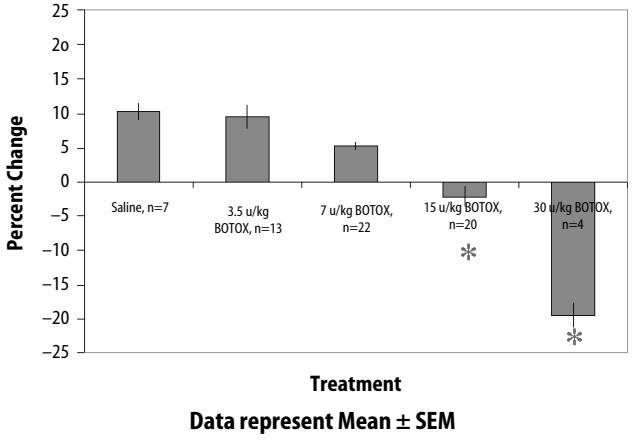

Fig. 3 The weight change produced by Botox®. Five days after s.c. injection of Botox® into the rat paw, rats treated with 15 and 30 u/kg showed a significant weight loss. However, rats treated with lower doses, 3.5 and 7 u/kg, did not produce a significant weight loss comparing to control animals. Asterisk (*) indicates a p value < 0.05 determined by ANOVA.

duce the central sensitization associated with chronic pain. This effect on the nociceptive neurons could work in concert with the other well-known effects of botulinum toxin on the cholinergic motor neuron innervating the extrafusal and intrafusal fibers.

A preclinical investigation on the local antinociceptive efficacy of BTX-A (Botox®) was reported at the recent Society for Neuroscience annual meeting [75]. A rat model of inflammatory pain was used to demonstrate that a subcutaneous injection of BTX-A prevented the classical behavioral pain response to a subplantar injection of formalin. BTX-A was administered subcutaneously to the plantar surface of the rat 5 days before the formalin challenge in the same area. The classic two-phase pain response in this model was observed by the rat's behavior (Fig. 1). BTX-A produced a dose related (7, 15, 30 units/kg) inhibition of both phases of the pain response. The highest dose caused a significant inhibition of the acute pain response (phase I) as well as the secondary inflammatory pain associated with phase II (Fig. 2). However, the 15 and 30 units/kg doses caused a systemic effect, as measured by the reduced weight gain of the rats (Fig. 3). Further studies with lower doses demonstrated local antinociceptive activity without changes in the rat weights, demonstrating a local effect. Other measures of muscle weakness (behavioral and histological) supported these observations.

The preclinical (in vitro and in vivo) evidence coupled with the clinical observations strongly suggests that botulinum toxin (especially BTX-A) may have a separate antinociceptive effect from its well-known effect on the neuromuscular junction and other cholinergic nerves. Further studies are needed to elucidate the mechanism of this important observation.

In summary, each botulinum neurotoxin product demonstrates a unique efficacy and safety profile and should not be considered generic equivalents. When used responsibly, botulinum toxin therapy can provide physicians with a therapeutic tool to localize a treatment and provide patients symptomatic relief for several weeks and positively impact their quality of life.

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