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Tremor and Other Hyperkinetic Movements

Review

Diffusion of Botulinum Toxins

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Abstract

Background: It is generally agreed that diffusion of botulinum toxin occurs, but the extent of the spread and its clinical importance are disputed. Many factors have been suggested to play a role but which have the most clinical relevance is a subject of much discussion.

Methods: This review discusses the variables affecting diffusion, including protein composition and molecular size as well as injection factors (e.g., volume, dose, injection method). It also discusses data on diffusion from comparative studies in animal models and human clinical trials that illustrate differences between the available botulinum toxin products (onabotulinumtoxinA, abobotulinumtoxinA, incobotulinumtoxinA, and rimabotulinumtoxinB).

Results: Neither molecular weight nor the presence of complexing proteins appears to affect diffusion; however, injection volume, concentration, and dose all play roles and are modifiable. Both animal and human studies show that botulinum toxin products are not interchangeable, and that some products are associated with greater diffusion and higher rates of diffusion-related adverse events than others.

Discussion: Each of the botulinum toxins is a unique pharmacologic entity. A working knowledge of the different serotypes is essential to avoid unwanted diffusion-related adverse events. In addition, clinicians should be aware that the factors influencing diffusion may range from properties intrinsic to the drug to accurate muscle selection as well as dilution, volume, and dose injected.

Keywords: Botulinum toxin, diffusion, spread, injection technique

Citation: Brodsky MA, Swope DM, Grimes D. Diffusion of botulinum toxins. Tremor Other Hyperkinet Mov 2012; 2: http://tremorjournal.org/article/view/85

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Editor: Elan D. Louis, Columbia University, United States of America

Received: December 3, 2011 **Accepted:** June 3, 2012 **Published:** August 6, 2012

Funding: Funding for editorial support was provided by Merz Pharmaceuticals, LLC, Greensboro, NC.

Financial Disclosures: Dr. Brodsky has served as an advisor to Merz Pharma, Ipsen, Allergan, Inc., UCB, and Teva. Dr. Swope has received grant/research support from Allergan, Inc., Teva, Synosia/Biotie, and St Jude Medical Center; has served as an advisor for Allergan, Inc., Ipsen, Merz Pharma, and Teva; and has received fellowship support from Merz. He also is a member of the speakers' bureau for Allergan, Inc. and is affiliated with its Injection Training Center. Dr. Grimes has served as an advisor to Merz Pharma, Allergan, Novartis, and Teva; he has received grant/research support from NIH, Michael J. Fox Foundation, CIHR, Parkinson Research Consortium, Impax Pharmaceuticals, Merz Pharma, Allergan and Schering-Plough.

Conflict of Interest: The authors report no conflict of interest.

Introduction

Botulinum toxin (BoNT) preparations act by binding presynaptically to high-affinity recognition sites on the cholinergic nerve terminals and decreasing the release of acetylcholine, causing a neuromuscular blocking effect. This inhibition of acetylcholine release leads to muscle weakness and the potential for the relief of undesirable muscle contraction, hence serving as an effective treatment for a wide range of muscle disorders including strabismus, blepharospasm, hemifacial spasm, cervical dystonia, and spasticity.[1]

There are seven serologically distinct types of BoNT, designated A through G, which are antigenically and serologically distinct but structurally similar. All have similar neurotoxic properties resulting in flaccid muscle paralysis; however, only the A (onabotulinumtoxinA [ONAA], abobotulinumtoxinA [ABOA], and incobotulinumtoxinA [INCOA]) and B (rimabotulinumtoxinB [RIMAB]) forms have been approved for clinical use. Each botulinum product is purified and manufactured using proprietary processes, resulting in unique agents that differ in such features as molecular weight, uniformity of toxin complex size, protein content, and the presence of inactive ingredients, all of which can impact performance characteristics including potency, duration of effect, and adverse event (AE), and diffusion or migration profile.

The AEs associated with BoNT are generally of three types: those due to expected effects of the neurotoxin (e.g., excessive local muscle weakness), those due to diffusion of the neurotoxin to nearby, uninjected muscles, and those resulting from systemic distribution of the toxin.[2] Diffusion of BoNT beyond the target muscle is of clinical concern because of the potential for local and systemic effects that result in muscle weakening away from the desired site. BoNT may diffuse across fascial planes to adjacent muscles or be spread hematogenously.[3] At its extreme, the possible leakage of BoNT into the systemic circulation may manifest as clinical botulism[4,5] leading to respiratory failure and death.[6]

Methods

In this review, published data on the variables affecting diffusion, including those pertaining to protein composition and molecular size of a given botulinum toxin and also factors such as injection volume and dose, and injection method (e.g., needle gauge used, speed of injection, and target muscle localization), are discussed. In addition, findings on diffusion from comparative studies in animal models and human clinical trials that illustrate differences between available, approved botulinum toxin products are elucidated. While this is not a systematic review, a prespecified protocol was followed for the literature search. Potentially relevant publications were obtained from a PubMed search conducted during October of 2010 by Linnéa Elliott and Maria Vinall of The Curry Rockefeller Group under the direction of the authors. The search focused on English language publications with the terms botulinum toxin, botulinum neurotoxin, and diffusion or migration. The results of this search were reviewed by the authors, who added additional publications they considered noteworthy but which were not identified by the search. Published abstracts from recent medical conferences in relevant fields were also searched, and pertinent abstracts from those were included in the review. The final choice of references was made by the authors.

Results and discussion

The diffusion characteristics of BoNT have been well studied in humans[7–10] and animals[11,12] using a variety of techniques, including compound muscle action potentials (CMAPs) and motor-evoked potentials,[7] histological determination of glycogen-depleted muscles,[13] acetylcholine esterase staining,[14] muscle fiber diameter variability,[15] and quantitative electromyography (EMG) measures of muscle activity.[16]

Evidence for diffusion comes from both animal and human studies. In a study using muscle biopsy to identify spread, Borodic et al.[15] reported a diffusion gradient of BoNT/A over a distance of 30–45 mm from the point of injection into latissimus dorsi muscle of rabbits.[14] The extent of denervation gradient or diffusion was dose dependent. Another study used neural cell adhesion molecule (N-CAM) staining to assess the diffusion of activity of equipotent doses of three BoNT/A formulations from the point of injection along the mouse hind limb. The results showed a similar time course of paralysis, and minimal but comparable diffusion in the anatomical area adjacent to the site of injection that decreased over time in a similar manner.[17] Results of an electrophysiologic study in patients with blepharospasm and facial hemispasm treated for the first time with BoNT/A in the orbicularis oculi muscle showed a significant effect in untreated muscles with different peripheral innervation that could not be explained by axonal diffusion from the terminal nerve endings of the facial nerve and which the investigators concluded was related to local diffusion of the BoNT/A.[7] A study that investigated whether the volume of solution used to inject equivalent units of botulinum exotoxin A affects the diffusion of toxin and areas of rhytid diminution in the treatment of dynamic forehead lines found that injection of botulinum exotoxin A in low concentration and higher volume resulted in greater diffusion and a larger affected area. The pattern of toxin spread was altered by muscular contraction in the injected sites.[10]

Although most clinicians agree that diffusion of BoNT occurs, its extent and clinical importance has been disputed. In a study of patients receiving BoNT to treat hemifacial spasm, Lorenzano and colleagues[18] assessed the nearby untreated muscles of patients, both clinically and neurophysiologically, and concluded that diffusion did not occur to any significant extent. This finding was echoed by Carli and colleagues,[17,19] who reported that intramuscular injections of BoNT/A to the tibialis anterior muscle of mice exhibited only limited diffusion to adjacent muscles. In another animal study using radio-labeled BoNT and autoradiography, Tang-Liu and colleagues[20] showed no detectable systemic effects or generalized botulinum neurotoxin toxicity, indicating that most of the toxin remained at the injection site.

Pickett et al.[21] has suggested that the confusion regarding the extent and clinical relevance of diffusion among the different botulinum toxins can be attributed to incorrect extrapolations of information obtained from animal studies to clinical settings, the inappropriate testing of products with different dose ratios, the incorrect suggestion that products with larger complex molecular size migrate less, and, in some cases, poor study design.

Depending on the clinical indication for which it is used, diffusion of BoNT may be advantageous. Clinicians may capitalize on effects of diffusion when giving injections for palmar and axillary hyperhidrosis.[22,23] When treating larger muscles with BoNT, most often seen in patients with spasticity, many investigators now recommend trying to increase the diffusion characteristics of the toxin by using high-dilution volumes.[24]

Variables that may affect diffusion

It has been suggested that diffusion of BoNT is influenced by a number of factors such as dose, concentration, volume, rate of injection, needle size, distance of needle tip from the neuromuscular junction, number of injections, target muscle selection, the presence of muscular fascia, the presence of tissue damage at the injection site, muscle contraction following injection, and the protein composition and molecular size of the BoNT formulation.[2,10,11,25,26] However, dose, concentration, and volume are probably the greatest contributors, in that the greater the dose, concentration, or volume, the greater the risk of diffusion (Table 1).[10,15,27]

Table 1. Factors Thought to Affect Diffusion

Factor	Does It Affect Diffusion?
Protein composition	No
Molecular size	No
Injection volume	Yes
Injection dose	Yes
Injection concentration	Yes
Injection method	Maybe

Protein composition and molecular size

Based on the principle that larger proteins diffuse more slowly through an identical aqueous medium than smaller proteins, Foster and colleagues[2] predicted that a BoNT of greater size or molecular weight would be less likely to diffuse outside the target tissue than those of smaller size. Thus, ONAA would be less likely to diffuse outside the target tissue than ABOA, RIMAB, or INCOA. However, Carli and colleagues[17] found the diffusion characteristics of the three products to be indistinguishable. In addition, in a long-term clinical study,[28] the diffusion profile of ONAA and INCOA (which is free of complexing proteins) did not differ, suggesting that the complexing proteins are neither necessary for BoNT's therapeutic effect nor relevant for tissue diffusion. This is consistent with earlier findings that the 150-kDa neurotoxin molecule is released from the 900-kDa complex in less than a minute when exposed to physiological pH values.[29] There is also no difference between purified neurotoxin alone or toxin complex in terms of localization at the site of injection or subsequent migration into body tissues.[20] Other studies have also reported that smaller size or the absence of complexing plays no role in toxin diffusion,[30,31] and that INCOA has an *in vivo* diffusion profile comparable with that of preparations retaining the complexing proteins.[1,32]

Injection factors

Diffusion of botulinum toxin may be dose dependent, and specific complications may be related to the choice of injection site. In a retrospective analysis of 26 patients with adult onset idiopathic spasmodic torticollis treated with BoNT/A for a mean of 1.1 years, Borodic et al.[14] noted that treatment with a median of 150 IU of ONAA resulted in a significantly ($p=0.026$) higher incidence of dysphagia than a median dose of 100 IU when the treatment was administered via the sternomastoid muscle but not when the posterior cervical muscle group was injected alone. When the investigators conducted a prospective study in the same patient population and limited the dose at the sternomastoid muscle to 100 IU, they noted a substantial decrease in the incidence of dysphagia.[14] Participants in a study evaluating the safety and efficacy of two doses of ONAA (50 units and 100 units) in the treatment of essential hand tremor reported hand weakness that was dose dependent (30% of participants in the low-dose group and almost 70% in the high-dose group).[33] Injection site was not identified as a contributing factor in this study.

Injection volume has also been implicated as a factor in diffusion. In one study, a fivefold increase in volume resulted in an ~50% increase in affected area.[10] In another study, the diffusion gradient around the site of injection increased with the concentration of BoNT injected.[15] At BoNT/A doses of 5–10 IU, a gradient of denervation occurred throughout the entire muscle with no apparent endpoint, suggesting that both the magnitude of denervation and the extent of the gradient are dose dependent. A possible consequence of greater volume leading to more diffusion into surrounding tissue may be diminution in duration and magnitude of effect.[10]

It is most likely that both dose and volume are important determinants of the effects on the target muscle. Results of a dose-ranging, electroneurographic study investigating the dose equivalence, diffusion characteristics, and safety of ABOA and ONAA in 79 volunteers showed significant and similar reductions in compound muscle action potential amplitude in the extensor digitorum brevis 2 weeks after injection, with effects persisting to the 12-week time point. For both products, the reduction in amplitude increased with increasing doses and with increasing concentration.[27]

Other injection-related factors that may influence diffusion but to a lesser extent include needle gauge and speed of injection, since too large a gauge needle and/or too fast an injection could lead to trauma to the target tissue with the result that toxin uptake in the target area is decreased, leaving more toxin to spread to adjacent areas.[26] Spread can also be influenced by the distance between the tip of the needle and the neuromuscular junction (NMJ), with uptake being enhanced and spread reduced when the needle tip is close to a cluster of NMJs.[26,34] Rosales and colleagues[11] have suggested that muscle architecture (e.g., whether and how the individual muscle units are arranged in compartments) may also influence the spread of BoNT.

Limiting diffusion

Perhaps the most useful technique to limit diffusion is target muscle localization. Several techniques using EMG and endoscopic or imaging guidance are purported to increase the accuracy of targeting and thus reduce diffusion. Use of EMG, electrical stimulation (ES), or ultrasound guidance is employed in children for difficult-to-locate individual muscle groups.[26] EMG is also commonly used to confirm appropriate localization of the injection needle in specific muscles immediately before injection. Molloy and colleagues[35] examined the accuracy of muscle localization in patients with focal hand dystonia without EMG guidance and found that only 37% of needle placement attempts reached the target muscles or muscle fascicles, demonstrating the need for EMG guidance for correct localization of desired muscles. In contrast, superficial, easily targeted muscles can be injected directly, without a need for special techniques.[36]

Geenen and colleagues[37] studied 12 patients who received BoNT for focal hand dystonia: eight patients under passive EMG guidance and four with ES. Although the limited study concluded that ES was at least as good as EMG monitoring, both injection techniques resulted in

weakness of non-targeted muscles. EMG guidance may be used with (active) or without (passive) ES. Passive EMG guidance can be used for the treatment of cervical and laryngeal dystonia, as well as strabismus. Active EMG guidance may be applied when treating writers', musicians', and typists' cramps, spasticity, and other conditions where it is difficult to accurately target the muscle using voluntary contraction. EMG guidance may allow more precise injections and the identification and treatment of deep cervical muscles, and, indeed, the magnitude of improvement in cervical dystonia may be greater with EMG guidance.[38]

Ultrasound is a relatively convenient, painless, and less time-consuming procedure. Sconfienza and colleagues[39] have reported that the use of ultrasound to guide needle positioning prior to injecting BoNT into the iliopsoas muscle in 10 patients with spinal lesions allowed for easy and exact needle placement. The use of ultrasound guidance has also been shown to produce results that are superior to manually guided injections in the treatment of sialorrhea in patients with Parkinson's disease.[38]

Comparative studies

For each of the botulinum toxins the volume, dose, and accuracy of the toxin placement appear to have the most effect on the clinical outcome.

Animal studies of diffusion

The diffusion profiles of BoNT/A and BoNT/B products have been studied in numerous animal studies. In one study, equipotent doses of ONAA, ABOA, and INCOA caused a similar duration of paralysis with no difference in diffusion when injected into the tibialis anterior muscle of mice.[17] However, in two other studies in which ONAA, ABOA, and RIMAB were injected into the gastrocnemius muscle of mice, ONAA displayed less diffusion than either ABOA or RIMAB.[40,41] Different results were seen in a study that examined the effect of ONAA and RIMAB injected into the abductor pollicis brevis muscle of juvenile monkeys. In that study, the authors noted dose-dependent diffusion into both nearby and relatively remote muscles with ONAA but not RIMAB.[11] The results from these studies confirm that some BoNT products are clearly not interchangeable and indicate that, at clinically effective doses, side-effect rates may be different.[2]

Human clinical trials suggesting diffusion differences

ABOA versus ONAA. In a crossover study in which 212 patients with blepharospasm were randomized to receive double-blind ONAA or ABOA (ONAA/ABOA ratio of 1:4 IU), ABOA was associated with a significantly ($p<0.05$) greater incidence of AEs than ONAA, specifically ptosis (three cases with ONAA versus 14 cases with ABOA; $p<0.01$). The authors hypothesized that the reason for the difference in AEs might be related to the diffusion profile of the two products.[42] Results from a double-blind, randomized, three-period, crossover study involving 54 patients with cervical dystonia indicated that ABOA (ONAA/ABOA ratios of 1:3 and 1:4) is more efficient than ONAA for both impairment and pain in cervical dystonia; however, the number of AEs was higher with both ABOA treatments. The most frequent AE was dysphagia, found in 3%, 15.6%, and 17.3% (ONAA, ABOA 1:3, and 1:4, respectively) of the patients.[43] The study results, reported by Ranoux and colleagues,[43] differed from the only other study to compare the conversion factor between ONAA and ABOA units for the treatment of cervical dystonia[44] in several respects. The earlier study was a parallel design, while the study by Ranoux and colleagues used a crossover design that allowed patients to serve as their own control, thus eliminating some of the individual differences that might contribute to an AE (e.g., a thin neck). In addition, in the study by Ranoux and colleagues, a standardized protocol for injections was used and the same volume was injected for each of the three treatments. In light of these controls, the authors suggested that the higher AE profile of ABOA may in some way be related to its efficacy and its greater tendency to diffuse within the tissues.[43]

In a review of clinical and preclinical studies evaluating the diffusion properties of ONAA, ABOA, and RIMAB, de Alemeida and colleagues[25] concluded that higher doses of ABOA are needed to achieve efficacy similar to ONAA and that these higher doses are associated with an increase in diffusion-related AEs. The authors suggested ONAA has the least potential for diffusion, followed by ABOA, then RIMAB. These results are in accord with prior studies noting a lack of dose equivalence between ONAA and ABOA. Sampaio and colleagues[45] noted that 3–5 units of ABOA is required to achieve the same therapeutic or aesthetic effect as 1 unit of ONAA. Lowe and colleagues[46] have suggested that when doses are titrated to provide similar efficacy, the result is a ratio of ED_{50} (e.g., effective dose for 50% of the population receiving drug) values of approximately 1:5 (ONAA:ABOA), noting that at this ratio ONAA has a lower risk of diffusion than ABOA.

INCOA versus ONAA versus ABOA). INCOA and ONAA have been found to have comparable efficacy and safety in large phase 3 clinical trials in blepharospasm[47] and cervical dystonia.[48] According to Frevert,[49] the similar AE profiles seen in these studies are indicative of similar diffusion profiles. In a phase 1B study in 32 healthy volunteers, after injection of INCOA or ONAA into the extensor digitorum brevis muscle, CMAP analysis of two adjacent muscles (abductor hallucis and abductor digiti quinti) revealed no reduction of the muscle activity caused by diffusion after injection of either toxin.[1]

After intramuscular injection into the forehead, the diffusion profiles of INCOA and ONAA were not significantly different, while ABOA produced a significantly greater area of diffusion versus INCOA at comparable doses and identical volumes of injection.[50,51]

RIMAB versus ONAA. In a small study that investigated the diffusion of ONAA relative to RIMAB, RIMAB consistently produced a greater radius of toxin diffusion, as measured by the wrinkle reduction area, calculated using a digital micrometer on traced scanned images.[9] Other, larger studies also suggest that RIMAB diffuses differently from the other botulinum toxins. In a multicenter, randomized, double-blind, parallel-arm study comparing ONAA and RIMAB for

treatment of cervical dystonia, both dysphagia and dry mouth were more frequent with RIMAB. Dysphagia was reported by 48% of subjects treated with RIMAB versus 19% treated with ONAA (p=0.0005); 80% of subjects treated with RIMAB reported dry mouth compared with 41% of ONAA-treated subjects (p=0.0001).[52] In another report, mild (but not moderate/severe) dry mouth was also significantly (p=0.0005) more frequent with RIMAB than with ONAA.[53] A systematic review and analysis of the published literature comparing rates of dysphagia and dry mouth showed clear differences with RIMAB. Among the 70 published articles included in the analysis, RIMAB had the highest number that reported an association with rates ranging from 3.2% to 90%.[54] This would suggest that RIMAB has the highest local and systemic diffusion properties compared with the other toxins.

Conclusions

It is generally accepted that containment of BoNT diffusion is a desirable goal after injection. An accumulating body of evidence suggests that some of the botulinum agents have different diffusion characteristics. Meticulous placement of the toxin using correct dosing exactly targeted to the right muscle to produce a precise treatment effect offers the best chance of a good outcome. Techniques such as EMG guidance can also help to control the effects of diffusion by increasing the accuracy of the injection.[35,55,56]

Although BoNT serotypes are structurally and functionally similar, specific differences in neuronal acceptor binding sites, intracellular enzymatic sites, and species sensitivities suggest that each serotype is its own unique pharmacologic entity, sometimes due to distinct purification and manufacturing procedures. Physicians must have a working knowledge of the different serotypes, different doses used for each formulation of each serotype, and the side-effect profile of each product in order to insure against diffusion-related AEs. One should also be aware that a number of factors influence comparative data on efficacy, diffusion, and spread. These factors may range from properties intrinsic to the drug to accurate muscle selection and to the dilution, volume, and doses injected. In particular, the results of the clinical trials must be considered within the context that there are still no data on the conversion rate among the various treatments. Thus, too high or too low concentrations may have been used in the individual studies, making it difficult to draw too firm a conclusion from any one trial.

Acknowledgment

Editorial support, consisting of substantive editing, copy editing, and styling of the authors' draft, as well as assistance with literature searches, was provided by Linnéa Elliott and Maria Vinall of The Curry Rockefeller Group, LLC, Tarrytown, NY.

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