

New Concepts in Botulinum Toxin Therapy

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Over the past 10 years, botulinum toxin has evolved from a dreaded poison to being the basis of one of the most important new therapeutic agents for treatment of regional human involuntary movement disorders. When injected in highly diluted unit doses into striated muscles, the toxin produces a reproducible temporary state of regional denervation by inhibiting acetylcholine release at the neuromuscular junction.^[1] Neurogenic muscular atrophy ensues,^[2-4] resulting in regional decreased tone and contractility of injected muscles.^[5,6]

In clinical studies, botulinum toxin has proven useful in the treatment of certain cases of strabismus,^[7] involuntary blepharospasm, blepharospasm associated with Meige syndrome,^[8,9] adult onset spasmodic torticollis,^[10-14] spasmodic dysphonia,^[15] occupational limb spasms^[16] and, most recently, in the treatment of cerebral palsy.^[17] Case reports have provided preliminary evidence of efficacy in temporal mandibular joint disease,^[18] myofascial pain syndromes,^[19] anismus,^[20] and spastic urethral obstruction associated with spinal cord injury. Other applications may arise with further experience with this therapeutic technology.

Relative to other pharmacological therapy useful in treating dysphonias and spasticity, botulinum toxin has no known adverse central nervous system effects. The drug produces a dose dependent regional muscular denervation over an

area injected.^[14] Side effects relate primarily to excessive weakness of the injected muscle or contiguous muscles.

1. Applications and Administration Techniques

Botulinum toxin preparations are directly injected into hyperactive muscle groups to produce the partial denervation necessary to reduce involuntary movement. The active neurotoxin binds to its receptor on the presynaptic membrane of cholinergic nerve terminals at the neuromuscular junction. The toxin enters the nerve terminal by endocytosis. Once inside the neuron, the toxin proteolytically cleaves a protein needed for normal neurotransmitter release. Treatment with the toxin produces partial denervation that lasts generally between 4 and 16 weeks.^[21,22] The long duration of action is pharmacologically unique relative to other spasmolytic drugs.

Once injected, the active neurotoxin diffuses from the point of injection over a given region, producing a denervation gradient over this region.^[14] The area in which significant denervation occurs has been defined as the denervation field.^[5,6,14,22,23] The intensity of denervation and the size of the denervation field detected by histochemical staining and muscle fibre morphometric studies has been found to be dose dependent when dose is quantified as mouse LD₅₀ units (dose lethal to 50% of animals).^[22]

Injection locations and techniques vary according to the region of the body being treated. For the treatment of involuntary eyelid spasms, the injections are given into multiple locations to the protagonist muscle of eyelid closure, the orbicularis oculi. Care is needed to keep injection locations away from muscle groups which, if weakened, would cause undesirable effects or complications.^[14,24] For instance, in the treatment of blepharospasm, injection at the mid position of the upper lid should be avoided, as this area is close to the muscular portion of the levator palpebrae superioris muscle. Weakening of this muscle can cause ptosis, which can adversely affect the efficacy of the toxin therapy (see below for other examples of the diffusion phenomena).^[5,6]

Generally, drug efficacy is also dependent on injection location.^[5,6,10] For simple movements such as eyelid closure, the orbicularis oculi is clearly the muscle responsible for the movement, and hence is targeted for injection.^[24] However, for the treatment of more complex movement patterns, such as seen with spasmodic torticollis and occupational hand-finger movement disorders, identifying the muscles involved in the abnormal movement or posture becomes more challenging. With abnormal head movements, multiple muscle groups are involved, and injections should be given in accordance with which muscles are contributing to the abnormal posture or movement, based on basic anatomic considerations.

In situations in which there needs to be monitoring of the needle tip to assure needle placement within muscle tissue (e.g. spasmodic dysphonia) or a specific muscle accounting for an abnormal movement (e.g. finger movement disorders, strabismus), teflon coated needles are used, along with electromyogram (EMG) monitoring.^[16] The EMG monitor ensures the placement of botulinum toxin directly within the muscle accounting for the pathological state in certain circumstances. For muscles located close to the skin surface or for large muscles, the EMG direction is not necessarily needed and actually may not be desirable.



Fig. 1. A patient with severe intractable blepharospasm despite forcetel attempts by the examiner to open her eyelids. The involuntary movement is so strong as to cause forced closure. Examination of her eyes was impossible at this particular point in time. Blepharospasm is often associated with other involuntary movements of the face, head and neck. Patients with blepharospasm may have a family history of cranial cervical involuntary movement disorders.

When larger muscles are injected, the technique of toxin injection has been a subject of interest. The distribution of neuromuscular junctions within larger muscles (innervation zone) has not been carefully anatomically defined. However, paraspinal muscles in rabbit (*longissimus dorsi*) and the upper portion of the orbicularis oculii have been found to have a diffuse distribution.^[24] When larger muscles involved in abnormal head and neck movements associated with adult onset spasmodic torticollis were treated, a small increase in benefit was shown in a clinical study evaluating multiple injections per muscle versus single injection points per muscle.^[10]

Others have found the EMG helpful in directing toxin injection into larger muscles to sense hyperactive regions.^[25] However, deep probing of the cervical region with an EMG needle may be associated with increased risk to the administration procedure if important anatomic structures become impaled (e.g. lung cavity, vessels). Regardless of the exact technique used, the diffusion of botulinum toxin producing regional denervation around point injections into muscles, and the toxin effect on contiguous muscles, is important in understanding both efficacy and compli-



Fig. 2. Adult onset spasmodic torticollis characteristically involves multiple muscle groups of the cervical region. In this particular patient, the sternomastoid muscles, scalene, levator scapuli, splenius capitus and cervicis muscle and trapezius are all involved on the same side, causing head tilt and shoulder elevation. These patients often have pain, posture deformity, limited range of motion of the cervical spine and involuntary movement.

cations associated with this useful therapeutic technology.

We have found that point injections of botulinum toxin used to treat cervical dystonia produced diffuse denervation through fascial planes at higher doses, and containment of denervative effect at lower doses.^[14,22] Diffusion appears to be directly related to the amount of mouse LD₅₀ units used at an injection point.^[14,22] This latter observation, coupled with clinical experience, has led to the conclusion that dose-related diffusion and appreciation of the muscular anatomy involved with the particular disease are the most important determinants in directing therapy.^[13]

2. Systemic Effects of Regional Injections of Botulinum Toxin

Initially, there was concern about the possibility of systemic weakness or possible botulinum intoxication after local injections. Although the exact lethal dose level for humans is unknown, primate experimentation has indicated that a dose in excess of 2500 mouse LD₅₀ units (35 to 40 U/kg) are needed to produce systemic weakness.^[26] To date, there have been no reports of systemic botulism poisoning after local injection of these preparations.

3. Efficacy in Major Indications

Leading indications for the use of botulinum toxin include blepharospasm (fig. 1) and spasmodic torticollis (fig. 2). Botulinum A toxin is approved in the US and UK for the treatment of blepharospasm and strabismus.

Approximately 80 to 90% of patients with essential blepharospasm and hemifacial spasm demonstrate improvement after a single injection. The duration of benefit averages 12 weeks for essential blepharospasm and 16 weeks for hemifacial spasm. Biopsies in human muscle tissue after 6 months have shown no residual botulinum toxin effects.^[27]

Spasmodic torticollis produces morbidity by causing painful spastic contractions of cervical muscles, posture deformity, involuntary movements of the head, decreased range of motion of the cervical spine, and activity limitations. The pain is the most responsive aspect of the torticollis syndrome, with 90% of patients treated responding. Posture deformity and decreased range of cervical motion is improved 80% while involuntary movements are improved by 60 to 70%. Increase in activity endurance has been noted in over 80% of patients.^[13]

4. Complications Associated with Botulinum Toxin Application for Two Common Indications

When the eyelids are injected for the treatment of blepharospasm, the major complications are



Fig. 3. Ptosis can result from injection of botulinum toxin. The cause is toxin diffusion from injection sites to other muscle groups. Here it is the levator palpebra superior muscle that has been influenced by toxin diffusion, causing transient ptosis. Ptosis is reversible within several weeks after injection.

ptosis, exposure keratitis, diplopia and epiphora (tearing). Ptosis is caused by excess spread of the denervating effect into the superior orbit with attendant weakening of the elevating muscle of the eyelid (fig. 3).

Diplopia results from diffusion of botulinum toxin into the orbit affecting the extraocular muscles which govern eyeball movement. Exposure keratitis results from excess weakening of the orbicularis oculii, with attendant decreased blink reflex. Epiphora results from weakening of the portion of the orbicularis muscle involved in lacrimal sac function.

Dysphagia is the major immediate complication associated with the treatment of spasmodic torticollis. Dysphagia has resulted in upper airway obstruction in at least 2 patients.^[14] The cause of the dysphagia has been shown to be excessive dose application to the sternomastoid muscle in a retrospective and small prospective clinical study.^[14] The sternomastoid muscle directly overlies the peripharyngeal musculature. Diffusion of the denervating effects of botulinum toxin from the sternomastoid muscle to peripharyngeal musculature can easily occur because of this anatomic proximity.

Injection of excessive amounts of toxin may result in peripharyngeal muscular weakness

causing dysphagia. The original incidence of this complication has been reported at 15 to 17% with the US toxin ('Botox'). Higher incidences have been reported with the UK toxin ('Dysport'). Limiting the injection site to the sternocleidomastoid muscles has clearly lowered the incidence of this complication to less than 2% with the American toxin.

Although the clinical indications for botulinum toxin are expanding and further information on technique and anatomic considerations will emerge in other body regions, the above indications give examples of important principles in the application of this technology.

5. Sensitisation after Repeated Injections of Botulinum Toxin

Increasingly, concern is growing regarding the development of neutralising antibodies after repetitive injections of botulinum toxin. As many of the movement diseases treated are chronic, indefinite repeated injections can be necessary. With repeated injections, however, a small but significant number of patients develop neutralising antibodies. When present, neutralising antibodies render further botulinum toxin injections ineffective. This complication of therapy is much more common with high dose indications (spasmodic torticollis) and much less common with low dose indications (blepharospasm, strabismus, spasmodic dysphonia). This complication has been reported to occur in 3 to 5% of patients after 3 years of therapy in high dose indications, but the incidence may actually be greater because of insensitive assay techniques and the necessity for repeated injections over time.

The true incidence of antibody formation over many years and comparative evaluations with various formulations remain to be determined for high dose applications. Although neutralising antibodies have been reported in a patient with blepharospasm,^[28] patients with blepharospasm can be treated for many years without developing resistance to this low dose application.

Using the standard mouse neutralisation assay, Hathaway and associates^[29] reported that 13 of 198 (6.5%) serum samples tested were positive for neutralising antibodies between the years 1984 and 1988. However, between the years 1988 and 1992, 48 of 167 (29%) specimens tested were positive for neutralising antibodies.^[29] The incidence of antibody formation was retrospectively correlated to dose exposure. Antigenicity is an important consideration in the long term use of botulinum toxin preparations. When neutralising antibodies are present in patients receiving botulinum toxin A, injected muscles fail to develop weakness and patients become unresponsive to subsequent injections of that immunotype.

The formation of botulinum toxin antibodies appears correlated to: (i) dose given per injection; (ii) quantity of botulinum protein given per injection; (iii) number of injections administered; and (iv) frequency of injections.

Many physicians are not giving the injection any more frequently than at 3-month intervals. Issues relating to drug antigenicity are important in the future development of this technology.

6. Other Botulinum Toxin Immunotypes

There are 7 distinct serotypes of botulinum toxin (types A to G). However, the clinical literature has been focused primarily on therapy with type A toxin. Botulinum toxin type F has been studied in adult onset spasmodic torticollis.^[37] It has become apparent in relatively early clinical studies that the F toxin has a shorter duration of action than the A toxin at equivalent mouse LD₅₀ units. The shorter duration of action of the F toxin is a distinct disadvantage in its use for the treatment of chronic diseases because more injections are necessary, resulting in more frequent procedures and inconvenience to the patient, and because more frequent exposures may be associated with a higher rate of sensitisation.

Botulinum B toxin has been investigated in preclinical studies and has been found to produce regional denervation similar to botulinum A

toxin.^[30] Although the B toxin produces similar histological effects, increasing evidence exists that the B toxin binds to different receptors on presynaptic membranes and target protein substrates within nerve terminals. At equivalent LD₅₀ mouse units of A and B serotypes, the B serotype has a shorter duration of action (data from a rabbit model, personal observations). Although the histological changes produced by botulinum B toxin are similar to the A toxin, further studies are needed to assess the duration of action of botulinum B toxin, as well as its relative potency.^[30]

7. Specific Activity and Immunological Resistance

Since the introduction of botulinum toxin to the practice of medicine, the measurement of activity has been exclusively quantitated using mouse lethality units.^[7,31,32] This measure of biological activity was preferred because of ease of end-point measurement as well as its pertinence to the toxicological effect, the clinically significant attribute of botulinum toxin prior to its use as a therapeutic agent.

Measurement of toxin activity in biological units instead of nanograms is essential, as different preparations of toxin have varying degrees of bioactivity per nanogram of protein (specific activity). In fact, as development of botulinum A antibody formation can be correlated to injection number and duration of exposure, and total protein exposure, knowledge of specific activity of preparations is an important consideration in pharmacological preparations. The higher the specific activity of the preparation, the lower the botulinum protein exposure per injection. Recently, in studies of small numbers of animals, repeated injections with high activity botulinum A toxin preparations were associated with no antibody formation, whereas low activity preparations produced sensitisation (Goodnough & Johnson, personal communication).

8. The Problem with the Mouse Lethality Unit

Although specific activity is important in assessing the relative purity of botulinum toxins, there are independent factors important in the potency of the preparations. This observation is most apparent when comparing the dose-response relationships in the clinical literature for neurological blepharospasm and adult onset spasmodic torticollis, the current leading indications for the use of botulinum toxin.

There are 2 sources of botulinum A toxin used in clinical studies in the world literature, 'Botox' (US Botulinum A toxin) and 'Dysport' (UK botulinum A toxin). For example, the dose range used to treat the orbicularis muscle in blepharospasm ranges between 10 and 35 mouse lethality units per eye when 'Botox' is used, and 100 and 125 units when 'Dysport' is used.

Likewise, for the treatment of torticollis, significant differences in dose requirements per injection have been observed. The dose range for 'Dysport' is between 600 and 1200 mouse lethality units^[32-36] compared with only 150 to 200 such units for 'Botox'.^[10-14] Such differences in dose requirements cannot be explained by individual techniques or disease variations, as these differences have been reported at multiple centres. They are obvious when comparing package inserts, and have recently been acknowledged in the clinical literature as potentially dangerous if confusion occurs as to which dose is associated with which toxin type.^[35,36]

Clinicians using different preparations of botulinum toxin should be aware that different relative potencies between botulinum A toxins can have substantial implications. If mouse units are used interchangeably between the British and American forms of botulinum A toxin, serious adverse effects may occur.^[35,36] As the occurrence of most adverse effects is dependent on diffusion, and diffusion is dose and potency dependent, diffusion dependent complications may arise, such as dysphagia when treating torticollis,^[14] or diplopia and ptosis when treating blepharospasm.

The differences in potency of equivalent mouse LD₅₀ units of botulinum toxin have been recognised comparing other immunotypes of botulinum toxin. Botulinum toxin type B, which has been recently introduced into clinical studies, has been found to have different denervating properties at equivalent mouse unit doses.^[42] In early clinical studies, botulinum F toxin was found to have a substantially shorter duration of action relative to botulinum A toxin.^[37] Additionally, botulinum G toxin also has differences in biological activity when assessed using regional denervation models.^[42]

9. Regional Denervation – Reason for Potency Differences between Botulinum Toxin Preparations

Various immunotypes of botulinum toxin may have substantial differences in their mechanism of action and chemical structure.^[30,31,38,39] To date, all methods of standardising botulinum toxin have involved using a mouse lethality unit (LD₅₀) as a standard unit of measure.^[7,31] The lethality unit was originally favoured because of clear endpoint analysis in the bioassay. Although this assessment of bioactivity simulates botulinum when evaluated as a toxin, there are limitations to this method of bioassay when evaluating botulinum toxin as a drug.

The essential pharmacological effect produced by botulinum toxin injections is *regional chemodenervation*. A unit quantity of botulinum toxin is injected into hyperkinetic, hypertonic or hypertrophied muscles or muscle groups temporarily creating decreased tone, amplitude of contraction, and neurogenic muscular atrophy over a defined anatomic region.^[5,6,14] The area within which botulinum toxin produces clinical effects has been termed the denervation field.^[5,6,14] When regional denervation is used as a measure of biological activity, there are important differences recognised in biological activities between the various types of botulinum toxin. Potency and duration of action differences at equivalent mouse units can be demonstrated in animal models.

These observations are useful to alert clinicians to the fact that the mouse unit is not a precise measure of potency when comparing various forms of botulinum toxin, and that further work needs to be accomplished in material preparation for this important therapeutic technology. These observations also may be significant in understanding differences in batch production equivalency and clinical variations noted with different batches of botulinum material used in clinical studies. For instance, differences in potency have occasionally been noted with the same dosages of the same product. Perhaps the mouse units may be equivalent as labelled, but regional denervation effects may not be equivalent. In fact, even with respect to conformity of LD₅₀ measurements, there can be significant variation in activity measurements per laboratory.^[31] More work is clearly needed in the applying the concepts of regional denervation to drug standardisation and clinical application.

Recently, a regional denervation unit has been defined to quantitate the clinically important biological effect of botulinum toxin. This unit has been used to explain potency differences found in clinical studies using US and British botulinum toxin as well as other immunotypes.^[40,41]

10. Conclusions

Botulinum toxin has become an important drug for the treatment of regional movement disorders. It clearly is the drug of choice for the treatment of neurological blepharospasm, hemifacial spasm, adult onset spasmodic torticollis and spasmodic dysphonia. Other applications are being explored in spasticity, cerebral palsy, jaw pain syndromes, myofascial pain syndromes and facial nerve disorders. Future indications and applications will develop because of the unique pharmacological capabilities of this technology.

Current problems in the technology involve immunogenicity of materials, biological activity evaluation (potency and diffusion potential) and the need for more rigorous clinical studies. Sensitisation to repeated injections is the most

immediate concern for those being treated for chronic diseases such as torticollis. Factors important in understanding causes of immunological resistance need to be identified and corrected. In future years, applications of this valuable therapy will no doubt increase in the practice of medicine and surgery.

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