

Botulinum toxin therapy, immunologic resistance, and problems with available materials

Gary Borodic, MD; Eric Johnson, ScD; Mike Goodnough, PhD; and Edward Schantz, PhD

Botulinum toxin injections have become an extremely useful therapeutic modality in the treatment of certain segmental movement disorders such as blepharospasm, adult onset spasmodic torticollis, spasmodic dysphonia, and oral mandibular dystonia. Clinical efficacy is substantially better, and complications of this therapy are significantly lower than alternative approaches for many of these disorders. Short-term complications have often been the result of undesirable diffusion away from the injection location, resulting in transient weakness. The study reported by Jankovic et al¹ deals with immunologic resistance, the major long-term complication of repeated injections.

Historic concern becomes a current reality.

Botulinum toxins have long been known to be antigenic proteins, with different serotypes characterized by the use of neutralizing antibodies. Because botulinum toxin is used to treat chronic diseases and repeated injections are necessary over a long period of time, sensitization after repeated injections is an ongoing concern of both clinicians and regulators from initial studies to the present.

Concern regarding antibody production was present in the original formulation for clinical studies. Initial emphasis was placed on the drying to achieve preservation to prevent contamination. However, the original method of drying had serious problems because of 80 to 90% loss of activity of the material in this process. In 1979, Schantz² and Scott were perfectly aware that the inactivated botulinum toxin may be as antigenic as active toxin; however, there was insufficient clinical experience to assess the significance of this observation. This formulation approved in the United States has become accepted over the past decade. By altering the drying process, it is now possible to prevent botulinum toxin inactivation, hence lowering incidental toxoid accumulation within the vials.³

Alternative immunotypes: the issue of bioequivalency and efficacy.

Once neutralizing an-

tibodies are present to immunotype A, the clinical results of subsequent injections are obliterated.⁴⁻⁷ Although other immunotypes of botulinum toxin are under development, clinical studies indicated that distinct biological property differences between immunotypes are important in medicinal application.⁸⁻¹¹ For instance, botulinum toxin type F has demonstrated some efficacy in botulinum A toxin-resistant patients yet has a short duration of action (3 weeks) compared with botulinum toxin type A.⁷ Botulinum type B also has been shown to have a shorter duration of action than botulinum type A in animal models.^{10,11}

Clearly, when used to treat chronic diseases, botulinum toxin preparations with the longest duration of action would be preferable to limit the frequency of injections. Additionally, recent studies indicated differences in regional denervation potency between various immunotypes of botulinum toxin, with certain preparations of botulinum toxin type A having the highest potency.⁸⁻¹¹

With the clinical and laboratory data suggesting that certain preparations of botulinum toxin type A may be unique in its duration of action and denervation potency, the issue of sensitization becomes even more relevant. Because of these observations, it is important to understand the factors important in preventing this complication: (1) clinical experience regarding dose and methods of administration; (2) better understanding of the sensitivity of the assay for the botulinum toxin antibodies; and (3) scientific issues relating to formulation of the botulinum neurotoxin protein.

Clinical observations and experience. As indicated by Jankovic et al,¹ sensitization is a major cause of decreased or absent clinical response after repeated injections. Jankovic et al related resistance to dose exposure per injection cycle. This finding is consistent with past experience comparing the incidence of sensitization between blepharospasm and torticollis, the two wide-scale clinical indications for botulinum toxin injection over the past 10 years.

From the Department of Ophthalmology (Dr. Borodic), Harvard Medical School and Massachusetts Eye and Ear Infirmary, Boston, MA; and Department of Microbiology (Drs. Johnson, Goodnough, and Schantz), University of Wisconsin, Madison, WI.

Received March 24, 1995. Accepted in final form May 30, 1995.

Address correspondence and reprint requests to Dr. Gary Borodic, 90 Kennington Road, Canton, MA 02021.

Blepharospasm therapy usually involves the injection of 15 to 50 LD₅₀ units into four to six locations to each orbicularis muscle. In comparison, torticollis therapy usually involves injections of 150 to 300 LD₅₀ units into dystonic cervical muscles, often in multiple locations. Retrospective review of the literature reveals the incidence for sensitization in the blepharospasm indication to be small using the standard mouse bioassay technique,^{12,13} whereas the sensitization has been significantly higher for the larger dose application, spasmodic torticollis (3 to 10%).⁶ The findings of Jankovic et al¹ within just the dystonia population further reinforces the notion that sensitization is a dose-dependent complication of long-term repetitive botulinum toxin injections. Their findings are further substantiated by previous evaluations made by Hathaway and Dang.¹⁴ The authors believe that these data as well as the results reported by Jankovic et al actually underestimate the problem.

Antibody test. The clinical significance of circulating botulinum toxin antibodies has been discussed.^{6,12-19} The work of Jankovic et al¹ indicates that the presence of neutralizing botulinum toxin antibodies has significant predictive value for results obtained with subsequent injections. These findings have been the general consensus for those using the therapeutic technology for some time. Recently, other investigators using a nonbioassay method for antibody testing (microsphere technique) did not find correlation with clinical results.¹⁷ Only the bioassay can detect neutralizing antibodies at this time. Other techniques such as ELISA need to be validated for neutralizing antibody determination before interpreting the results, because these techniques may detect antibodies to inert protein (e.g., hemagglutinin) within the botulinum toxin formulation, which may have no therapeutic significance. In short, the distinction between neutralizing and nonneutralizing antibodies needs to be clearly defined in subsequent clinical studies.

Another important issue relative to antibody testing is the sensitivity of the test. The mouse bioassay for botulinum antitoxins was developed to advance toxoid research for various botulinum toxin immunotypes.¹⁸ The assay was set at a sensitivity that would confer immunity to multiple lethal challenges of botulinum toxin.¹⁸ Because of this, the sensitivity of the mouse bioassay assay reported in neurologic and ophthalmologic literature to date is low. In practice, the quantity of neutralizing antibody must be sufficient to inactivate small amounts of botulinum toxin used to create regional denervation over a given injection area. Such small antitoxin levels may not be picked up by a bioassay that has been set at a threshold sensitivity that detects antitoxin levels capable of resisting a 10,000 to 100,000 times LD₅₀ challenge.¹⁸ If the assay's sensitivity threshold is low, its specificity may be quite high. This interpre-

tation of the current mouse bioassay can be used to explain three clinical phenomena noted in practice.

1. Inconsistent positivity of the mouse bioassay when patients are repetitively tested over time. Antibody titers may vary above and below assay sensitivity thresholds at varying times since last antigen exposure.

2. Very strong concordance between positive bioassay result and subsequent clinical results (100% as reported by Jankovic et al¹).

3. A series of patients are reported who are seronegative yet show no muscular atrophy to botulinum type A but do develop muscular atrophy when injected with another serotype, botulinum type F (personal communication, S. Fahn).

Because the clinically important level of antitoxin is that level sufficient to neutralize regional denervating doses and not lethal doses of botulinum toxin, the reported mouse bioassay should be considered a very specific but not a necessarily sensitive test for circulating antitoxin in patients receiving therapeutic injections.

To further understand resistance after repetitive injections of botulinum toxin, efforts have been made to evaluate the problem using different methods. One clinical method has been the remote point injection.¹⁹ Initial data on this method appear to indicate higher sensitivity for resistance detection, and the test has predictive value for subsequent clinical response to therapeutic botulinum toxin injections.

Given the limitations of the botulinum toxin antibody test to date, it is probable that the true incidence of sensitization to botulinum toxin preparation is significantly underestimated in the clinical literature.

Specific activity and botulinum toxin. Of all published medical literature on botulinum toxin therapeutic technology and immunologic resistance, little consideration has been given to preparation, formulation, and specific toxicity of botulinum toxin for human use. Specific activity is defined as follows:

specific activity = biological activity/nanograms of botulinum neurotoxin.

Biological activity has been conventionally measured in LD₅₀ units. Nanograms of botulinum toxin are measured with spectrophotometry in early formulation or ELISA in the final preparation. The specific activity index gives a direct measurement of both purification grade and quality of protein preparation to produce a desired biological effect. In clinical terms, biological effect is synonymous with therapeutic effect. The LD₅₀ units or other bioassay measurements are conceptual based on the protein ability to produce a certain effect. Nanograms in the specific activity formula merely reflect the quantity of protein used in the therapeutic preparation. The study by Jankovic et al,¹ as well as retrospective review of the literature, indicates that antigenicity of botulinum toxin preparations is not only directly re-

Table Dose per injection cycle*

	Dose in LD ₅₀ units	Dose in nanograms	Sensitization
Spasmodic Torticollis	150-300	38-150	Significant problem
Blepharospasm	15-80	4-40	Very uncommon

* Doses apply only to BOTOX botulinum toxin type A (Allergan, Inc., Irvine, CA). Substantial differences exist when other formulations of botulinum toxin type A are used.

lated to dose in LD₅₀ mouse units but directly related to quantity of botulinum toxin in nanograms used per injection. In fact, nanograms per injection cycle is probably a much more relevant unit because immune response will occur to mass of protein antigen exposed per inoculation (therapeutic botulinum toxin injection) probably irrespective of other biological effects of that protein.

Given the above definition of specific activity and given that the measured specific activity of BOTOX botulinum toxin type A is between 2 and 4 LD₅₀ units per nanogram of neurotoxin, the table represents the usual doses per injection cycle for two large therapeutic applications of botulinum toxin.

The table applies only to BOTOX botulinum toxin type A (Allergan, Inc., Irvine, CA). Substantial differences exist when other formulations of botulinum toxin (e.g., DYSPORT, Porton Products Ltd., Berkshire, England) are used in therapy.²⁰ From the table, it can be derived that incidence of sensitization is not only related to dose exposure in LD₅₀ units per injection cycle but also nanograms of botulinum toxin exposure per injection cycle (protein load). Specific activity is the measurement which indicates how much protein exposure a patient receives both cumulative and with each botulinum toxin injection hence represents an important aspect of drug evaluation process.

Botulinum toxin used in clinical practice in the United States is from a single batch produced in 1979 by Edward Schantz (lot 79-11). A sample of this toxin kept in Schantz's laboratory indicates that it has lost considerable activity, having a specific activity of 2.3 LD₅₀ units/ng. Freshly prepared botulinum A toxin has a specific activity of about 33 LD₅₀ units/ng, which is consistent from lot to lot.

High specific activity botulinum A toxin. The most consistent prospective and retrospective clinical data indicate that sensitization is related to dose in nanograms and LD₅₀ units per injection cycle, methods that can be useful to decrease antigen exposure per injections cycle and may offer improvements to mitigate this problem for high-dose applications of therapeutic botulinum toxin injections, such as those used to treat adult onset spasmodic torticollis. Altering the specific activity in the botulinum toxin preparation to yield the highest possible significant bio-

logical effect with the least amount of neurotoxin protein would be preferable.

Because it is possible to improve the specific activity of the currently US licensed form of botulinum toxin A from 2.3 LD₅₀ units/ng to 25 to 30 LD₅₀ units/ng, it is possible to reduce the protein exposure from the current 38 to 150 nanograms per injection cycle to 5 to 12 nanograms per injection cycle for large dose indications. Essentially, the higher specific activity botulinum toxin reduces the protein exposure for high-dose indications such as those currently used to spasmodic torticollis to low-dose indication currently used to treat blepharospasm. If a quantity of neurotoxin protein exposure is a prime determinant of immunogenicity as suggested by clinical experience and study, then this formulation improvement should be a considerable advance to reducing sensitization after repeated therapeutic injections. However, prospective clinical evaluation of sensitization rates with high specific activity botulinum toxin in clinical practice remains to be reported.

Interim recommendations. Retrospective analysis of ophthalmic and neurologic literature and studies on antibody positive cases against negative cases indicate dose per injection cycle is linked to antibody formation. Limiting total dose per injection cycle at this point until a higher specific activity botulinum A toxin becomes available would be a logical step in preventing sensitization. As ophthalmic applications are only rarely associated with sensitization and almost never are associated with use of >100 LD₅₀ units, limiting total dose to ≤100 LD₅₀ units per injection cycle at 3-month intervals may be very helpful in preventing this complication.

Summary. Botulinum toxin is a valuable technology for the treatment of regional movement disease. High-dose applications (>100 LD₅₀ units per injection cycle) have been associated with sensitization that renders further therapeutic injections ineffective. The true incidence of sensitization is probably underestimated by the mouse bioassay. Other immunotypes of botulinum toxin have been effective in producing some therapeutic benefit; however, duration of action (botulinum toxin type F) and lower potencies may make these less attractive alternatives than botulinum type A. Increased specific activity botulinum toxin may be a method to reduce antigen exposure and mitigate against immunoresistance associated with dystonia therapy. Limiting the dose to ≤100 LD₅₀ units per injection cycle may limit this complication in the interim.

References

1. Jankovic J, Schwartz K. Response and immunoresistance to botulinum toxin injections. *Neurology* (in press).
2. Schantz E. Historic perspectives. In Jankovic J, and Hallett M, eds. *Therapy with botulinum toxin*. New York: Marcel Dekker, 1994.

3. Johnson E, Goodnough M. Stabilization of botulinum toxin type A during lyophilization. *Appl Environ Microbiol* 1992;58:3426-3428.
4. Borodic GE, Pearce LB, Johnson E. Antibodies to botulinum toxin. *Ophthalmology* 1994;101:1158-1159.
5. Hambleton P, Cohen HE, Palmer BJ, Melling J. Antitoxins and botulinum toxin treatment. *BMJ* 1992;304:959-960.
6. Jankovic J, Schwartz K. Clinical correlates of response to botulinum toxin injections. *Arch Neurol* 1991;48:1253-1256.
7. Green P, Fahn S. Use of botulinum type F injections to treat torticollis in patients with immunity to type A. *Mov Disord* 1993;8:479-483.
8. Pearce LB, Borodic GE, First ER, MacCallum RD. Measurement of botulinum toxin activity: evaluation of the lethality assay. *Toxicol Appl Pharmacol* 1994;128:69-77.
9. First E, Pearce LB, Borodic GE. Dose standardization with botulinum toxin for human use. *Lancet* 1994;343:1035.
10. Borodic GE, Johnson E, Goodnough M. Myogenic ptosis model for evaluation of botulinum toxin activity. *Ophthal Plast Reconstr Surg* (in press).
11. Pearce LB, Borodic GE, Johnson EA, First ER, MacCallum R. The median paralysis unit: a more pharmacologically relevant unit of biologic activity for botulinum toxin. *Toxicon* 1995;33:217-227.
12. Biglan AW, Gonnering R, Lockhart LB, Rabin B, Fuerste FH. Absence of antibody production in patients treated with botulinum A toxin. *Am J Ophthalmol* 1986;101:232-235.
13. Gonnering RS. Negative antibody response to long-term treatment of facial spasms with botulinum toxin. *Am J Ophthalmol* 1988;105:313-315.
14. Hathaway C, Dang C. Immunogenicity of the neurotoxin of clostridium botulinum. In Jankovic J, Hallet M, eds. *Therapy with botulinum toxin*. New York: Marcel Dekker, 1994.
15. Tsui JK, Wong NLM, Wong E, Calne DB. Production of circulating antibodies to botulinum-A toxin in patients receiving repeated injections for dystonia [abstract]. *Ann Neurol* 1988;23:181.
16. Zuber M, Sebald M, Bathien N, et al. Botulinum antibodies in dystonic patients treated with type A botulinum toxin, frequency and significance. *Neurology* 1993;43:1715-1718.
17. Siatkowski RM, Tyutyunikow A, Biglan AW. Serum antibody production to botulinum A toxin. *Ophthalmology* 1993;100:1861-1866.
18. Cardella MA. Botulinum toxoids. In Lewis KH, Cassel K Jr, eds. *Botulism: proceedings of a symposium*. Washington, DC: Government Printing Office 1964:113-130. [PHS Publ. No. 999-FRI.]
19. Borodic GE, Pearce B, Duane D, Johnson E. Antibodies to botulinum toxin [letter]. *Neurology* 1995;45:204.
20. Brin M, Blitzer A. Botulinum toxin, dangerous terminology errors. *J R Soc Med* 1993;86:493-494.