Discussion Regarding Botulinum Toxin, Immunologic Considerations with Long-term Repeated Use, with Emphasis on Cosmetic Applications

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MINIMAL RISK OF ANTIBODY FORMATION AFTER AESTHETIC TREATMENT WITH TYPE A BOTULINUM TOXIN

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The article by Dr. Borodic, on immunological considerations for the long-term use of botulinum toxin A in aesthetic medicine, raises a number of interesting issues. However, one important point that must be borne in mind, in any such discussion, is that the total protein content of the product being administered (toxin complexes with various protective non-toxin proteins) is not relevant for the production of neutralizing antibodies (NAb) to the toxin. The original Botox formulation, produced from bulk toxin batch 79–11 as provided by Dr. Schantz in Wisconsin, contained about 90% inactivated toxin due to both the age of the material and the salt precipitation and lyophilization method used for final product preparation. This product correspondingly had a higher immunogenic potential than one without the inactivated toxin. Changing the bulk toxin batch used in this formulation reduced the immune challenge and, consequently, the rate of NAb formation.

The protective protein complex itself is not believed to be relevant for the production of NAb to the neurotoxin molecule. The complex is pH sensitive and rapidly dissociates under physiological conditions, releasing the neurotoxin molecule. This sensitivity has been described in Schantz and Johnson’s classic review papers as a means of obtaining pure toxin. In vitro experiments have shown this dissociation to be both complete and rapid. Any antibodies to other proteins in the complex will not be NAb; any Western blot or other analytical test that cannot distinguish between these other antibodies and NAb is not clinically useful. Currently, no in vitro method can distinguish this difference. Therefore, in vivo or ex vivo tests must be used, such as the mouse protection assay (MPA) and the mouse phrenic hemidiaphragm assay (MPHA). Alternatively, pragmatic tests on patient wrinkles, the extensor digitorum brevis muscle, or sweat glands have been used by clinicians but are only applicable to the individual concerned and are not suitable for screening or epidemiological studies.

In contrast to Dr. Borodic’s view, the authors have found the MPA to be very sensitive for the assessment of patient sera, the method being routinely capable of determining the neutralization of 1 median lethal dose (LD50) unit of type A toxin (equivalent to 0.0001 units/mL of type A botulinum antitoxin) or lower. The MPHA is able to detect NAb titers to a sensitivity of 0.0003 units antitoxin/mL, which is, in turn, approximately 30 to 300 times more sensitive than in vitro methods such as an ELISA assay.

Dr. Borodic states that there are no long-term studies of NAb formation in patients treated for facial wrinkles. However, there is a considerable body of relevant data from indications using significantly higher doses of the two main commercial preparations, Dysport and Botox. These are especially relevant when considering the overall rate of antibody formation to toxin treatment. In Germany, two long-term studies have been carried out with...

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Dysport, which has been marketed in the same formulation since 1990: one vial of Dysport contains 500 Ipsen mouse LD50 units of toxin in 4.35 ng of protein complex. An earlier study reported data from 303 patients with cervical dystonia (CD) treated for a mean of 3.2 years (mean 10.2 treatments), of whom 17 showed secondary nonresponse, and 9 (3%) were NAb-positive according to the MPHA. NAb formation was significantly correlated with so-called booster injections at short intervals in this early 1999 study. Booster injections are no longer carried out, for this very reason. A 2004 study on 100 CD patients, treated with Dysport for a mean of 5.1 years and including a subgroup of 32 patients treated for a mean of 10 years in one clinic, identified just three secondary nonresponse patients, none of whom showed NAb in the MPHA test.

Long-term data in high dose indications for patients treated only with the present formulation of Botox, introduced around 1998, are available. In post-stroke spasticity (111 patients treated for a mean period of 54 weeks) and CD (326 patients with a median of 9 treatments), NAb were detected in 0.6% and 1.23% of patients respectively, using the MPA. Taken together, these data suggest that the risk of NAb formation, even after several years of use at high therapeutic doses, is low with either Dysport or the present formulation of Botox, at least in adults. The corresponding risk in low-dose use for the treatment of wrinkles must be significantly reduced. The case reported by Dr Borodic is, to our knowledge, the only one in the literature—despite the fact that injections of botulinum toxin type A to treat facial wrinkles are now the most common procedure in aesthetic medicine. Also, the published long-term data on the aesthetic use of Dysport and Botox do not suggest any cause for concern. Dr Borodic is correct to draw attention to the potential consequences of NAb formation due to the use of botulinum toxin type A in aesthetic medicine, but the risk of this happening appears to be very low.

RESPONSE RELATIVE TO PHARMACEUTICAL COMPOSITION OF BOTULINUM TOXIN FOR HUMAN INJECTION, CLINICAL EXPERIENCE WITH INCIDENCE OF ANTIBODY FORMATION, THE ACTUAL ANTIBODY TESTS, AND SOME ETHICAL CONSIDERATIONS REGARDING THE EVOLVING USES AS A THERAPEUTIC AND COSMETIC PHARMACEUTICAL

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As Dr Pickett states, the composition of the 79-11 batch of botulinum toxin used in the United States between 1983 and 1998 did contain a much higher protein content and correspondingly low specific activity. This preparation contained between about 40 ng per 100 U vial (specific activity 2.5 u/ng). The large amount of toxin based protein in the vials included:

1. Active neurotoxin (active ingredient)
2. Inactivated neurotoxin from stored source materials or formulation deterioration during lyophilization

The large inactivated neurotoxin protein component of the original formulation was the major concern, as deactivated neurotoxin still can have immunogenic properties. As considerable amounts of both inactive neurotoxin protein was present in earlier formulations, the incidence of antibody production with secondary resistance was 17% in higher-dose cervical dystonia studies using the mouse-neutralizing detection method over a period of 3 to 5 years. Lower-dose indications receiving lower neurotoxin protein load per injections cycle were associated much less commonly with antibody formation. The differences in the rate of neutralizing antibody development between high dose indications such as cervical dystonia (higher neurotoxin protein exposures per injection) and low-dose indications such as hemifacial spasm and blepharospasm (lower neurotoxin protein exposure per injection cycle) provided motivation to reduce the neurotoxin protein per vial, which was accomplished in the botulinum type A toxin (BOTOX) product in 1998. The originators of botulinum toxin manufacturing at the University of Wisconsin were concerned about the quality of neurotoxin in the early BOTOX-Oculinum preparations. Since 1998, the commercially available type A botulinum toxin complex in the United States, BOTOX, was modified by its commercial manufacturer to a much higher specific activity with attendant reduction in total protein to approximately 5 ng per 100 U vial. As Dr Pickett has accurately pointed out, this formulation change clearly reduced, but did not eliminate, the incidence of secondary resistance. The impact of the formulation change on reduction of antibody formation rate was most notable for cervical dystonia, the prototype high-
Clinical significance of neutralizing antibodies to botulinum toxin is conventionally measured with a mouse protection assay which uses one fixed amount of patient’s serum, a lethal dose of botulinum toxin to the reference mouse, and the recipient mice who either survive (positive assay) or are killed by injections of the mixture within 3 to 4 days (negative assay). This assay was originally designed to measure effectiveness of botulinum toxins (vaccines) with respect to conferring immunity. In this application, the sera of scientist’s immunized with botulinum toxoid originally testing positive can drop significantly, indicating the need for booster vaccinations. From the physician and surgeons’ perspective, there are also problems with this assay. Patients who have developed secondary resistance achieving no weakness in targeted or remote point muscles may be negative to this assay. Even patients with well-documented resistance originally positive to neutralizing mouse assay can show negative results over time. For this reason, the remote point clinical testing for weakness became popular over the last 15 years. Patients testing negative for mouse neutralizing antibodies can have positive remote point tests. From a physiologic perspective, the mouse assay also may lack correlation to tissue pharmacokinetics. When the surgeon injects botulinum toxin into a muscle, the toxin becomes in transient, binding to presynaptic membrane receptors, internalization, ultimately reacting with the cytoplasmic substrate. During this transient time before receptor mediated internalization, the molecule is vulnerable to antitoxin (from hours to about 1–2 days). The interstitial fluid perfusing the muscle may be much greater than the amount gathered within small-volume serum specimen used in the mouse bioassay. The serum volume concentration multiple may be the reason for the strong predictive value and possible higher sensitivity of the remote point type tests than the mouse assay. This tissue kinetic may explain the higher confidence in remote point “individual” assessment tests referred to by Dr Pickett and colleagues.

Regional hindlimb paralysis assays and tissue bath nerve-muscle preparation may suffer some of the same criticisms, although they probably have a small increased toxin sensitivity over the mouse assay. As neutralizing antibodies may differ in quantity and binding affinities to the neurotoxin, low-level or low-affinity antibodies in the test tube with a small sera specimen may still be clinically significant. Newer in vitro assays that demonstrate the highest toxin sensitivity system may offer the best chance for a more sensitive in vitro antibody test. In this respect, the cultured neuronal cell assay, which detects both botulinum toxin membrane receptor binding and cytoplasmic substrate-SNAP 25 cleavage, is under evaluation and may offer an improved method of assay. The assay has been reported to have a toxin sensitivity to 0.1 U, about 10 X that of the mouse detection assay sensitivity in the mouse detection system referred to by Dr Pickett.

Appropriately, the major supplier of BOTOX has made it clear on the package insert that long-term studies in the cosmetic use of botulinum toxin are lacking. Many patients using botulinum toxin for cosmetic purposes do so for many years and many will receive ongoing injections over a period of decades going forward. For many, the treatment is a lifelong endeavor. Secondary immunity in the past has usually required a number of years to develop (usually 3–6 years for cervical dystonia). Studies involving 3 to 9 injection cycles may not be adequate exposure paradigms to make any firm conclusions, particularly in light of a low-sensitivity mouse assay. From a clinical perspective, the cosmetic patient who may become immune may be less likely to report the secondary resistance because they judge the injections as ineffective and become “lost to follow-up.” In contrast, the therapeutic patient would continue to suffer and to seek relief from chronic disease. Since publication of the original articles, I have still another cosmetic case demonstrating both neutralizing antibodies to the mouse assay and neuronal cell assay. In my practice, a fraction of the patient population with neurologic blepharospasm who has received repeated injections for 1 to 2 decades relate a reduced response over time, without clear explanation. These patients often receive the same dose range as the cosmetic population. Other physicians have noted secondary resistance even with lower doses of the newer preparations.

Wide-scale use of the toxin as a cosmetic will probably lead to many cosmetic recipients needing future therapeutic injections if they develop stroke, spasticity, prostatic hypertrophy, certain headaches, or many of the other therapeutic indications known to be responsive to toxin technology. Although it is clear that the risk in the short term is small, the long term is still unknown.
Given the weaknesses inherent in current assay techniques, the cross-usage of the medication, the higher standard of safety inherently needed for cosmetic applications for acceptable risk–benefit assessments, the need for accurate informed consent, further assay development, knowledge of compositional pharmacology, and clinical study—assessment of antibody formation rates are needed for the cosmetic patient, as has been conducted for other indications.

As Dr Pickett and colleagues have brought up the issue regarding fractional inactivation and denaturation of botulinum toxin production during lyophilization in the original 79-11 preparation, it would be helpful if manufacturers revealed percent biologic-activity loss during drying and preparation process. Creation of inactive and potentially immunogenic neurotoxin can occur during this step of the process, as Dr Pickett has pointed out. The loss of biologic activity during the drying process indicates the conversion of active neurotoxin to inactive neurotoxin. Drying recovery data may be useful in comparing preparations of the same immunotype for purity that has been linked to immunogenicity. Additionally, each manufacturer should reveal the total protein quantity and compositions in the vials inclusive of an estimated mass of botulinum neurotoxin, complexing proteins (if present), method of LD 50 bioassay, gel protein electrophoresis, and albumin concentrations so that the many clinicians using these materials can better understand and evaluate the differences between available preparations from a pharmacologic perspective, and make their own judgments.

REFERENCES: PICKETT AND CAIRD DISCUSSION: MINIMAL RISK OF ANTIBODY FORMATION AFTER AESTHETIC TREATMENT WITH TYPE A BOTULINUM TOXININ


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