Neutralizing Botulinum Toxin Type A Antibodies: Clinical Observations in Patients with Cervical Dystonia

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Local injections of botulinum toxin type A (BoNT/A) may be used in the treatment of diseases associated with increased muscle tension (eg, dystonia; Tsui et al, 1985; Tsui et al, 1986; Greene et al, 1990), spasticity (Snow et al, 1990), involuntary cocontractions (Rollnik, Hierner, et al, 2000), and pain syndromes like tension-type headaches (Rollnik, Tanneberger, et al, 2000).

Therapeutic doses of BoNT/A are usually too low to stimulate the immune system. Nevertheless, formation of BoNT/A antibodies may be observed in a substantial number of patients (Anderson et al, 1992; Greene and Fahn, 1992; Göschel et al, 1997). This immune response may result in a secondary nonresponse to BoNT/A and necessitate termination of treatment (Göschel et al, 1997). Nonresponse can be defined as a primary or secondary therapeutic failure (clinically defined) to BoNT/A treatment (Dressler, 1995). Specific antibodies pose a problem for 3% of patients with cervical dystonia (CD) after 15 months of therapy (Anderson et al, 1992) and for 8% after 20 months of therapy (Greene and Fahn, 1992). We must mention, however, that marginal titers of neutralizing antibodies may also be observed among responders (Göschel et al, 1997). In addition, nonneutralizing antibodies do not affect the therapeutic outcome (Göschel et al, 1997). Some patients may revert from immunoresistance (Ab+) to antibody-negative (Ab−) status (Sankhla et al, 1998). Nevertheless, repeated injections can boost antibody formation in this subgroup of patients (Sankhla et al, 1998).

The conditions under which BoNT/A antibodies may form are still a matter of discussion. Siatkowski et al (1993) found no significant differences between Ab+ and Ab− patients with respect to duration of treatment, number of injections, or cumulative dose of BoNT/A. These findings might be explained by a huge variation in data from a heterogeneous group of patients (blepharospasm, facial hemispasm, CD). Nevertheless, a few studies support the hypothesis that cumulative BoNT/A dose is a major factor in the formation of neutralizing antibodies: Low-dose treatment of CD does not induce antibody formation (Brans et al, 1995). Our recent investigation involving 115 patients treated with low-dose BoNT/A (Rollnik, Matzke, et al, 2000) supports this finding.

Subjects and Methods

We studied 6 Ab+ CD patients exhibiting a secondary nonresponse to BoNT/A (Dysport®) treatment. Mean age was 41.3 years (SD, 5.9 y). Secondary nonresponse was defined as the first ineffective treatment (at which point in time an antibody assay was performed). These Ab+ patients were compared with 12 Ab− patients (mean age, 56.8 y; SD, 15.3 y) matched for severity of clinical symptoms, duration of treatment, and number of treatments (Table 1).
Neutralizing BoNT/A antibodies were detected and quantified using an in vitro toxin-neutralizing assay based on a nerve-muscle preparation (Göschel et al, 1997). Statistical analyses used $t$ tests for independent samples (Ab+ vs Ab−) and bivariate Pearson correlations.

**RESULTS**

In Ab+ patients, secondary nonresponse occurred after an average of 11.7 treatments (SD, 4.9 treatments) over an average of 31.5 months (SD, 10.2 mo). Mean antibody titer in the first assay was 2.36 mU per mL (SD, 4.27 mU/mL; range, 0.30–10.00 mU/mL). Cumulative BoNT/A dose until secondary nonresponse was 5984 mouse units (MU; SD, 3151 MU). (See Table 1.)

In Ab− patients, cumulative BoNT/A dose after 8.8 injections (SD, 3.7 injections) over 29.6 months (SD, 9.7 mo) was 3143 MU (SD, 2125 MU).

Ab+ and Ab− patients differed in cumulative BoNT/A dose ($t = −2.31, df = 16, P = .037$) and age (Ab+ mean age = 41.3 y, SD = 5.9 y; Ab− mean age = 56.8 y, SD = 15.3 y; $P = .032$).

When neutralizing antibodies were detected, BoNT/A therapy was stopped for approximately 6 months. After this interruption, patients profited from an average of 2.7 additional treatments (SD, 2.0 treatments; range, 1–6 treatments) before a definite nonresponse occurred. After an average of 9.6 months (SD, 5.5 mo), a second antibody assay was performed. This assay showed BoNT/A antibody titer of 3.45 mU per mL (SD, 4.27 mU/mL; range, 0.40–10.00 mU/mL). Increase in antibody titer correlated significantly with the BoNT/A dose given between the first and second assays ($r = .97, df = 5, P = .006$), which suggests a booster effect. Mean BoNT/A dose before the second assay was 578 MU (SD, 678 MU; range, 115–1750 MU). In 1 case, Botox® was used instead of Dysport; with Botox, the titer increase was also considerable (Fig. 1).

**Conclusions**

Formation of neutralizing BoNT/A antibodies poses a problem for 3% to 8% of patients with CD after 15 to 20 months of treatment with regular doses of BoNT/A (Anderson et al, 1992; Greene and Fahn, 1992). Presence of these antibodies may lead to a secondary nonresponse to BoNT/A application, necessitating termination of treatment (Göschel et al, 1997). The conditions leading to antibody formation, however, are still a matter of discussion. Some authors (eg, Siatkowski et al, 1993) have hypothesized that the cumulative BoNT/A dose is not of major importance; others (eg, Brans et al, 1995; Rollnik, Matzke, et al, 2000) have indicated that low-dose treatment may be a successful strategy in the prevention of antibody formation.

**Table 1** Group Characteristics

<table>
<thead>
<tr>
<th>BoNT/A Antibody Positive (Ab+)</th>
<th>BoNT/A Antibody Negative (Ab−)</th>
<th>Statistic</th>
</tr>
</thead>
<tbody>
<tr>
<td>N</td>
<td>6</td>
<td>12</td>
</tr>
<tr>
<td>Female/male (n)</td>
<td>3/3</td>
<td>5/7</td>
</tr>
<tr>
<td>Age (y)</td>
<td>41.3 (5.9)</td>
<td>56.8 (15.3)</td>
</tr>
<tr>
<td>Duration of treatment (mo)</td>
<td>31.5 (10.2)</td>
<td>29.6 (9.7)</td>
</tr>
<tr>
<td>No. treatments until nonresponse</td>
<td>11.7 (4.9)</td>
<td>8.8 (3.7)</td>
</tr>
<tr>
<td>Cumulative BoNT/A (Dysport®) dose until nonresponse (MU)</td>
<td>5984 (3151)</td>
<td>3125 (2126)</td>
</tr>
</tbody>
</table>

Data are means (SDs), except where noted otherwise. BoNT/A = botulinum toxin type A; NS = not significant; MU = mouse units.

![Figure 1](image-url)
In the present study, we investigated which factors might contribute to antibody formation. Of cumulative dose, duration of treatment, and number of treatments, only cumulative dose was a significant predictor of antibody formation. In addition, Ab+ patients were significantly younger than Ab− controls. This finding could be explained by the fact that immune function declines with age (Lesourd and Mazari, 1999). Therefore, younger patients might have a higher risk for forming neutralizing antibodies.

In addition, our results suggest that Ab+ patients may profit from a treatment break and then approximately 3 additional injections (Sankhla et al, 1998). Nevertheless, repeating BoNT/A injections creates a vicious circle. From our experience, we know that therapy fails for these patients after 2 or 3 treatments. A change in medication (from Dysport to Botox, or vice versa) is useless; only a change in neurotoxin subtype (to BoNT/B or BoNT/F) might be promising (Sankhla et al, 1998).

In line with other studies, we recommend that the BoNT/A dose should be as low as possible in order to prevent formation of neutralizing antibodies (Brans et al, 1995; Rollnik, Matzke, et al, 2000), as cumulative dose does seem to play a major role. After a nonresponse to BoNT/A treatment, an antibody assay should be performed in order to identify Ab+ patients. When neutralizing antibodies begin forming, some patients may profit from a break of approximately 6 months and then approximately 3 additional BoNT/A treatments.

Further studies on neutralizing BoNT/A antibodies are strongly encouraged.

References
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