Prospective, Double-Blind, Randomized, Parallel-Group, Dose-Ranging Study of Botulinum Toxin Type A in Men with Glabellar Rhytids

ALASTAIR CARRUTHERS, MD,* AND JEAN CARRUTHERS, MD†

Departments of *Dermatology and †Ophthalmology, University of British Columbia, Vancouver, British Columbia, Canada

BACKGROUND. The effective dose for treating glabellar lines with botulinum toxin type A in men has not been studied adequately. OBJECTIVE. To compare the safety, efficacy, and duration of response of four doses of botulinum toxin type A on glabellar rhytids in men. METHODS. Eighty men were randomized to receive a total dose of either 20, 40, 60, or 80 U of botulinum toxin type A (BOTOX, BOTOX Cosmetic, or Vistabel, Allergan, Inc., Irvine, CA, USA) in the glabellar area. Glabellar lines were assessed at rest and maximum frown by a trained observer at baseline, 2 and 4 weeks, and monthly thereafter. Patients provided self-evaluations at the same visits. Adverse events were monitored throughout.

RESULTS. The 40, 60, and 80 U doses of botulinum toxin type A were consistently more effective in reducing glabellar lines than the 20 U dose (duration, peak response rate, improvement from baseline). There was a dose-dependent increase in both the response rate at maximum frown and the duration of effect assessed by the trained observer. In addition, the participants reported a dose-dependent reduction in the ability to frown, improvement in their global assessment, and increased feelings of attractiveness, self-confidence, and satisfaction. The incidence of adverse events was not increased with higher doses.

CONCLUSION. Male participants with glabellar rhytids benefit from starting doses of at least 40 U of botulinum toxin type A.

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THE EFFICACY and safety of botulinum toxin type A for the esthetic treatment of hyperfunctional facial lines, including glabellar lines, have been demonstrated in numerous trials. In two multicenter, double-blind, randomized, placebo-controlled trials in which more than 500 participants were treated, 20 U of botulinum toxin type A was found to be a highly effective dose in a mostly female study population.1–3 Dose titration and its effect on treatment intervals have not, however, been standardized, nor have recommendations for dose adjustments on the basis of gender been developed.

The efficacy and safety of varying doses of botulinum toxin type A in the treatment of glabellar lines in women were recently characterized in a double-blind, randomized, dose-ranging study.4 In that study, a 10 U total dose was less efficacious than total doses of 20, 30, and 40 U. The peak response to treatment and the duration of the benefit were dose dependent, but there were no dose-related differences in adverse effects, supporting the use of at least 20 U total dose of botulinum toxin type A in female participants.

Clinical experience suggests that men require a higher total dose. Because no more than approximately 20% of participants in clinical trials have been male, the optimal dosing in men remains to be determined. Therefore, we conducted this trial of botulinum toxin type A to compare the safety and efficacy of four doses of botulinum toxin type A in men and to evaluate whether the duration of response was dose dependent. The lowest total dose of 20 U was selected based on the results of the dose-ranging study in women and on our clinical observations in men.

Methods

Study Design

A single-center, prospective, double-blind, randomized, parallel-group, dose-ranging study of up to 52 weeks’ duration.

Patients

Men between 18 and 65 years of age were eligible for the study. Glabellar rhytids were judged by a trained observer as moderate or severe on the Facial Wrinkle Scale (FWS) during maximum attempted muscle contraction.

Key exclusion criteria included the use of any agent (eg, aminoglycoside antibiotics) that could interfere with neu-
romuscular transmission or any condition (eg, Eaton-Lambert syndrome, myasthenia gravis, excessive weakness, or atrophy of target muscles) that could amplify the effects of treatment with botulinum toxin type A. Individuals were also excluded if they had any known allergy or sensitivity to any component of the study medication; had prior cosmetic procedures, soft tissue augmentation, or visible scars on the treatment area; or had received treatment with botulinum toxin within 1 year of baseline evaluation.

Any subject could be withdrawn from the study at any time in the event of a serious adverse event. They could also be withdrawn if any exclusion criteria became apparent or on voluntary discontinuation. Subjects who were withdrawn before the initiation of treatment could be replaced.

The study complied with the Declaration of Helsinki recommendations for biomedical research involving human subjects. Institutional Review Board approval was obtained at the site before the start of the study. The design of the study, objectives, and potential risks were explained to potential subjects, and written informed consent was obtained prior to enrolment.

Protocol and Injection Technique

For the double-blind trial, participants were randomly assigned into one of four possible treatment groups using a block-of-eight design. On day 0 (baseline) of the trial, participants were treated with a total dose of either 20, 40, 60, or 80 U botulinum toxin type A. The total dose was divided among seven intramuscular injections into the muscles contributing to glabellar frown lines: 20% of the total dose into the procerus muscle, 15% into each corrugator muscle, and 50% over four sites in the orbicularis oculi (15% into each of two sites above the medial canthus and 10% into each of two sites above the midpupillary line; Figure 1). This injection pattern was identical to the injection pattern used in our similar study in females and was our standard glabella injection pattern at the time of these studies.

Masking

To maintain the blind, vials were prepared by a registered nurse who took no further part in the study. Identical-appearing syringes were marked only with the participant number. Injection volumes for a given site were constant across doses to preserve masking. Total injected volume was 0.4 mL for each participant (procerus 0.08 mL and corrugator and medial orbicularis 0.06 mL each on both sides, and midpupillary orbicularis 0.04 mL on each side). The vials were reconstituted with nonpreserved saline to final dilutions of 50 U/mL (20 U total dose), 100 U/mL (40 U total dose), 150 U/mL (60 U total dose), and 200 U/mL (80 U total dose). A different vial was used for each participant, and injections were made within 1 hour of reconstituation. Nonpreserved saline was used because this is the method of reconstitution recommended in the package insert, and this was the diluent we used in the similar female study.

Follow-Up and Outcome Measures

The study comprised a screening/baseline visit and post-injection follow-up visits at weeks 2 and 4 and then every 4 weeks for the duration of the study. At screening, the trained observer assessed severity of glabellar rhytids using the FWS: none = 0, mild = 1, moderate = 2, and severe = 3. Eligible subjects provided a medical history, including previous and concomitant medication use, and underwent an abbreviated physical examination.

The trained observer evaluated glabellar rhytids at each follow-up visit using the FWS. Subjects evaluated their glabellar rhytids at maximum attempted muscle contraction using the FWS. They also completed a self-evaluation questionnaire that assessed feelings of attractiveness, self-confidence, and satisfaction with appearance on a scale of 0 (not at all) to 6 (extremely) and rated the degree of improvement on a scale ranging from −4 (complete improvement, 100%) to +4 (very marked worsening, at least 100%).

Adverse Events

Signs and symptoms of adverse events were monitored throughout the study. Investigators rated adverse events for severity, seriousness, and relationship to treatment. Severity was graded as mild, moderate, or severe. Serious adverse events were those that were life threatening and resulted in death, hospitalization, or persistent or signifi-
cant disability or incapacity. The relationship to study treatment was assessed as unrelated, possible, probable, definite, or unknown.

**Data Analysis and Statistical Methods**

The selected sample size of 20 participants per treatment group was based on an α level of 0.05, a power of 0.8 to 0.9, and a potential dropout rate of 15%. All analyses of efficacy and safety were conducted on the intent-to-treat population, which included all participants assigned randomly to receive one of the four doses of botulinum toxin type A and who underwent at least one efficacy assessment while on treatment with the study drug.

**Baseline Comparability**

To evaluate baseline comparability, demographic data and the pertinent medical history of participants were tabulated for each of the four treatment groups. A chi-square test was used to analyze all categorical variables. Analysis of variance was used for continuous variables.

**Efficacy**

The primary outcome measurements were the trained observer’s assessment of the severity of wrinkles at maximum frown compared with baseline. Secondary efficacy measures included the maximum treatment effect (greatest change in category of FWS achieved as evaluated by the trained observer), the response rate (percentage of participants with a rating of none [0] or mild [1]), the percentage of participants with improvement from baseline, and the subjects’ self-evaluations of response to treatment. The primary efficacy data analysis was based on the duration of effect or relapse rate, defined as a return to the baseline value on the FWS at two consecutive visits and by comparison with baseline photographs, as evaluated by the trained observer. The categorical data were analyzed using a chi-square test; one-way analysis of variance was used to evaluate changes in mean scores on the FWS. A survival analysis of the time to relapse across treatment groups was evaluated using the log rank and Wilcoxon tests.

**Safety**

Safety analyses were undertaken for the incidence and severity of adverse events. The frequency of adverse events by treatment group was assessed using a chi-square test.

**Results**

**Demographics and Baseline Characteristics**

Eighty participants were enrolled in the study. The four treatment groups did not differ significantly in any baseline characteristics, including age, race, findings on physical examination, and FWS score at maximum attempted contraction (Table 1). Of the 80 participants, 77 completed the study. Two participants withdrew consent, and one discontinued without providing information.

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**Table 1. Patient Characteristics and Study Distribution**

<table>
<thead>
<tr>
<th>Treatment Group</th>
<th>Dose</th>
<th>Age (mean ± SD)</th>
<th>Race</th>
<th>Baseline FWS (C); mean (SD)</th>
<th>Category at baseline, with contraction (n)</th>
<th>Category at baseline, at rest (n)</th>
<th>Completed study</th>
<th>Withdrew consent</th>
<th>No information</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>20 U (n = 20)</td>
<td>44.2 ± 14.6</td>
<td>White</td>
<td>20</td>
<td>1</td>
<td>None</td>
<td>19 (95%)</td>
<td>1 (5%)</td>
<td>0 (0%)</td>
</tr>
<tr>
<td></td>
<td>40 U (n = 20)</td>
<td>38.6 ± 8.2</td>
<td>Hispanic</td>
<td>0</td>
<td>2</td>
<td>Mild</td>
<td>19 (95%)</td>
<td>1 (5%)</td>
<td>0 (0%)</td>
</tr>
<tr>
<td></td>
<td>60 U (n = 20)</td>
<td>44.0 ± 12.8</td>
<td>Asian</td>
<td>0</td>
<td>1</td>
<td>Moderate</td>
<td>19 (100%)</td>
<td>0 (0%)</td>
<td>0 (0%)</td>
</tr>
<tr>
<td></td>
<td>80 U (n = 20)</td>
<td>39.6 ± 13.2</td>
<td>Other</td>
<td>0</td>
<td>3</td>
<td>Severe</td>
<td>19 (95%)</td>
<td>1 (5%)</td>
<td>1 (5%)</td>
</tr>
</tbody>
</table>

C = maximum contraction; FWS = Facial Wrinkle Scale.
Efficacy: Trained Observer Assessments

Responder Rate
The maximum effects on glabellar lines obtained on the FWS at full contraction are shown in Table 2. Across all groups, 70 of the 80 participants (87.5%) experienced a reduction in the severity of their glabellar lines from severe to mild or none. In the 20 U group, 65% of participants had an improvement in the FWS from severe to mild or none; 90% and 95% of the participants in the 40 U and 60 U groups, respectively, and 100% of the participants in the 80 U group had similar changes. Differences between groups were statistically significant (p < .0001). In the 80 U group, 17 participants (85%) had a reduction in their lines from severe to none. These differences in maximum effect achieved by treatment group are reflected in the changes in the mean FWS scores for each treatment group over the course of the study (Figure 2). Patients in the 20 U dose consistently exhibited less of a decrease in mean FWS scores throughout the study. Statistically significant differences between groups were observed at weeks 2 through 12.

Responder rates are shown in Figure 3. For all treatment groups, the peak responder rate was observed between weeks 2 and 4 and was 65% in the 20 U group compared with 100% in the other dosage groups (p < .0001). At 2 months, the responder rate was maintained at 80% in the 80 U group and 90% in the 60 U group. It fell to 50% in the 40 U group and 25% in the 20 U group. The differences between groups were statistically significant (p = .0001). At the 3-month visit, 35% of the participants in both the 40 U and 60 U groups and 50% of participants in the 80 U group continued to be classified as responders. In contrast, only 15% of participants in the 20 U group were classified as responders at 3 months.

Duration of Effect: Relapse Rate and Survival Analysis
A dose-response relationship was apparent in the duration of the effect on the FWS at maximum attempted contraction. The mean time to relapse was 17.6 weeks for the 20 U dose, 21.7 for the 40 U dose, 22.8 for the 60 U dose, and 24.2 for the 80 U dose. The percentage of participants relapsing at each visit is shown in Figure 4.

Based on the categorical analysis, one participant in the 20 U dose group relapsed by 2 months and five patients relapsed by 3 months. In contrast, none of the participants in the other three treatment groups had relapsed by 3 months (p = .0009). A significant difference among the treatment groups was also detected at 4 months (p = .004), which began to disappear by month 5 (p = .079). At month 5, 79% of participants receiving the 20 U dose had relapsed compared with 63% at the 40 U dose, 50% at the 60 U dose, and 40% at the 80 U dose. At 6 months, the relapse rate was 90% for the 20 U dose, 74% for the 40 U dose, 75% for the 60 U dose, and 60% at the 80 U dose. Of all participants, 26% (20 of 78) had not relapsed at 6 months.

Improvements from Baseline
The percentages of participants in each treatment group showing any improvement from baseline, based on the trained observer’s assessment at maximum contraction, are shown in Figure 5. At 1 month, 85% of participants in the 20 U group were improved over baseline compared with 95% in the 40 U group and 100% in the 60 U and 80 U groups. By 2 months, 60% of the 20 U group, 90% of the 40 U group, 100% of the 60 U group, and 95% of the 80 U group were rated as improved (p = .001). Statistically significant differences were maintained at month 3, when 90% of the 80 U group continued to be rated as improved (p = .0027).

Table 2. Maximum Effect Achieved on the Facial Wrinkle Scale at Maximum Concentration (Trained Observer Evaluations)

<table>
<thead>
<tr>
<th>Effect Achieved</th>
<th>Number of Patients (%) by Dose</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>20 U</td>
</tr>
<tr>
<td>No change</td>
<td>1 (5)</td>
</tr>
<tr>
<td>Moderate–none</td>
<td>1 (5)</td>
</tr>
<tr>
<td>Severe–moderate</td>
<td>5 (25)</td>
</tr>
<tr>
<td>Severe–mild</td>
<td>12 (60)</td>
</tr>
<tr>
<td>Severe–none</td>
<td>1 (5)</td>
</tr>
</tbody>
</table>

Figure 2. Mean scores on the Facial Wrinkle Scale (FWS) at maximum contraction. *p < .0001; †p = .0234.
Efficacy: Patient Self-Evaluations

**Responder Rate**

At the 2-week visit, 75% of participants in the 80 U dose group rated themselves as responders on the FWS at maximum attempted contraction. In contrast, 35% of participants in the 20 U dose group were self-evaluated responders. At the 1-month visit, 75% of participants in the 80 U group continued to rate themselves as responders versus 20% in the 20 U group ($p = .0027$).

**Improvements from Baseline**

Patients’ self-evaluations generally mirrored those of the trained observer (Figure 6). By week 2, 45% of participants in the 20 U group reported improvement compared with 85% in the 40 U group and 90% in each of the other two groups ($p = .001$). At 3 months, 75% of the participants in the 80 U group still reported an improvement compared with 45% in the 60 U group, 65% in the 40 U group, and 30% in the 20 U group ($p = .023$). At month 4, 45% of the participants in the 80 U and 60 U groups continued to report improvements. In the 40 U group, 40% reported improvement from baseline, whereas only 15% in the 20 U group reported improvements.

**Global Improvement**

Mean scores on the participants’ Global Assessment of Improvement Scale (9-point scale) differed significantly among groups at weeks 4 to 12. Generally, participants who received the 20 U dose perceived the least degree of improvement on the Global Assessment of Improvement Scale. Those who were in the 80 U group had the highest scores on the Global Assessment of Improvement Scale (Figure 7).

**Patient Questionnaire**

Patients reported increases in feelings of attractiveness, self-confidence, and satisfaction with appearance on a scale of 0 (not at all) to 6 (extremely). Significant differences between treatment groups in participants’ ratings of attractiveness ($p = .047$) and feelings of satisfaction were observed at week 2 ($p = .0193$). At month 1, treatment group differences in ratings of attractiveness ($p = .0052$), self-confidence ($p = .0225$), and feelings of satisfaction ($p = .0064$) were detected.
Safety

Botulinum toxin type A was generally well tolerated in all participants, and there were no significant differences between treatment groups in the number of participants per group reporting an adverse event or in the number of adverse events per group. Twelve adverse events were considered to be possibly or definitely treatment-related (Table 3). All of these were transient and mild to moderate in severity. No association between botulinum toxin type A dose and the occurrence of adverse events was detected. Three severe adverse events were reported; none were considered to be drug related.

Discussion

In male participants, botulinum toxin type A was highly effective in reducing the appearance and severity of glabellar lines. On all measures, higher doses were more effective and tended to provide more durable responses than the 20 U dose. Supporting a dose-response relationship on the duration of the effect, the mean time to relapse was 21.7 weeks in the 40 U group, 22.8 weeks in the 60 U group, and 24.2 weeks in the 80 U group. In contrast, the mean time to relapse was 17.6 weeks for participants receiving the 20 U dose. At 6 months post-treatment, the relapse rate was 90% for the 20 U dose group compared with 74% for the 40 U dose group, 75% for the 60 U dose group, and 60% for the 80 U dose group. Even so, 26% of all participants had not relapsed at the 6-month visit. The percentage of participants with improvements from baseline according to both participant and trained observer ratings was greater for the higher doses than for the 20 U dose, and these differences persisted for up to 3 months. Similarly, the peak responder rate (the percentage of participants with a rating of none or mild) was greater in the higher-dose treatment groups according to both participant self-evaluations and trained observer ratings. Overall, the benefits of treatment were most apparent at maximum attempted contraction of the treated muscles.

There are minor inconsistencies in the results of this study that we believe are due to the difficulties of performing esthetic studies with standardized treatment procedures. A much larger participant population would be required to achieve greater consistency in the results; however, this is impractical, and the dose-response relationship that we have demonstrated in many areas of the study is sufficient. The major area in which the dose-response relationship is not observed is later in the study as participants have relapsed; therefore, the number of individuals analyzed is smaller and more subject to variability. The inadequacy of the 20 U dose is clear, whereas the advantage of higher doses is not as well demonstrated. We have therefore emphasized the superiority of the 40 U dose and higher doses rather than the overall dose-response relationship.

The low rate of adverse events seen in the present study further confirms the excellent safety profile of botulinum toxin type A in esthetic use. The incidence of treatment-related adverse events was similar in all groups, and none of these were severe or serious.

Table 3. Treatment-Related Adverse Events

<table>
<thead>
<tr>
<th>Treatment Group</th>
<th>20 U</th>
<th>40 U</th>
<th>60 U</th>
<th>80 U</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>(n = 20)</td>
<td>(n = 20)</td>
<td>(n = 20)</td>
<td>(n = 20)</td>
<td>Total</td>
<td></td>
</tr>
<tr>
<td>Adverse events</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Bruise under</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>left eye</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Headache</td>
<td>0</td>
<td>3</td>
<td>0</td>
<td>0</td>
<td>3</td>
</tr>
<tr>
<td>Brow/lid twitch</td>
<td>0</td>
<td>2</td>
<td>1</td>
<td>0</td>
<td>3</td>
</tr>
<tr>
<td>Heaviness in</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>brow/forehead</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Forehead spasm/</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>muscle contraction (frontal area)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>All events</td>
<td>1</td>
<td>5</td>
<td>2</td>
<td>4</td>
<td>12</td>
</tr>
</tbody>
</table>

Figure 7. Mean scores on the participants’ Global Assessment of Improvement Scale.
This is the first systematic dose-ranging study undertaken in male participants. Although botulinum toxin type A has been used in numerous trials, dosing was not consistent in earlier trials of botulinum toxin type A and has not been standardized. Moreover, the majority of participants have been female.

A double-blind, randomized, dose-ranging study on the treatment of glabellar lines in women demonstrated that a 10 U total dose was less efficacious than total doses of 20 U, 30 U, and 40 U. The peak response to treatment and the duration of the benefit were dose dependent, but there were no dose-related differences in adverse effects, thus supporting the use of at least a 20 U total dose of botulinum toxin type A in females.

Taken together, the results from these two prospective, double-blind, randomized, parallel-group trials support previous clinical observations that the treatment of glabellar lines in men requires higher treatment doses of botulinum toxin type A than in women. In the current study, the 40 U, 60 U, and 80 U doses were consistently more effective than the 20 U dose in regard to both the extent and the duration of effect, without any increase in the risk of drug-related adverse events. Clinical benefits can typically be expected to last at least 3 to 4 months in most participants and up to 6 months in perhaps 25% of participants. Based on these findings, male participants with glabellar rhytids appear to benefit from starting doses of at least 40 U of botulinum toxin type A and probably higher. At present, we start men at a dose approximately twice that which we use in women (60–80 U).

The results of this study raise broader questions regarding the treatment of the male face. For example, should all areas of the male face be treated at twice the dose used for females? This question needs further study because the sensitivity of muscles to botulinum toxin type A can differ markedly, and the results in one group of muscles, such as the glabellar complex, should not be transposed to another muscle or muscles. In addition, despite the very similar responses of these male participants to the questions on attractiveness, self-confidence, and satisfaction to the responses of females, there are definite differences in the male esthetic. Treatment of men should be adjusted accordingly. This area requires further study and clinical experience to ensure that we are giving our clients the optimal results from their botulinum toxin type A injections.

The results of this study refer to the Allergan formulation of botulinum toxin type A (BOTOX, BOTOX Cosmetic, Vistabel, Irvine, CA, USA) and cannot be generalized to any other formulations or serotypes of botulinum toxin type A.

References