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One-year, randomised, multicenter, two-period study of the safety and efficacy of repeated treatments with botulinum toxin type A in patients with glabellar lines

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Summary

A randomised, multicenter, 1 year study of patients with moderate or severe glabellar lines at maximum frown was conducted to evaluate the safety and efficacy of botulinum toxin type A (BOTOX®; Allergan, Inc, Irvine, CA, USA). Period one consisted of two identical 4-month placebo-controlled studies. Period two consisted of a follow-on, 8-month, open-label study. Patients received 20 U of botulinum toxin or placebo during period one. Patients with at least mild glabellar lines at maximum frown were given 20 U of botulinum toxin at initiation of period two and at 4 months. Botulinum toxin was superior to placebo in reducing glabellar line severity during period one. Progressive improvement with repeated treatment cycles was noted in all efficacy measures during period two. The incidence of blepharoptosis decreased with successive treatments. Botulinum toxin injections did not induce neutralising antibodies. Botulinum toxin type A treatment for glabellar lines was safe and effective in reducing glabellar lines.

Key words: botulinum toxin type A, BOTOX® Cosmetic, glabellar lines*

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*BOTOX® is a registered trademark of Allergan, Inc, Irvine, CA, USA.

Introduction

The use of botulinum toxin type A to improve the appearance of hyperfunctional facial lines is one of the most common cosmetic procedures performed today[1]. In the medical community, confidence in the widespread use of this unique therapy for aesthetic purposes requires adequate documentation of both its long-term efficacy and safety in this application. In this regard, it is also important to demonstrate that long-term, repeated use of botulinum toxin for aesthetic indications does not lead to antibody-induced reduction in the effects of this drug, which is also widely used in a variety of therapeutic indications.

When botulinum toxin is injected intramuscularly in small amounts, it produces a localised, dose-dependent, long-lasting, but ultimately reversible decrease in muscle activity. This reliability and reversibility has led to the successful use of botulinum toxin in the treatment of over a dozen different conditions[2]. It is recognised as the treatment of choice for most focal dystonias[3,4], is used to treat spasticity in both children and adults[5–8] and shows promise as a prophylactic treatment for tension and migraine headaches[10–13]. Botulinum toxin has a long and impressive safety record in those conditions for which botulinum toxin is most useful (particularly the dystonias); it is often the safest and most effective treatment option available[2,14].

The doses of botulinum toxin used in aesthetic applications are very small and the risks of adverse effects related to its mechanism of action are concomitantly low. The low doses may also reduce the formation of botulinum toxin neutralising antibodies, which, although occurring infrequently[15], may lead to a reduction in efficacy.

Goals of this study were to assess the safety and efficacy of repeated botulinum toxin treatment versus placebo for decreasing the severity of glabellar lines in a large cohort of typical facial aesthetics patients. This study also assessed the incidence of antibody formation with repeated treatment.

Patients and methods

Study design

This was a 1-year, repeated-treatment evaluation conducted at 30 sites (29 US and 1 Canadian), consisting of two independent, but identical, 4-month, randomised, double-blind, placebo-controlled studies (period one), followed by an 8-month, open-label follow-up study (period two).

The two studies in period one shared the same protocol, which consisted of one treatment with either botulinum toxin (BOTOX®*) or placebo (vehicle). These trials have been published separately[16,17]. Patients from both trials were eligible to be considered for entry into the second phase of the study (period two), during which patients could have received up to two additional botulinum toxin treatments, 4 months apart.

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Study participants

There were 537 patients enrolled into period one. Enrollment began in February 1999 and was completed in June 2000. Approval was obtained by the governing Institutional Review Board (IRB) at each study site prior to study initiation. The study design, purpose and potential risks of participation were discussed with each patient prior to enrollment and written informed consent was obtained. All aspects of this study complied with the Declaration of Helsinki recommendations regarding biomedical research involving human patients.

To be eligible for enrollment into period one, patients had to be between 18 and 75 years of age with glabellar lines of at least moderate severity at maximum frown. Severity was graded by the investigator using a 4-point scale (0 = none; 1 = mild; 2 = moderate; and 3 = severe), assisted by a photoguide showing severity examples on which all raters were trained prior to the initiation of the study. There was no requirement for a minimum severity rating for glabellar lines at rest, although this severity was also recorded. Prior botulinum toxin treatment was allowed. Patients also had to be medically stable, able to complete the entire study, and able to comply with study instructions. Key exclusion criteria included any disorder (such as myasthenia gravis, Eaton-Lambert syndrome) or agent (such as aminoglycoside antibiotics) that might interfere with neuromuscular function, or any other condition or situation that might put the patient at significant risk, confound the study results (such as significant pre-existing brow or eyelid ptosis), or interfere with the patient's participation in the study. Also excluded were individuals who had glabellar lines that were so severe that they could not be lessened by spreading them apart with the fingers, had a known allergy or sensitivity to any study component, had participated in another clinical study within 30 days of the study start date, were planning other facial cosmetic procedures during the study period, or were pregnant, breastfeeding, or planning a pregnancy during the study.

Patients were eligible for participation in period two if they successfully completed period one and had glabellar lines of at least mild severity ($\geq$ 1 on investigator-rated scale) during maximum frown at the end of period one. Patients also had to continue to meet all of the inclusion and exclusion criteria described for period one regarding concurrent medical conditions or treatments.

Patients could have been discontinued from the study at any time for adverse events or administrative reasons (inability to continue, lost to follow-up, or withdrawal of consent). All patients were instructed to continue their standard at-home facial skin care regimen, without change, throughout the study.

Randomisation/blinding

During period one, the randomisation schedule at each study center was stratified by age group ($\leq$ 50 years and $\geq$ 51 years). Within each age group stratum, patients were assigned in a ratio of three botulinum toxin patients to one placebo patient. Vials of botulinum toxin and placebo were identical, identified only by patient

number and study number, and required identical dilution and injection procedures. Only one patient was treated from each vial. To help maintain blinding, randomisation block size was not divulged to the physician investigators.

To further ensure adequate blinding, two evaluators co-assessed each patient on day 0, one of whom then performed the day 7 assessment while the other performed the day 30 assessment. This prevented the evaluation on day 30, the key time point, from being influenced by the patient's appearance on day 7.

Study protocol

During period one, patients received one intramuscular injection of either placebo or botulinum toxin. During period two, patients received two botulinum toxin injections, the first on day 0 (120 days after the start of period one) and the second on day 120 (240 days after the start of period one). Day 120 of period 1 was day 0 of period two. Thus, there was a maximum of three treatments administered 4 months apart throughout the study.

Study medication

During period one, all vials of study medication were masked as described above. Each vial of botulinum toxin type A (BOTOX®, Allergan, Inc, Irvine, CA, USA) contained 100 units (U) of botulinum toxin, 0.5 mg albumin (human), and 0.9 mg sodium chloride in a sterile, vacuum-dried form without preservatives. Each vial of placebo (BOTOX® vehicle) was identical to the botulinum toxin vials, except for the omission of the active ingredient, botulinum toxin. All vials were reconstituted with 2.5 ml of 0.9% sterile, preservative-free saline for a final dilution of 40 U/ml for the botulinum toxin vials.

During period two, all botulinum toxin vials were labeled as BOTOX® and were identical in content to botulinum toxin containing vials used in period one.

Injection procedure

On day 0 of period one, patients received intramuscular injections of either placebo or 20 U botulinum toxin at five injection sites in the glabellar area: two in each corrugator muscle bilaterally and one in the procerus muscle. Each injection was 0.1 ml, for a total injection volume of 0.5 ml. Investigators were instructed to place the single injection into the procerus muscle at the midline, and to inject each corrugator muscle at its inferomedial aspect, near the origin of the supratrochlear nerve, and superolaterally into the superior middle aspect of the muscle at least 1 cm above the bony orbital rim.

On days 0 and 120 of period two, patients received botulinum toxin injections identical to those administered to the botulinum toxin group during period one.

Outcome measures

Baseline characteristics

At the day 0 visit of period one, demographic characteristics, medical history, history of botulinum toxin use, medication history, and physician's assessment of glabellar line severity were recorded.

Efficacy

Physician's assessment

Physicians graded glabellar line severity at every visit, both at maximum frown and at rest, on an investigator rating scale of none (0), mild (1), moderate (2), or severe (3). A standardised photoguide, which gave photographic examples of each glabellar line severity grade both at maximum frown and at rest, was provided to each study center to help in the grading of the severity of glabellar lines across study sites.

Patient's global assessment of change

Patients graded the change in appearance of glabellar lines at every follow-up visit by responding to the question "How would you rate the change in the appearance of your glabellar lines compared with immediately before your injection?" The patient scored the change on a 9-point scale ranging from +4 (100% improvement) to 0 (no change) to –4 (100% worse).

Safety

Adverse events were monitored throughout the study and were rated by the physician for severity, seriousness, and relationship to study treatment. Severity was graded as mild (awareness, but tolerable), moderate (interferes with normal activity), or severe (incapacitating). Adverse events were considered serious if they were life-threatening or resulted in death, hospitalisation, or a persistent or significant disability/incapacity. Relationship to study treatment (none, possible, probable, or definite) was assessed by the presence or absence of another plausible cause and the temporal relationship of the adverse event to the administration of the study treatment.

Vital signs, standard hematology, and blood chemistry analysis were also assessed. All laboratory testing of blood samples was performed by Covance Central Laboratories Inc (Indianapolis, IN, USA), except analysis for the presence of serum antibodies to botulinum toxin type A, which was performed by Biological Test Center (BTC; Irvine, CA, USA).

Antibody testing

The presence of neutralising antibodies to botulinum toxin was assessed using the mouse protection assay (MPA)[18]. The assay was performed by mixing 2 ml of patient serum in a tube with 0.5 ml of botulinum toxin (50 U/ml) and incubating the mixture at 20 +/- 2° C for 1 hour. After incubation, a syringe was filled with 2 ml of the mixture and 0.5 ml injected into each of four mice. The MPA was considered negative (neutralising antibodies absent) if three or more mice died, positive (neutralising antibodies present) if three or more mice survived, and inconclusive if two mice died and two survived.

The MPA is currently considered the "gold standard" for detecting the presence of specific antibodies that neutralise the biological activity of botulinum toxin. An antibody-positive MPA test has been demonstrated to correlate well with a lack of clinical response to botulinum toxin injections and is considered highly specific for predicting treatment failure secondary to antibody resistance[19].

Timing of assessments

During period one, all efficacy variables and adverse events were assessed at baseline and at every follow-up visit (days 7, 30, 60, 90, and 120 postinjection). During period two, all efficacy variables and adverse events were assessed at 30, 60, 90, and 120 days following each injection. Vital signs were collected at baseline and at the end of period one, at every visit in period two, and at the end of period two. Standard hematology and blood chemistry assessments, and antibody testing were conducted on blood samples drawn on days 0 and 120 of period 1 and on days 120 and 240 of period two (or exit visit if exit occurred earlier).

Data analysis and statistics

During period one, efficacy analysis was intent-to-treat and included all patients who were randomised for treatment (placebo and botulinum toxin). The safety analysis included all randomised and treated patients.

Only those patients who received all three botulinum toxin treatments were analysed for between-treatment changes in efficacy across periods one and two. These patients had been in the botulinum toxin group during period one and received both botulinum toxin treatments during period two.

A subanalysis was performed on adverse event data by total number of botulinum toxin injections received (1, 2 or 3) during periods one and two.

All data were summarised with descriptive statistics and/or frequency tables. All statistical tests performed were two-sided. No interim analyses were performed.

Baseline characteristics

Continuous variables (age, time since first botulinum toxin treatment, time since last botulinum toxin treatment, frequency of botulinum toxin treatment, and most recent botulinum toxin dose) were summarised by sample size, mean, median, standard deviation, minimum, and maximum. Categorical or qualitative variables (race, sex, medical history, history of treatment for facial lines, and prior medication use) were summarised by frequency counts and percentages.

Efficacy measures

A frequency distribution of data at baseline and change from baseline data at each follow-up visit was generated for the physician's rating of glabellar line severity at maximum frown and at rest. A frequency distribution of the scores for the patient's assessment of change in glabellar line appearance was generated for each follow-up visit. The equality of the distributions of the botulinum toxin and placebo groups was evaluated using the exact Smirnov test. Two-sided, 95% confidence intervals for mean change from baseline for each treatment group were based on the *t*-distribution.

The responder rate for the physician's assessment of glabellar line severity at maximum frown was defined as the percentage of patients whose glabellar line severity changed from moderate or severe (2 or 3) at baseline to none or mild (0 or 1) at

follow-up. Although there was a baseline inclusion requirement for a maximum frown severity score of moderate to severe, there was no such requirement for the at-rest score. For this reason, the responder rate for the physician's assessment of glabellar line severity at rest was analysed only for the subgroup of patients who had a baseline glabellar line severity score of moderate or severe.

The responder rate for the patient's global assessment of change was defined as the percentage of patients who reported an improvement of ≥ +2 (moderate/definite improvement; ≈ 50%). For each measure, a Mantel Haenszel test stratified by age group (≤ 50 years and ≥ 51 years) was performed to evaluate the equality of the proportions of responders between treatment groups. Relative risks and two-sided 95% confidence intervals were calculated. For each age group and each study center, simple relative risk estimates were calculated.

Missing data in each of the three studies that comprised this report (two double-blind and one open-label) were replaced by the mean of all non-missing data at the appropriate visit in that study. Missing data were imputed only for patients injected in the individual treatment cycle.

Safety measures

The Fisher's exact test was performed to test for between-group differences in adverse events. For laboratory variables, blood pressure, and heart rate, the Wilcoxon signed-rank test was performed for within-group analyses and the Wilcoxon rank-sum test was used for between-group analyses of data at exit.

Determination of sample size

A total sample size of 200 patients entering period one was required to have an 85% chance of detecting a 25 percentage point difference (specifically 50% versus 25%) between the treatment groups in the proportion of patients with a patient's global assessment score ≥ +2, significant at the 0.05 level. This sample size also gave a > 85% chance of detecting a 30 % difference between treatment groups in the proportion of patients with a physician's assessment of none or mild at maximum frown, also significant at the 0.05 level.

Results

Patient population

Prior botulinum toxin use for facial lines was reported in 14.3% (58/405) of those who received botulinum toxin in period one and in 13.0% (17/131) of those who received placebo in period one among the patients. Among these patients, the mean interval since the prior botulinum toxin treatment was 9 months, the mean interval between prior botulinum toxin treatments was 5.8 months, and the mean dose of the most recent treatment was 26.3 U. There were no statistically significant differences in patient demographic characteristics in period one between the botulinum toxin group and the placebo group (Table 1). The flow of patients through periods one and two is presented in Figure 1 and Table 2.

Table 1. Patient demographics (all patients entering period one)

	Botulinum toxin group n = 405		*Placebo group n = 132*		*Total patients n = 537*	
Mean age in years	46.2		45.5		46.0	
Sex	*Number*	*Percentage*	*Number*	*Percentage*	*Number*	*Percentage*
Female	334	(82.5%)	106	(80.3%)	440	(81.9%)
Male	71	(17.5%)	26	(19.7%)	97	(18.1%)
Race						
Caucasian	341	(84.2%)	109	(82.6%)	450	(83.8%)
Black	21	(5.2%)	7	(5.3%)	28	(5.2%)
Asian	9	(2.2%)	4	(3.0%)	13	(2.4%)
Hispanic	30	(7.4%)	11	(8.3%)	41	(7.6%)
Other	4	(1.0%)	1	(0.8%)	5	(0.9%)

Figure 1. Study profile

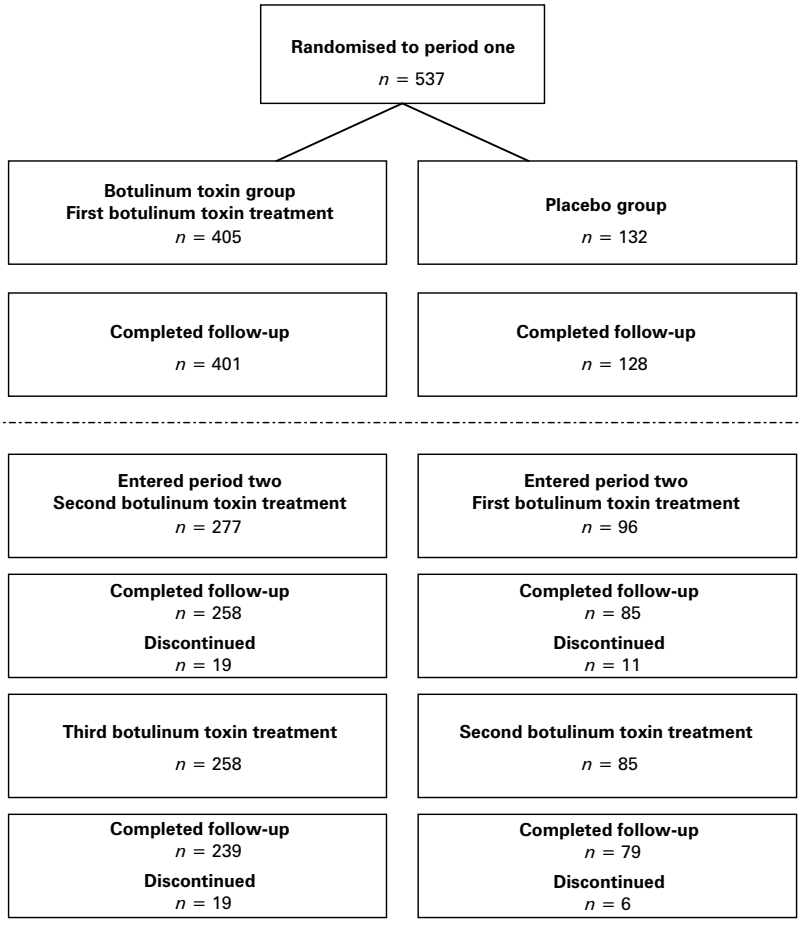


Table 2. Summary of patient exit status

Period one

	Botulinum toxin group	*Placebo group*
Enrolled	405	132
Discontinued		
Lost to follow-up	2	1
Personal reasons	1	2
Other*	2	2
Completed	401	128

Period two

	From the botulinum toxin group	*From the placebo group*
Enrolled	277	96
Discontinued		
Adverse events[a]	2	0
Lost to follow-up	14	7
Personal reasons	16	9
Glabellar line severity < mild	3	1
Other[b]	3	0
Completed	239	79

[a] Unrelated to study treatment.

[b] Other includes relocation, scheduling confusion.

A total of 537 patients (botulinum toxin: 405; placebo: 132) were enrolled in period one. The majority of the subjects were Caucasian (450/537; 84%) and female (440/537; 82%). During period one, four patients withdrew from the botulinum toxin group (2 were lost to follow-up, 1 moved from area, and 1 withdrew for personal reasons) and 4 patients withdrew from the placebo group (1 was lost to follow-up, 2 withdrew for personal reasons, and 1 was enrolled in violation of exclusion criteria and not treated).

Of the 529 patients who completed period one, 72 patients were not offered enrollment into period two for reasons detailed in Table 3. Of the 457 eligible for period two, 84 chose not to participate, and the majority of these gave no reason. Only one patient declined further study participation because of dissatisfaction with the effect of botulinum toxin in the previous study.

The patients receiving the second and third botulinum toxin treatments are those who completed 120 days of double-blind treatment, had glabellar lines of at least mild severity at maximum frown, and were willing and able to complete the entire

Table 3. Enrollment disposition of 529 patients who completed period one

Reason not offered enrollment into period two	
From study centers that elected not to participate in period two	38
Failed to meet exclusion/inclusion criteria	9
Moved from the study area	4
Exited preceding study after open-label study enrollment cut-off	21
Total	72/529 (14%)
Reason declined enrollment into period two	
Gave no reason for their decision	57
Too busy	12
Wanted to participate in an excluded activity (e.g. pregnancy, elective surgery, participation in a different study)	5
No compensation was offered	8
Did not like the effects of botulinum toxin during the previous study	1
Concerns about adverse effects	1
Total	84/457 (18%)

course of the study. A total of 373 patients (277 from the botulinum toxin group and 96 from the placebo group) entered period two and 318 completed both periods one and two. The patient population was predominantly Caucasian (317/373; 85%) and female (315/373; 84.5%). During period two, 55 patients withdrew from the study prior to the final follow-up visit. Most of the patients who withdrew left due to administrative reasons, but four patients did not receive a second injection because their glabellar lines had not returned to at least mild severity and consequently did not qualify for a second injection. Of the 373 patients who entered period two, 11 patients received only one botulinum toxin treatment, 104 received two botulinum toxin treatments, and 258 received all three botulinum toxin treatments.

Efficacy

Physician's assessment at maximum frown

Period one

At baseline, the mean scores for the physician assessment at maximum frown were nearly identical in the botulinum toxin and placebo groups (botulinum toxin: 2.59; placebo: 2.58). Following treatment, the decrease in mean score was significantly greater in the botulinum toxin group than the placebo group at every follow-up visit ($p<0.001$). The mean score in the botulinum toxin group fell by more than 1.5 grades to 1.02 (mild severity) on day 7, and was 0.87 on day 30. Mean scores in the botulinum toxin group gradually increased throughout the remainder of period one, but remained below 2 (moderate severity) on day 120. In contrast, mean scores in the placebo group never fell below 2.5 (moderate to severe).

The responder rate for the physician's assessment at maximum frown (percentage of patients with a rating of none or mild at follow-up) was significantly greater for the botulinum toxin group than for the placebo group at all time points in period one ($p<0.001$; Figure 2a). In the botulinum toxin group, the responder rate was 73.8% (299/405) on day 7, and peaked at 80.2% (325/405) on day 30. The responder rate gradually declined during the rest of period one, but slightly more than 25% of patients were still rated as responders at day 120. The responder rates in the placebo group remained at or below 6.1% throughout period one.

Across periods one and two

For patients who received all three botulinum toxin treatments, there was progressive improvement in the treatment response rate for the physician's assessment at maximum frown (from treatment 1 to treatment 3; Figure 2b), though the magnitude of improvement was similar across treatment periods. Responder rates were significantly ($p \leq 0.002$) higher after the third treatment than after the first treatment at days 30, 60, and 90. Significant progressive improvements were also seen between treatments at other time points.

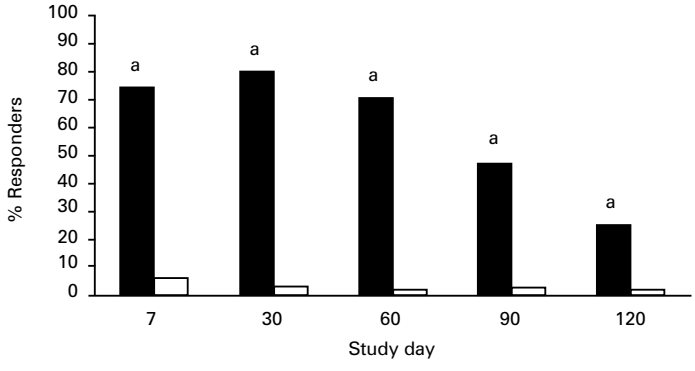
Physician's assessment at rest

Period one

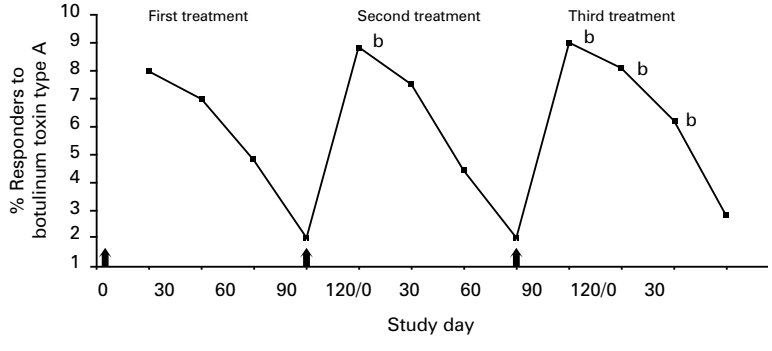
The mean baseline scores for the physician's assessment at rest for all patients were similar in the botulinum

Figure 2. Physician's assessment of glabellar line severity at maximum frown. Responder rates (percentage of patients with a rating of none or mild at follow-up). (a) Period one. (b) Periods one and two. Only the results from the 258 patients receiving all three botulinum toxin treatments are illustrated. ■, botulinum toxin type A; □, vehicle.

a. Period one



b. Periods one and two



[a] Indicates significantly different from vehicle ($p < 0.001$).

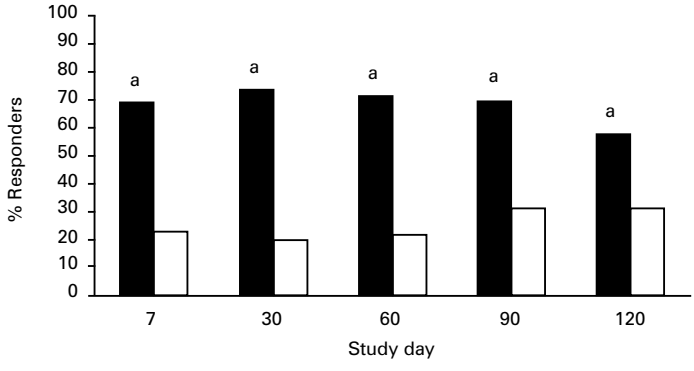
[b] Indicates that the value is significantly greater than the value at the same time point after one or both previous botulinum toxin treatments ($p \leq 0.028$).

Arrow indicates time of botulinum toxin treatment.

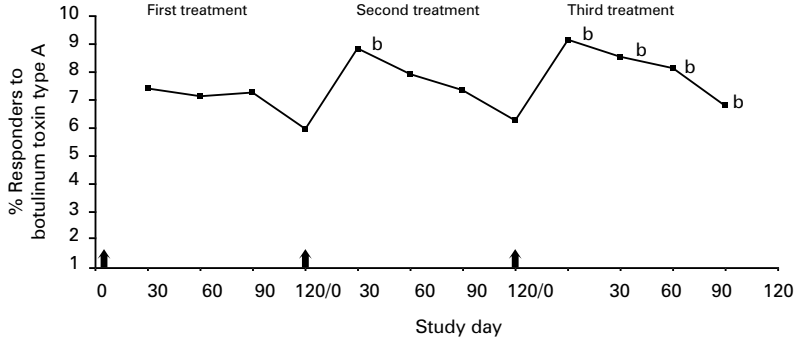
toxin (n = 405) and placebo (n = 132) groups (botulinum toxin: 1.32; placebo: 1.29). Following treatment, the decrease in mean score was significantly greater in the botulinum toxin group than in the placebo group at every follow-up visit (p<0.001). The mean score in the botulinum toxin group fell to 0.69 on day 7 and to 0.58 on days 30 and 60. Mean scores in the botulinum toxin group gradually increased throughout the remainder of period one to 0.67 on day 90 and to 0.83 on day 120 but remained below 1 (mild severity). In contrast, the mean score in the placebo group stayed at or above 1.13 through days 7, 30, 60, and 120.

Figure 3. Physician's assessment of glabellar line severity at rest. Responder rates (percentage of patients with a rating of moderate or severe at baseline and none or mild at follow-up). (a) Period one. (b) Periods one and two. Only the results from the 106 patients receiving all three botulinum toxin treatments and whose glabellar lines were moderate or severe at rest at baseline are illustrated. ■, botulinum toxin type A; □, vehicle.

a. Period one



b. Periods one and two



[a] Indicates significantly different from vehicle ($p < 0.007$).

[b] Indicates that the value is significantly greater than the value at the same time point after one or both previous botulinum toxin treatments ($p < 0.007$).

Arrow indicates time of botulinum toxin treatment.

The subgroup of patients with moderate or severe glabellar lines at baseline also had similar mean scores for the physician's assessment at rest in the botulinum toxin ($n = 161$) and placebo ($n = 49$) groups (botulinum toxin: 2.19; placebo: 2.16).

Following treatment, the decrease in mean score was significantly greater in the botulinum toxin group than in the placebo group at every follow-up visit ($p<0.001$). The mean score in the botulinum toxin group fell by more than 1.0 grade to 1.18 on

day 7, and to 1.07 (mild severity) on day 30. Mean scores in the botulinum toxin group gradually increased throughout the remainder of period one to 1.36 (mild to moderate severity) on day 120. In contrast, the mean score in the placebo group stayed at or above 1.85 through day 60 and was 1.69 on day 120.

The responder rate for the physician's assessment at rest (percentage of patients with a rating of moderate or severe at baseline and none or mild at follow-up) was determined for the subset of patients with baseline scores at rest of moderate or severe. In these patients, the responder rate was significantly greater for the botulinum toxin group than for the placebo group at all time points in period one ($p<0.007$; Figure 3a). In the botulinum toxin group, the responder rate was 68.3% (110/161) on day 7 and peaked at 73.9% (119/161) on day 30. Responder rates in the botulinum toxin group declined slightly during the remainder of period one, but the majority of patients (59%; 95/161) were still rated as responders at day 120. The responder rates in the placebo group remained below 35% throughout period one.

Across periods one and two

For patients who received all three botulinum toxin treatments, there was progressive improvement in the treatment response rate for the physician's assessment at rest from treatment one to treatment third (Figure 3b), though the magnitude of improvement was similar across treatment periods. The responder rate was significantly ($p<0.007$) higher after the third treatment than after the first treatment at days 30, 60, 90, and 120. Significant progressive improvements were also seen between treatments at other time points.

Patient's global assessment

Period one

The mean scores for the patient's global assessment were significantly greater in the botulinum toxin group than the placebo group at every follow-up visit ($p<0.001$). The mean score in the botulinum toxin group was 2.70 on day 7 and rose to 2.99 on day 30 (≈ 75% improvement). The mean score in the botulinum toxin group was 1.95 (≈ 50% improvement) on day 90 and 1.26 (≈ 25% improvement) on day 120. In contrast, mean scores in the placebo group never exceeded 0.31 at any time during period one.

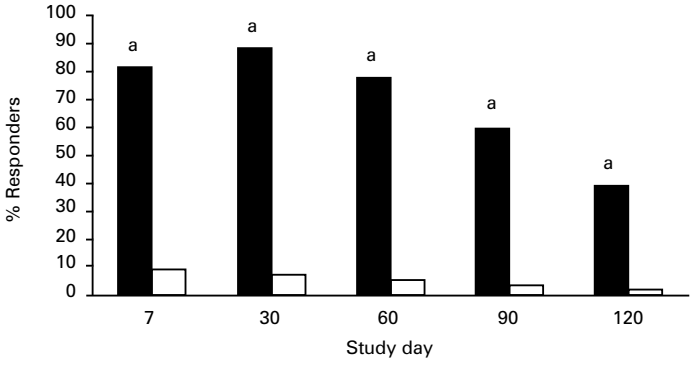
The responder rate for the patient's global assessment (percentage of patients reporting moderate or greater improvement; change in score ≥ +2) was significantly greater for the botulinum toxin group than for the placebo group in period one ($p<0.001$; Figure 4a). The responder rate was 82.5% (334/405) at day 7 and peaked just below 90% (89.4%; 362/405) at day 30. Responder rates in the botulinum toxin group gradually declined throughout the remainder of period one, but 39% of patients were still rated as responders at day 120. In the placebo group, the responder rate was <10% at all assessments.

Across periods one and two

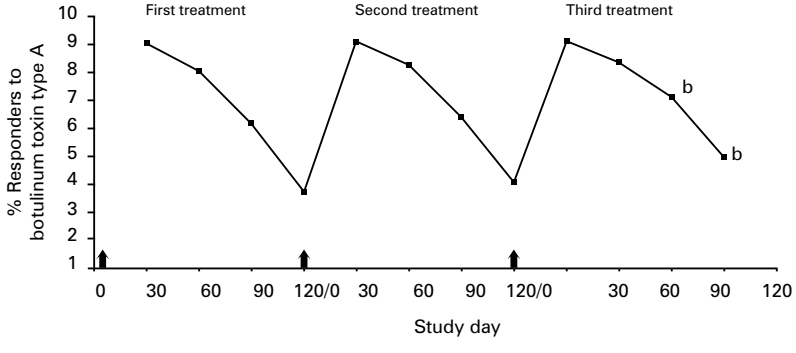
For patients who received all three botulinum toxin treatments, the

Figure 4. Patient's global assessment of glabellar line severity. Responder rates (percentage of patients reporting moderate or greater improvement; score ≥ +2). (a) Period one. (b) Periods one and two. Only the results from patients receiving all three botulinum toxin treatments are illustrated. ■, botulinum toxin type A; □, vehicle.

a. Period one



b. Periods one and two



[a] Indicates significantly different from vehicle ($p<0.001$).

[b] Indicates that the value is significantly greater than the value at the same time point after one or both previous botulinum toxin treatments ($p<0.005$).

Arrow indicates time of botulinum toxin treatment.

responder rates following each successive treatment were similar to or greater than those seen after the first treatment (Figure 4b), though the magnitude of improvement was similar across treatment periods. The responder rate was significantly ($p<0.005$) higher after the third treatment than after the first and second treatments at days 90 and 120. The proportion of patients rated as responders at day 30 after all three treatments was 79.8%.

Table 4. Most common[a] adverse events during period one regardless of causality

Adverse event	*Botulinum toxin (n = 405)*	*Placebo (n = 132)*	*p value*
Headache	13.3%	17.7%	0.250
Respiratory infection	3.5%	3.8%	0.789
Blepharoptosis	3.2%	0.0%	0.045
Facial pain	2.2%	0.8%	0.464
Flu syndrome	2.0%	1.5%	>0.999
Nausea	3.0%	2.3%	>0.999
Muscle weakness	2.0%	0.0%	0.209
Ecchymosis	1.7%	2.3%	0.711
Injection site edema	1.5%	2.3%	0.459
Back pain	1.0%	2.3%	0.369
Acne	0.5%	3.1%	0.033
Diarrhea	0.5%	2.3%	0.095

[a] Incidence ≥ 2% in any group.

Safety

Adverse events

Botulinum toxin treatment was well tolerated. Across periods one and two, 12 patients experienced 19 serious adverse events, none of which was considered to be related to study medication. During period one, no patients discontinued due to adverse events. During period two, two patients from the botulinum toxin group were discontinued due to adverse events, neither of which was considered related to the study medication (one patient was diagnosed with breast cancer and one had an unplanned pregnancy and delivered a healthy baby at term after study exit).

During period one, the most common adverse events (regardless of causality) were headache, respiratory infection, and blepharoptosis (Table 4). Only blepharoptosis occurred with significantly greater frequency in the botulinum toxin group than in the placebo group (p=0.045) and was considered to be related to botulinum toxin treatment. Headache, which occurred with similar frequencies in the botulinum toxin and placebo groups, was considered to be related to the injection procedure rather than to the specific study medication.

Across periods one and two, the most common treatment-related adverse events were headache and blepharoptosis (Table 5), both of which decreased markedly in frequency with repeated treatment. The incidence of headache was 8.2%, 0.6%, and 0.8% after the first, second, and third treatments, respectively, and the incidence of blepharoptosis was 3.0%, 2.2%, and 0.8% after the first, second, and third treatments, respectively.

Overall, a total of 23 patients (4.6%) experienced blepharoptosis at some point during either period one or two. Ptosis

Table 5. Most common[a] treatment-related adverse events across periods one and two

Adverse event	*1st Botulinum toxin (n=501)*	*2nd Botulinum toxin (n=362)*	*3rd Botulinum toxin (n=258)*
Headache	8.2%	0.6%	0.8%
Blepharoptosis	3.0%	2.2%	0.8%
Face pain	1.8%	0.0%	0.0%
Edema at injection site	1.4%	0.8%	0.4%
Pain at injection site	1.4%	0.8%	0.0%
Nausea	1.2%	0.0%	0.0%
Ecchymosis	1.2%	0.0%	0.0%
Muscle weakness	1.4%	0.3%	0.0%
Erythema	1.6%	0.0%	0.0%

[a] Incidence >1% in any treatment cycle.

was unilateral in 20 patients and bilateral in three patients. Of the 26 eyes affected, ptosis was considered mild in severity for 19 (73.1%) with an average duration of 27 days (range 2 to 55 days), and moderate for 7 (26.9%), with an average duration of 29 days (range 7 to 60 days). No patients reported severe ptosis. The majority of the reports of ptosis (14/23; 61%) occurred after the first treatment. Among the 23 patients who experienced ptosis, 13 received three botulinum toxin treatments, four received two botulinum toxin treatments, and six received one botulinum toxin treatment. Only one patient reported ptosis after more than one botulinum toxin treatment. Although this patient reported ptosis throughout the entire study, a diagnosis of ptosis was not supported by physical examination or review of facial photographs. All the other cases resolved without sequelae.

Laboratory values and vital signs

Although there were statistically significant changes for many laboratory variables during both periods one and two, the mean changes were small and not clinically relevant. There were no consistent, clinically significant changes in laboratory values or vital signs.

Neutralising antibodies

Serum samples were collected on days 0 and 120 of period one and day 120 (prior to injection) of period two, and at study exit (day 240 of period two or earlier). Of the 258 patients who received all three botulinum toxin treatments, 159 patients had antibody-evaluable samples at the beginning of period one and at the end of period two. At the end of period two, none of the 159 tested positive for neutralising antibodies, including the patients who at the beginning of period one had positive samples (3) or inconclusive samples (8) (Table 5). Of the botulinum toxin patients, 283 (70%) had antibody-evaluable pretreatment and posttreatment samples at the end of the double-blind treatment period. Of these, 88% (248/283) tested negative at both time points. Four patients

Table 5. Antibody results of patients who received three botulinum toxin treatments and had analysable samples

Pretreatment samples (Day 0 of double-blind studies)	*Posttreatment samples (Day 240 of open-label study)*		
	Positive	Negative	Inconclusive
Positive	0	3	0
Negative	0	141	7
Inconclusive	0	8	0

(1.4%) tested positive at one or more time points, but all four patients were considered responders.

Of 373 patients who received the first injection in the open-label treatment phase, 241 (65%) had antibody-evaluable pretreatment and posttreatment samples. Of these, 90% (216/241) tested negative at both time points. Four patients (1.7%) tested positive at either the pretreatment or the posttreatment time point, but again, all four patients were considered responders.

Of 343 patients who received a second injection in the open-label phase, 184 (54%) had antibody-evaluable pretreatment (day 0) and posttreatment (treatment cycles one and two) samples. Ninety percent (166/184) tested negative at both pretreatment and posttreatment assays in treatment cycle two, and two (1.1%) patients tested positive at either time point. Again, both patients were considered responders.

Discussion

The present study demonstrated that three treatments of botulinum toxin over 1 year were exceptionally effective and safe for the treatment of glabellar lines, in this large patient population. Progressive improvements in efficacy were observed following additional botulinum toxin treatments, while the incidence of treatment-related adverse effects declined.

Botulinum toxin was significantly more effective than placebo in reducing glabellar line severity, as assessed by both physicians and patients at all postinjection visits in the two 4-month, double-blind, placebo-controlled studies that comprised period one of the present study[16,17]. Our findings reinforce the results from a smaller, placebo-controlled study, which demonstrated that low doses of botulinum toxin effectively reduced glabellar lines[20].

Dramatic improvement in glabellar line severity, seen for the majority of patients as early as 7 days posttreatment, peaked at day 30, and remained well above baseline at day 120. Progressive improvement in response rates was observed in those patients who received two or three treatments, though the magnitude of response was similar across treatment periods. Significantly more patients were rated as responders after the second botulinum toxin treatment than after the first, and still more were responders after the third treatment than after the second.

The most dramatic demonstration of progressive improvement was noted in the physician's assessment of glabellar lines at rest. Among patients who received three botulinum toxin treatments, the responder rate at posttreatment day 30 increased from 74.5% after a single treatment to 90.6% after three treatments. Similarly, the responder rate at day 90 increased from 73.6% after one treatment to 85.8% after three treatments, and the rate at day 120 increased from 60.4% to 74.5%. These results demonstrate that a larger number of patients achieved a response with repeated treatment and suggest an increased duration of benefit with repeated treatment. This finding supports published observations that the benefits of aesthetic botulinum toxin treatments can last 7 months or longer in patients who have received previous botulinum toxin treatments for the same condition[21].

The duration of benefit at rest even after a single treatment is remarkable, since the subset of patients analysed for this measurement had resting scores of moderate to severe at baseline. Improvements in the appearance of frown lines at rest outlast the inhibitive effect of botulinum toxin on the ability of patients to actively frown.

While this study demonstrated a progressive improvement in efficacy with repeated botulinum toxin treatment, the frequency of adverse effects, particularly headache and blepharoptosis, declined with each treatment. Of the 84 patients who elected not to continue in the open-label period, most (57 patients) gave no reason, with one patient citing worry about the side effects of treatment. Of the 55 patients who discontinued the open-label phase of the trial, two were for adverse effects not related to treatment.

The declining incidence of adverse events may be due to an increase in the skill of the physician investigator as he or she gains experience in the administration of botulinum toxin. Subtle changes in injection site placement or depth may affect the development of ptosis. In addition, repeated treatment of an individual patient may give a physician additional insight into the best way to treat that patient. Finally, there is the possibility that tolerance to certain adverse events may develop over time.

The double-blind phase of the present study also supports previous findings that botulinum toxin treatment for glabellar lines is very safe[21–23]. The only adverse event that occurred more frequently in the botulinum toxin group than in the placebo group was blepharoptosis, which was infrequent (occurring in 3.2% of patients), mild to moderate in severity, transient, and reduced with subsequent injections.

In the present study, no patient tested positive for neutralising antibodies to botulinum toxin after three successive botulinum toxin treatments. Four patients who tested positive on the mouse protection assay prior to any treatment had negative results after 1 year of treatment. This supports the belief that the low and infrequent botulinum toxin doses used in facial aesthetics are highly unlikely to cause

the development of anti-botulinum toxin antibodies, even after repeated treatments. It is also possible that the risk of antibody development in the typical clinical practice may be even lower than was observed in this study, because the treatment interval in the present study was kept fixed at 4 months. Many patients in clinical practice exhibit a longer duration of benefit and have longer intertreatment intervals[21]. This could bring the risk of antibody formation down even further, as the relationship between botulinum toxin antibody formation and treatment interval is well known[15]. The MPA is the most widely used assay for detecting botulinum toxin antibodies and is the test specified by the US Food and Drug Administration (FDA), the Centers for Disease Control and Prevention, and the United States military. Its specificity for predicting clinical responses or treatment failures due to resistance is relatively high[19].

In conclusion, the present study demonstrates that botulinum toxin treatment for glabellar lines is remarkably effective, safe, and well tolerated. Furthermore, patients treated with botulinum toxin over the long-term showed a progressive improvement in efficacy with repeated injection, which is likely to result in increased injection intervals in clinical practice. The benefits of botulinum toxin treatment were also maintained through repeated injection cycles, supporting its use in long-term therapy. In addition, the overall incidence of adverse events was low, with the incidence of headache and blepharoptosis decreasing with repeated treatment to under 1% after the third treatment. Finally, the results reported in this study are based specifically on the BOTOX® formulation of botulinum toxin and cannot be generalised to other formulations of botulinum toxin or to other botulinum toxin serotypes.

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