STUDY

Efficacy and Safety of 3- and 5-Injection Patterns (30 and 50 U) of Botulinum Toxin A (Dysport) for the Treatment of Wrinkles in the Glabella and the Central Forehead Region

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Objective: To investigate the efficacy and safety of 2 injection site patterns (3- and 5-injection patterns [30 and 50 U]) of botulinum toxin A (Dysport; Ipsen Pharma, Ettlingen, Germany), in the treatment of glabellar and central forehead wrinkles.

Design: Multicenter, double-blind, placebo-controlled, randomized, 16-week trial.

Setting: Twenty-three German dermatologic centers.

Patients: Two hundred twenty-one patients with moderate or severe glabellar wrinkles when frowning maximally.

Intervention: Centers were randomly assigned to the 3-injection site pattern (3 injections of 10 U of botulinum toxin A or placebo) or 5-injection site pattern (5 injections of 10 U of botulinum toxin A or placebo). All centers used 3 sites in the procerus and corrugator muscles; the 2 additional sites were approximately 1-cm cranial from the corrugator sites.

Main Outcome Measure: Wrinkle severity was graded by 4 independent experts blinded to the treatment received using digital photographs and a standardized clinical scale (range, 0 [no wrinkles] to 3 [severe wrinkles]). A reduction of at least 1 point between weeks 0 and 4 was considered a therapeutic success (responder).

Result: One hundred ten patients (73 receiving botulinum toxin A vs 37 receiving placebo) received 3 injections; 111 patients (73 receiving botulinum toxin A vs 38 receiving placebo) received 5 injections. After 4 weeks, the proportions of responders were 86.1% vs 18.9% and 86.3% vs 7.9%, respectively ($P$/H11021$.001 for both). No major adverse effects were observed.

Conclusions: The 3 central injection sites are essential for the treatment of glabellar wrinkles. The 2 additional injection sites in the forehead region, targeting the frontalis muscle, did not significantly improve efficacy.

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OTULINUM TOXIN A HAS BEEN used successfully for esthetic purposes for almost 2 decades, although only a few clinical trials supporting its use have been published so far, and most of the evidence is in the form of case series. Dysport (Ipsen Pharma, Ettlingen, Germany), the brand of botulinum toxin A used in this study, has been available for more than a decade in Europe and is registered for various neurological indications. The product has been used “off label” in dermatology, a field in which there is considerable practical experience with its cosmetic use but few controlled trials reporting its efficacy and safety to date.

Botulinum toxin A is injected into the glabellar area, targeting the corrugator, procerus, and part of the frontalis muscles. The number and distribution of the injection sites, as well as the dose, vary among studies, with a minimum of 3 injection sites covering the corrugator and procerus muscles. An additional 2 sites may be injected laterally in the medial part of the corrugator muscles (shaped like a flying bird) or cranially in part of the frontalis muscle (shaped like an upside-down house), as in the present study. Because the muscles in this area are interwoven, 1 injection point might target several muscles.

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To our knowledge, this study represents the largest multicenter trial of Dysport for an esthetic indication to date. The objective of this study was to evaluate the efficacy and safety of 2 injection site patterns for botulinum toxin A in the treat-
ment of wrinkles of the glabella and the central forehead area. Both injection site patterns covered 3 central sites; one injection site pattern was limited to these 3 sites (3 injections of 10 U of botulinum toxin A or placebo), and another injection site pattern included 2 other more cranial sites (5 injections of 10 U of botulinum toxin A or placebo).

### METHODS

#### PATIENTS

Patients included in the study were aged 18 to 75 years; had moderate or severe vertical or diagonal glabellar wrinkles (scores of 2 or 3 on a standardized 4-point clinical scale ranging from 0 [no wrinkles] to 3 [severe wrinkles]) at maximum frown; and had mild, moderate, or severe (scores of 1, 2, or 3) vertical or diagonal glabellar wrinkles at rest. Women of childbearing potential with a negative pregnancy test result before enrollment in the study were included. All patients gave written informed consent.

#### STUDY DESIGN

This was a double-blind, placebo-controlled, randomized, 16-week trial. It was conducted in 23 dermatologic centers in Germany.

Centers were allocated randomly to 1 of 2 study arms: centers in study arm 1 treated wrinkles via 3 injection sites, and centers in study arm 2 treated wrinkles via 5 injection sites. Within each center, patients were randomized 2:1 to receive botulinum toxin A or placebo.

#### INTERVENTIONS

Each vial of 500 U of botulinum toxin A (Dysport) was diluted with 2.5 mL of saline. An injection volume of 0.05 mL containing 10 U of botulinum toxin A was used at each injection point, giving a total botulinum toxin A dose per patient of 30 U or 50 U, depending on study arm enrollment. An injection volume of 0.05 mL of saline was used for placebo injections. (The units of the 2 botulinum toxin A preparations on the market, Dysport and Botox [Pharm Allergan, Ettlingen, Germany], are not equivalent. In dermatologic practice, 3 U of Dysport is generally accepted as being equal to 1 U of Botox, and this ratio has recently been confirmed for use in the treatment of dystonia by a Cochrane systematic review.10)

In both study arms, 3 injection sites covered the medial parts of the corrugator muscles and parts of the procerus muscle. In study arm 2, there were 2 additional cranial sites covering part of the frontalis muscle (Figure 1).

#### ASSESSMENTS AND CLINICAL OUTCOME MEASURES

Assessments were performed at weeks 0, 2, 4, 12, and 16. The main efficacy assessments were based on a 4-point standardized clinical scale (0, no wrinkles; 1, mild wrinkles; 2, moderate wrinkles; and 3, severe wrinkles) that has been shown to have good to excellent reproducibility. Unlike other studies11-13 that have used 2 separate scales, only 1 scale was used to grade the wrinkles at maximum frown and at rest in this study.

The key evaluations were performed, using standardized digital photographs, by a committee of 4 of us (B.A., A.F., G.D.M., and S.T.) who had not been involved in the daily protocol of the study. The method used has been described elsewhere.11

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**Figure 1.** Injection sites (shown here on a cadaver) for 3-injection (A) and 5-injection (B) patterns of botulinum toxin A (Dysport; Ipsen Pharma, Ettlingen, Germany). Placebo was also injected into these sites.
A validated system was established to prevent tampering of the digital images before their transfer to the database and was monitored throughout the study. All patients were evaluated by another investigator among us during study visits.

**PRIMARY EFFICACY CRITERION**

A reduction of at least 1 point (as assessed by the committee) between weeks 0 and 4 for glabellar wrinkles at maximum frown was considered a therapeutic success (the patient was classified as being a responder). The number (rate) of responders was considered the primary efficacy criterion.

**SECONDARY EFFICACY CRITERIA**

The secondary efficacy criteria were the following: (1) the scores at maximum frown (evaluated by the committee) at weeks 0, 2, 4, 12, and 16 (data not shown); (2) the scores at rest (evaluated by the committee) at weeks 0, 2, 4, 12, and 16; (3) the scores at maximum frown and at rest (evaluated by the investigator) at weeks 0, 2, 4, 12, and 16 (data not shown); (4) the subjective assessment of improvement since the first visit (evaluated by the patient) at weeks 0, 2, 4, 12, and 16 (data not shown); and (5) the assessment of patients’ global satisfaction with the treatment at week 16.

**STATISTICAL ANALYSIS**

The primary analysis was based on the intention-to-treat data set. Differences between treatment groups for the primary efficacy criterion were tested using the Fisher exact test (2-sided) with \( \alpha = .05 \) in each study arm. Differences between treatment groups for the secondary efficacy criteria were tested on an exploratory basis. Based on the data set, the Fisher exact test or Cochran-Mantel-Haenszel test with table scores was used.

**RESULTS**

**PATIENT CHARACTERISTICS**

All 221 patients who were randomized into the study received study medication. One hundred ten patients (73 patients received 3 injections of 10 U of botulinum toxin A, and 37 patients received 3 injections of placebo) were included in study arm 1, and 111 patients (73 patients received 5 injections of 10 U of botulinum toxin A, and 38 patients received 5 injections of placebo) were included in study arm 2. All but 1 patient (in study arm 1) were included in the intention-to-treat analysis.

Women composed 89.9% (98/109) of the patients in study arm 1 and 90.1% (100/111) in study arm 2. The mean ± SD ages were 46.6 ± 9.2 years in study arm 1 and 46.4 ± 8.1 years in study arm 2. Only 1 patient, in study arm 2, was not white. There were no differences at baseline between the botulinum toxin A–treated and the placebo groups (data not shown). There were also no large differences in the mean wrinkle severity scores recorded by the committee vs those recorded by the investigator (data not shown).

**EFFICACY**

**Study Arm 1**

The responder rates at maximum frown at week 4 (primary efficacy criterion) based on the evaluation by the committee were 86.1% (62 of 72 patients) for 3 injections of botulinum toxin A and 18.9% (7 of 37 patients) for 3 injections of placebo. This difference was statistically significant (\( P < .001 \)) (Table 1, Table 2, and Figure 2). At week 16, 42 (61.8%) of 68 patients in the botulinum toxin A–treated group were at least moderately satisfied with the treatment. The remaining 26 patients (38.2%) in the botulinum toxin A–treated group and all patients in the placebo group were dissatisfied (\( P < .001 \), Fisher exact test) (Table 3).

**Study Arm 2**

The responder rates at maximum frown at week 4 based on the evaluation by the committee were 86.3% (63 of 73 patients) for 5 injections of botulinum toxin A and 7.9% (3 of 38 patients) for 5 injections of placebo. The difference between groups was statistically significant (\( P < .001 \)) (Table 4, Table 5, and Figure 3). At week 16, 49 (67.1%) of 73 patients in the botulinum toxin...
A–treated group were at least moderately satisfied with the treatment. The remaining 24 patients (32.9%) in the botulinum toxin A–treated group and all but 2 patients in the placebo group were dissatisfied ($P < .001$, Fisher exact test) (Table 6).

**SAFETY**

Only 1 serious adverse event occurred in the trial (acute depression after a bereavement in a patient in study arm 2), and this was not considered to be related to the study medication. Adverse events in which a causal relationship to the study drug could not be excluded occurred in 3 patients treated with 3 injections of botulinum toxin A (hypoesthesia, injection site discomfort, subjectively heavy eyelids, and Spock eyebrow), in 2 patients receiving placebo in the same study arm (headache and pyrexia), in 8 patients treated with 5 injections of botulinum toxin A (4 patients experienced headache, and 4 other patients experienced Spock eyebrow, hoarseness, dizziness, and eyelid ptosis), and in 4 patients receiving placebo in this study arm (headache, dizziness, blepharochalasis, and swollen face). Except for the case of Spock eyebrow in study arm 2, which was rated as moderate, all adverse events were considered to be mild. Only 1 case of eyelid ptosis was reported (in the group receiving 5 injections of botulinum toxin A). Ptosis of the right eyelid started 13 days after the injections, was considered to be mild, had improved by week 4, and was not visible by week 12. No treatment was required.

*Data are given as number (percentage) unless otherwise indicated.
†0 Indicates no wrinkles; 1, mild wrinkles; 2, moderate wrinkles; and 3, severe wrinkles.
This was not a simple dose-ranging trial to evaluate different doses, as in the study performed by Ascher et al, but rather a study of the number and distribution of injection sites. One injection site pattern (3 injections of 10 U of botulinum toxin A) involved only the glabella; the other injection site pattern (5 injections of 10 U of...
botulinum toxin A) involved the glabella and 2 additional cranial sites in the central forehead. The addition of these 2 sites targeting the frontalis muscle did not increase the efficacy. However, only wrinkles in the glabellar area were evaluated in this trial, and we cannot rule out the possibility that evaluation of central forehead wrinkles would have shown the 5-injection site pattern to be more effective.

Based on our results, 3 injections of 10 U of botulinum toxin A might be a sufficient dosage for the glabellar region for most patients. Other more lateral sites or an increased dosage might further improve the efficacy, as shown by Ascher et al for 25, 50, and 75 U total of botulinum toxin A.

Although patients’ satisfaction was generally good, 29.4% (in the group receiving 3 injections of botulinum toxin A) and 23.3% (in the group receiving 5 injections of botulinum toxin A) of patients reported only moderate satisfaction. Even for the group receiving 5 injections of botulinum toxin A, which showed a smaller proportion of moderately satisfied patients, this level of satisfaction might not be acceptable for this esthetic procedure. Increasing the dosage to the 3 central sites or adding 2 sites targeting the medial portion of the corrugator muscles might increase overall satisfaction. However, these would be the subjects of further study.

This trial also raised some methodological issues. What are the right outcome criteria for studies evaluating the efficacy of botulinum toxin A? Although a 4-point clinical scale ranging from 0 (no wrinkles) to 3 (severe wrinkles) has been used in almost all previous studies, most have in fact used one scale for the assessment of wrinkles at rest and another for the assessment of wrinkles at maximum frown. Consequently, a score of 2 (moderate wrinkles) does not represent the same depth of wrinkles in each case. In our study, only 1 scale was used. Therefore, a score of 2 represents the same depth of wrinkles at rest and at maximum frown, with the results that the mean scores at rest are, of course, lower than those at maximum frown and that changes between the botulinum toxin A and placebo study arms at rest are harder to measure. However, the advantage of using only 1 scale may be better reproducibility: the clinical scale used herein has good to excellent interinvestigator and intrainvestigator reproducibility.

In a clinical trial studying mimic wrinkles, effective blinding of the assessment of wrinkles is often difficult because it may be clear from patients’ muscle movements that botulinum toxin A has been used. On the other hand, a committee relying on photographs gets only a glimpse of the possible magnitude of wrinkles, which may bias the maximum range of possible scores toward the mean. However, the use of photographs is more likely to ensure that the investigators are completely blinded and are less biased about treatment efficacy.

Previously, most clinical trials studying the efficacy of botulinum toxin A have involved only a few centers; in fact, only 1 trial has included more than 10 centers. Larger studies, such as the present trial involving 23 centers and 42 investigators, better reflect clinical practice than those carried out in a few expert centers, and such trials are crucial to a full examination of safety. Given that the treatment of glabellar wrinkles is a cosmetic procedure, a high rate of adverse events such as eyelid ptosis would be unacceptable. In this study, both botulinum toxin A dosages proved to be safe. Only 1 case of eyelid ptosis was reported among our patients; the eyelid ptosis was mild and did not require treatment. Using Botox in the glabellar area, Carruthers et al reported an eyelid ptosis rate of 6.4% (13 of 203 patients) in a pilot study, declining to 1.0% (2 of 202 patients) in a subsequent study. Ascher et al meanwhile reported no eyelid ptosis in 102 patients treated with 25, 50, and 75 U total of Dysport. In this context, the position of the 2 extra injection sites may be important: the sites were distinctly more lateral in the studies conducted by Carruthers et al, compared with those in the present study and in the study by Ascher et al.

Although botulinum toxin A has been used for esthetic indications for a long time, rigorous clinical trials are needed to evaluate its efficacy and safety. This trial demonstrates the efficacy and safety of the European brand of botulinum toxin A, Dysport, for the treatment of glabellar and central forehead wrinkles.

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<th>Table 6. Assessment of Patients’ Global Satisfaction With the Treatment at Week 16 in Study Arm 2*</th>
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<td><strong>Rating†</strong></td>
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<td>Mean score</td>
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*Data are given as number (percentage) unless otherwise indicated. †0 Indicates not satisfied; 1, moderately satisfied; 2, satisfied; and 3, very satisfied.
gard Gundel-Leiter, MD, Innsbruck; Stefan Hammes, MD, Ludwigshafen; Gesina Hansel, MD, Dresden; Marc Heckmann, MD, Starnberg and Percha; Martina Hund, MD, Berlin; Susanne Jockenhöfer, MD, Berlin; Martin Jung, MD, Berlin; Sigrid Karrer, MD, Regensburg; Robert Kasten, MD, Ludwigshafen; Lars Kohler, MD, Ludwigshafen; Helga Konrad, MD, Dresden; Markus Krause, MD, Magdeburg; Tina Kuster, MD, Marburg; Andrea Misic, MD, Dortmund; Marion Moers-Carpi, MD, Hamburg; Franca Noack-Wiemers, MD, Leipzig; Ulrike Ortner, MD, Innsbruck; Claudia Pirker, MD, Dortmund; Lucie Rauch, MD, Düsseldorf; Anita Rüttner, MD, Münster; Konstanze Spieth, MD, Frankfurt a Main; Annette Stein, MD, Dresden; Rolf-Markus Szeimies, MD, Regensburg; Jörg Tittelbach, MD, Jena; Kerstin Trumper, MD, Dresden; Constanze Voigtlander, MD, Erlangen; Sabine Werfel, MD, München; Luitgard G. Wiest, MD, München; Petra Wörle, MD, Erlangen; and Wolf-Ingo Worret, MD, München.

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REFERENCES