ORIGINAL ARTICLE

Dosing, efficacy and safety plus the use of computerized photography for botulinum toxins type A for upper facial lines

NICHOLAS J. LOWE1,2,3, AADARSH SHAH1,2, PHILIPPA L. LOWE1,2 & RICKIE PATNAIK2

1Cranley Clinic, London, UK, 2Clinical Research Specialists, London, UK and Santa Monica, CA, USA, and 3UCLA School of Medicine, Los Angeles, CA, USA

Abstract
Introduction: Several studies confirm that botulinum toxins type A (BTX-A) are effective for reducing facial lines caused by hyperactive muscles. Two different commercial types of BTX-A currently available are BTX-A-1 (Botox™) and BTX-A-2 (Dysport™). This paper reports further comparison of dosing, efficacy and safety. Methods: Sites treated: glabellar, horizontal forehead lines and crow’s feet. Different dilutions and dosages were studied with BTX-A-1 and BTX-A-2. The reduction of facial lines was evaluated by investigators and patients. Computerized photographic numerical assessment was also studied in determining the efficacy of BTX-A for crow’s feet lines. Results: Study 1: Injecting glabellar lines at doses of BTX-A-1 (30 units) and BTX-A-2 (75 units) (2.5:1 ratio BTX-A2:BTX-A1) showed similar efficacy. Study 2: BTX-A-2 (256 units total) was significantly more effective than BTX-A-1 (64 units total) (i.e. a dose ratio of 4:1) for upper face lines. No differences in the side-effect profiles between the two toxins were observed in either study. Study 3: A computerized photographic numerical assay was an objective assessment of crow’s feet severity. Using a dose ratio of BTX-A-2 to BTX-A-1 of 3:1 showed a trend towards BTX-A-2 superiority. Conclusion: Two different botulinum toxins type A were shown to be effective and safe for hyperfunctional facial lines. The choice of dose, dilution and placement is critical for each individual toxin. Computerized photography gave numerical severity scores of crow’s feet severity.

Key Words: Botox, botulinum toxins A, BTX-A, Dysport, efficacy, facial line assay, safety

Introduction
The first medical use of botulinum neurotoxin was first described by Dr Alan Scott during the 1970s when he used a botulinum toxin type A (BTX-A) for reducing overactivity of selective periocular muscles in patients with strabismus (1,2). Following this observation, botulinum neurotoxins have been increasingly studied for a variety of therapeutic and aesthetic uses. Double-blind control studies using BTX-A for upper facial lines in the USA showed a reduction of these lines for approximately 4 months (3,4).

These initial observations were followed by double-blind placebo-controlled studies of several hundred patients performed in the USA confirming that BTX-A was safe and effective for reducing the severity of glabellar lines (5). Significant improvement was observed and the mean duration of treatment was in excess of 4 months using doses of BTX-A at a total of 20 units for the glabellar frown lines. Several other studies confirmed that BTX-A was effective for lateral periorbital/crow’s feet lines (6) and also lines on the infraorbital area (7).

There are two main serotypes of botulinum toxins used clinically at present, the most commonly used being BTX-A. Botulinum toxin type B (BTX-B) is active, but has a shorter duration of effect than BTX-A. It is occasionally used if therapeutic resistance is observed to BTX-A (8).

Other clinical studies have confirmed that both BTX-A and BTX-B are effective at reducing facial lines, as well as excessive sweating in areas such as the axillae (9). BTX-A treatment for axillary hyperhidrosis has been approved by the Medicines and Healthcare products Regulatory Agency (MHRA) in the UK and regulatory authorities in other countries including the Food and Drug Administration (FDA) in the USA. Several different botulinum toxins are approved in many countries; four are currently...
available in Europe. There are two commonly available BTX-A: Botox® now known as onabotulinum toxin A (Allergan, Inc., Irvine, CA, USA) and Dysport® now known as abobotulinum toxin A (Ipsen Ltd, Slough, UK), which we will refer to as BTX-A-1 and BTX-A-2, respectively, in this manuscript (Table I).

A number of different studies have looked at comparative dosages of these two different toxins in a variety of different indications; for example, cervical dystonia, blepharospasm, as well as upper facial lines. The comparative dosing of BTX-A-1 to BTX-A-2 has been between dose ratios of 1:1 and 1:6, respectively, in a variety of publications and indications (10–12). There is therefore a variation in agreement on the relative dosing requirements of BTX-A-1 and BTX-A-2 in different therapeutic areas.

One pilot, double-blind, randomized study performed by ourselves suggested that with a ratio of BTX-A-1 20 units and BTX-A-2 50 units, BTX-A-1 had a slightly better therapeutic effect in duration. These treatments were performed on mild-to-moderate facial lines. However, only one dose and dilution was studied and the small patient numbers may be criticized (13). Nevertheless, other pilot studies have predicted accurately multi-centre study outcomes (6,14).

The purposes of the studies reported here are extensions of that pilot study. The study is split into three parts. The first study compares the dosing and adverse events of the two botulinum toxins, BTX-A-1 and BTX-A-2, when treating severe glabellar lines. The second study compares the initial efficacy and adverse events of the two botulinum toxins at different dose ratios for facial lines of the upper face. The final study reports on the preliminary observations of a computer-assisted photographic method to provide numerical values of crow’s feet severity.

Materials and methods

The three studies were performed in a clinical research centre and clinic experienced in conducting dermatologic research. All the patients were female and were selected to have severe glabellar lines based on scoring used in previous published studies (5,13). Female patients of childbearing potential were required to have a negative urine pregnancy test result as well as be on reliable contraception.

Exclusion criteria for any part of the study include: pregnancy; past or planned facial cosmetic procedure, laser resurfacing in the previous 12 months or botulinum toxin use within the previous 6 months; facial asymmetry including brow ptosis, and atrophy or weakness of muscles in the target injection areas; visible scars that could effect evaluation of response; history of relevant allergies; myasthenia gravis and a history of other diseases that can interfere with neuromuscular function; systemic infection or infection at target injection sites; and treatment with amino-glycoside antibiotics or other agents interfering with neuromuscular function.

For study 1 the age range was 30–60 years inclusive and for study 2 it was 18–65 years. Studies 1 and 2 were double-blind randomized studies, with investigator observer blinding as well as patient blinding. Ethical approval according to the Declaration of Helsinki Principles was obtained. For study 3 there was patient blinding and informed consent was obtained.

Study 1: Severe glabellar lines BTX-A-1 (30 units) and BTX-A-2 (75 units)

Sixty patients were selected based on severe glabellar lines and assigned randomly into two groups. In one group of 30 patients (mean age 42.0 ± 5.7 years) 30 units of BTX-A-1 was injected bilaterally into the glabellar lines and in the other group of 30 patients (mean age 42.0 ± 6.0 years) 75 units of BTX-A-2 was used.

For BTX-A-1 a 100-unit vial was diluted with 1.67 ml of preserved saline from which 0.1 ml was injected at five glabellar sites (Figure 1), giving 6 units per site. A 500-unit vial of BTX-A-2 was diluted with 3.33 ml of preserved saline from which 0.1 ml was injected at the same five sites giving 15 units per site. The total units injected of each toxin was therefore higher than previously reported in our earlier pilot study of less severe glabellar lines (13), but remained at a dose ratio of BTX-A-1:BTX-A-2 of 1:2.5.

Patients who had been using topical facial products for at least 6 months prior to the start of the study were allowed to continue their use. They also refrained from applying facial cosmetics 4 hours preceding study visits.

Patients were examined and photographed (using a modified Canfield system; Canfield Scientific, Inc., Fairfield, NJ, USA) during maximum attempted

<table>
<thead>
<tr>
<th>Product</th>
<th>Toxin type</th>
<th>Toxin molecular weight (kD)</th>
<th>pH</th>
<th>Approved for cosmetic use in UK, Europe and USA</th>
<th>Approved for hyperhidrosis in UK, Europe and USA</th>
<th>Approved for medical indications (e.g. dystonia, blepharospasm) in Europe</th>
</tr>
</thead>
<tbody>
<tr>
<td>BTX-A-1 (Botox®)</td>
<td>A</td>
<td>900</td>
<td>−7</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td>BTX-A-2 (Dysport®)</td>
<td>A</td>
<td>500–900</td>
<td>−7</td>
<td>Yes</td>
<td>No</td>
<td>Yes</td>
</tr>
</tbody>
</table>
muscle contraction before injection and 2, 8, 16 and 20 weeks post-injection. Efficacy assessments at each visit were made by an investigator-assessed Facial Wrinkle Severity (FWS) score. The investigator-assessed FWS assessed the subject’s upper facial lines at maximum attempted muscle contraction, where none = 0, mild lines = 1, moderate lines = 2, and severe lines = 3. At week 20, patients were also asked to assess their own satisfaction with the study treatment, where 2 = very satisfied, 1 = satisfied, 0 = impartial, –1 = dissatisfied, and –2 = very dissatisfied. At each patient visit adverse reactions were also noted.

Study 2: Upper face BTX-A-1 (64 units) and BTX-A-2 (256 units)

The purpose of this study was to examine adverse events and initial efficacy over the first 4 weeks after injection of all upper facial lines with 64 units of BTX-A-1 or 256 units of BTX-A-2, giving a comparative dose ratio of BTX-A-1:BTX-A-2 of 1:4. It has been observed that virtually all adverse effects are observable with BTX-A during the first 4 weeks.

A total of 40 patients were selected with severe upper facial lines and were randomly assigned to two groups of 20 patients to receive BTX-A-1 or BTX-A-2.

For BTX-A-1 a 100-unit vial was diluted with 2.5 ml of preserved saline from which 0.1 ml was injected at 16 upper face injection sites giving 4 units per site and 64 units in total. A 500-unit vial of BTX-A-2 was diluted with 3.125 ml of preserved saline from which 0.1 ml was injected at the same 16 sites giving 16 units per site and 256 units in total. Injections were to the horizontal forehead lines, glabellar lines and lateral periorbital regions (crow’s feet) (Figure 1). The use of cosmetic facial products was regulated as in study 1.

Patients were examined pre-treatment, and 2 and 4 weeks post-treatment. FWS was utilized as in study 1 by a blinded investigator. Side effects evaluated were headache, bruising, brow drop, brow arching, and any systemic symptoms. One patient in the BTX-A-1 group dropped out prior to their first post-treatment assessment.

All data were analyzed on an intent-to-treat basis. Statistical evaluation used a Wilcoxon signed-rank test to evaluate between-group differences.

Study 3: Computerized photographic numerical assessment of crow’s feet

This was a treatment comparison, non-placebo study. A photographic wrinkle evaluation system, Visia (Canfield Scientific, Inc.), was used to evaluate the severity of crow’s feet lines at smile before and at times following either BTX-A-1 or BTX-A-2 injection.

Five patients with bilateral crow’s feet were selected and investigated for response to BTX-A-1 and BTX-A-2 at different time intervals. They were injected with a total of 16 units of BTX-A-1 over three sites to the left crow’s feet or with 48 units of BTX-A-2 to the right; a dose ratio of 1:3, respectively. Preparation of the toxins followed the same methods as in studies 1 and 2 to ensure that despite differences in concentration the injected volumes remained the same. Objective measurements were made using the photographic wrinkle evaluation system for all patients prior to treatment. Two patients were then photographed 5 days post-treatment and the other two patients at 4 weeks post-treatment. One patient who was photographed at 5 days was further photographed at 5 months. All photographs were taken at maximum smile.

Results

Study 1: Severe glabellar lines BTX-A-1 (30 units) and BTX-A-2 (75 units)

There was no significant difference at any point between BTX-A-1 and BTX-A-2 in the results of the

Figure 2. Graph showing no significant difference in glabellar lines between the BTX-A-1 and BTX-A-2 study groups as assessed by the Facial Wrinkle Severity (FWS) score (p > 0.05).
FWS score at each visit. The results are summarized in Figure 2.

The adverse events, summarized in Table II, show all adverse events to be mild. There were a total of 16 mild adverse events in the group treated with BTX-A-1 and 15 in the group treated with BTX-A-2. The most common adverse effect being a mild headache following injection. In neither group of patient was any brow ptosis observed.

Subject satisfaction with study treatment showed no significant differences between the BTX-A-1 and BTX-A-2 groups, with mean values of 0.96 ± 1.17 and 0.96 ± 0.96, respectively.

Study 2: Upper face BTX-A-1 (64 units) and BTX-A-2 (256 units)

The investigator-assessed FWS score showed that BTX-A-2 was statistically superior to BTX-A-1 in reducing glabellar lines at week 2 and week 4 at the dosages used (Figure 3). This was also seen on horizontal forehead lines at maximum contraction. The same trend as shown in glabellar lines was seen at the injection sites.

The adverse effect profile again showed no differences between BTX-A-1 at 64 units and BTX-A-2 at 256 units to the upper face (Table III). There was a similar incidence of headaches but there were no major differences in brow shape. One patient did notice excessive arching of the brow with BTX-A-1.

Study 3: Computerized photographic numerical assessment of crow’s feet

Variability of this assay was evaluated by taking three sequential photographs (Figures 4 and 5) of individuals and tabulating the computer grade scores. There was a close correlation with the three sequential scores.

It can be seen from Figure 6 that the initial response for BTX-A-2 was faster than BTX-A-1 in reducing crow's feet at 5 days and 4 weeks by ∼15%. However, BTX-A-1 showed a slightly superior long-term reduction in crow’s feet at 6 months. These results are of a very limited sample size, but they suggest computerized photographic numerical assessment to be useful for measuring the effects of agents on rhytids.

It appears that this type of computerized photographic measurement at maximum smile and rest may be an accurate comparison of potency of different toxins and also different doses and dilutions of the same toxin (Figure 4 and 5).

Discussion

Two different commercial forms of BTX-A, BTX-A-1 and BTX-A-2, have been available in Europe and other countries since about 1990. They are produced by different bacteria with different fermentation and purification processes. They have different molecular weights. They are both thought to act via the interruption of the same soluble N-ethylmaleimide sensitive factor attachment protein receptor (SNARE), SNAP25 protein.

Dilution and dosing have been investigated and several dose ratios of one of them to the other have been proposed (10–13). It is important to know the relative dosages of the different toxins, as it is sometimes required for a patient to switch to a different type A toxin for aesthetic and logistic reasons. Drug dilution, dosing, duration of benefit, and degree of improvement are all important clinical factors when considering the alternative toxin.

Table II. Study 1: The incidence of different adverse events.

<table>
<thead>
<tr>
<th>Type of adverse events</th>
<th>Number of patients suffering adverse events</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bruising</td>
<td>2</td>
</tr>
<tr>
<td>Headaches</td>
<td>9</td>
</tr>
<tr>
<td>Muscle twitch</td>
<td>2</td>
</tr>
<tr>
<td>Flu</td>
<td>3</td>
</tr>
<tr>
<td>Brow ptosis</td>
<td>0</td>
</tr>
<tr>
<td>Lid ptosis</td>
<td>0</td>
</tr>
<tr>
<td>Total</td>
<td>16</td>
</tr>
</tbody>
</table>

Table III. Study 2: The incidence of different adverse events.

<table>
<thead>
<tr>
<th>Type of adverse event</th>
<th>Number of patients suffering adverse events</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bruising</td>
<td>4</td>
</tr>
<tr>
<td>Headaches</td>
<td>2</td>
</tr>
<tr>
<td>Brow arching</td>
<td>1</td>
</tr>
<tr>
<td>Brow ptosis</td>
<td>4</td>
</tr>
<tr>
<td>Flu</td>
<td>2</td>
</tr>
<tr>
<td>Total</td>
<td>13</td>
</tr>
</tbody>
</table>
Ratios of the two toxins can be reevaluated once the injector has trained in dilution and dosing.

Side effects from BTX-A are usually mild at dosages that are used for aesthetic indications and occur usually within the first 4 weeks (4), the most frequent being discomfort/pain, bruising, headache, brow or eyelid ptosis or over-elevation (arching) of the eyebrows. Sometimes these are inherent unavoidable complications of any intramuscular injection. At other times, these may be the result of inexpert injection of the botulinum toxin (e.g. injections of too high a dose of BTX-A to the lateral and lower forehead area may result in brow as well as upper eyelid ptosis). Injections that are too far below the intra-orbital area may result in lower facial ptosis and facial asymmetry.

While these side effects are usually temporary they can be of understandable concern to patients. It has been proposed by others that the diffusion of BTX-A-2 is greater than that of BTX-A-1 (15,16). Most of this information is derived from both animal studies as well as isolated muscle study. In addition, one study by Trindade de Almeida et al. (15) showed that there was a greater diffusion pattern when foreheads were injected and the diffusion tracked by starch iodine marking of sweating. The diffusion of BTX-A-2 may be intradermal, intramuscular and dependent on dilution and concentration. Further studies are needed to clarify comparative diffusion and any clinical relevance.

With regard to the studies reported in our paper, we found that there were no statistical differences between efficacy or adverse effects for the treatment of severe glabellar lines in women when 30 units of BTX-A-1 or 75 units of BTX-A-2 was used (i.e. a dose ratio of BTX-A-1:BTX-A-2 of 1:2.5). This contrasts with the previous study (13) where the same dose ratio was used but lower dosages of both toxins were injected (20 units of BTX-A-1 and 50 units of BTX-A-2) and showed BTX-A-1 to have superior efficacy. One possibility is that higher dosages of BTX-A-2 increase the efficacy of this toxin compared with lower dosages. This may be related to a number of factors including receptor binding, neuromuscular endplate incorporation of toxin, as well as possible diffusion. As regards adverse effects from possible diffusion, it was noted that the side-effect profiles of both BTX-A-1 and up to 256 units of BTX-A-2 into the upper face were not significantly different. If diffusion were of clinical relevance, the dosages of BTX-A-2 used might be expected to have a greater side-effect profile.

The studies reported here must be viewed with some caution because of the relatively small numbers of patients in each group. Studies 1 and 2 were, however, double-blind randomized studies. Consistent injection techniques were followed with two experienced skilled injectors being involved in these studies.

When a higher dose of BTX-A-2 was used (256 units) compared with 64 units of BTX-A-1 (4:1 ratio), the initial efficacy strongly favoured BTX-A-2 in reducing glabellar and horizontal forehead lines without any difference in the side-effect profile.

The computerized photographic analysis system measuring numerically the severity of the wrinkle score in the crow’s feet area may be a potentially accurate assessment of line severity. Advantages of this technique are that it is independent of observer bias and it is quantitative – giving a numerical score. The assay can be utilized for crow’s feet evaluation.
as a bilateral paired double-blind assay, which has the advantage of requiring fewer patients to obtain preliminary data on comparative efficacy. Further validation studies are indicated with this and other computer measuring systems.

Recently suggested scales for facial lines, including crow’s feet, have been proposed. These relied entirely on clinical grading (17), while the photographs were validated by a group of physicians and surgeons. They used a 0–4 scale, which was a modification of a previously published 0–3 scale. These investigators commented that evaluation in a clinical trial should be performed on standardized photographs – not physical examination.

We also suggest a comprehensive evaluation would be (a) computer-graded standardized photographs; (b) physical examination and grading of the live patient; and (c) clinical photographs taken at the time of (a) and (b) above and graded independently. All of this should be preferably performed at rest and at maximum muscle activity (e.g. frowning or smiling).

In summary, these two botulinum toxins type A, BTX-A-1 and BTX-A-2, are both highly effective agents in the management of hyperfunctional upper facial lines. As a result of these investigations and 15 years of experience in their use, it is now our choice to utilize a dose ratio of 1 unit of BTX-A-1 to 2.5 or 3 units of BTX-A-2.

While it is not ideal to compare ratios, it is nevertheless helpful for dosing decisions when conducting comparative research studies.

Computer measurement of line severity once validated is likely to remove observer variability from this area of clinical research.

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