Subcutaneous vs Intramuscular Botulinum Toxin Split-Face Randomized Study

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IMPORTANCE Much has been published regarding rejuvenation of the upper face with botulinum toxin A injection; however, the optimal target tissue layer has not been specifically examined.

OBJECTIVE To seek a difference between subcutaneous (SC) and intramuscular (IM) administration.

DESIGN, SETTING, AND PARTICIPANTS Prospective, randomized study at a tertiary care university facial plastic surgery practice. Nineteen patients who underwent botulinum toxin A treatment to the forehead were randomized so that each patient received IM injection on one side of the face and SC injection on the contralateral side.

INTERVENTION Patients were assessed on the basis of eyebrow elevation before treatment, and at 2 weeks, 2 months, and 4 months following injection. Patients also completed a subjective questionnaire examining discomfort during injection, bruising, and tenderness, as well as their perception of their appearance after treatment.

MAIN OUTCOME AND MEASURE Eyebrow height measurements between SC and IM techniques.

RESULTS There was no difference in eyebrow height measurements between SC and IM techniques (0.00 [95% CI, −0.02 to 0.02]). Patients did report greater discomfort when receiving IM injections compared with SC injections (−0.76 [95% CI, −1.53 to 0.0005]). Patient satisfaction scores did not demonstrate a statistically significant difference between IM and SC techniques when measured on the first and second posttreatment visits; however, there was a trend toward significance on the final follow-up visit.

CONCLUSIONS AND RELEVANCE Subcutaneous injection of botulinum toxin A is equally effective in achieving paralysis of the underlying frontalis muscle as IM botulinum toxin A administration. In addition, the SC route may result in less pain to patients receiving botulinum toxin A injection for rejuvenation of the upper face.

LEVEL OF EVIDENCE 1.
Botulinum toxin was first discovered as a causative agent of food-borne illness in 1895 by Emile Pierre van Ermengem in Ellezelles, Belgium. Over half a century would pass before its mechanism of action would be uncovered as the inhibition of acetylcholine release from motor nerve endings. During this time, 2 variants of botulinum toxin would be classified and described by Burke in 1919 and several more in the intervening decades before the isolation of crystallized botulinum toxin A would finally occur in 1946.

Since the first reported study by Carruthers and Carruthers in 1992 of botulinum toxin A examining its utility in rejuvenation of the forehead, the injection of botulinum toxin A has become the most commonly performed cosmetic procedure in the United States. The use of botulinum toxin A for the treatment of facial rhytides has been demonstrated to influence others’ perception of a patient, mitigate the misunderstanding of an aging patient’s facial expressions, and improve patients’ moods while decreasing feelings of anxiety and depression.

Intramuscular (IM) administration of an injectable substance may result in increased pain when compared with application into the SC space, which may more readily accept a fluid bolus. While much has been published regarding the technique of botulinum toxin A injection in its use for rejuvenation of the upper face, the optimal target tissue layer has not been examined, and opinion varies as to whether injection need take place within the specific muscle body being targeted vs the immediately adjacent tissue planes. To prevent dilution and spread of effect, it is also recommended that higher concentrations be used in smaller volumes.

Injection of botulinum toxin A into the SC space has been reported anecdotally, although there is not, to our knowledge, a controlled study that examines whether or not a differential exists in terms of efficacy for botulinum toxin A injected intramuscularly vs subcutaneously. Therefore, we undertook a prospective, randomized, double-blind study, using a split face control, to ascertain whether the SC and IM routes possess equivalent effectiveness for facial rejuvenation of the forehead targeting the frontalis muscle.

Methods

This was a double-blind, randomized, prospective trial, performed on 19 participants from December 1, 2010, to June 1, 2011. Institutional review board approval was given by Thomas Jefferson University. All patients provided appropriate written informed consent prior to participation in the study. Each received a total of 12 U of botulinum toxin A (Botox; Allergan Inc) administered in a symmetrical fashion in 4 equal aliquots; 2 to the left forehead and 2 to the right. Each participant was randomly assigned a number, via a computer data-base, to determine which side of the face would receive an IM application of botulinum toxin, and which would receive an SC injection. Botox was diluted with injectable normal saline to a concentration of 4 U per 0.1 mL and injected through a 30-gauge, 0.5-in needle. No manipulation was performed of the injection sites following injection. If there was any bleeding, light pressure was briefly held with gauze.

Exclusion criteria for this study included individuals younger than 18 years as well as those older than 65 years, pregnant patients, patients with immunocompromised states, and patients with any neurologic conditions. In addition, individuals were not included in the study if they had received prior facial cosmetic procedures within the year prior to injection. These included skin peels, dermabrasion, injections of neuromuscular agents or fillers, as well as cosmetic surgery of any kind.

Prior to injection, all patients were photographed in the dedicated photography room within our department’s center for facial aesthetics and reconstructive surgery. All photographs were taken under identical lighting conditions using the same digital camera (model D90, Nikon). Patients were photographed in the standard fashion, positioned along the Frankford horizontal plane, in frontal, left, and right oblique, and left and right profile views. In all positions, patients were photographed in both a relaxed, neutral expression, as well as while exhibiting maximal voluntary eyebrow elevation.

Injections themselves were performed by the 2 senior authors (R.N.H. and H.K.) who are board-certified facial plastic and reconstructive surgeons. Injections were performed with the use of an Accuguide injection electromyographic (EMG) device (Xomed Surgical Products Inc) to aid in confirmation of the specific tissue layer being injected. When the needle was in position, participants were asked to raise their eyebrows, resulting in audible activity from the EMG probe when the needle tip was within the frontalis muscle body, and no change in activity when the needle was positioned in the SC plane (Figure 1).

Immediately following injections, patients were asked to answer a brief questionnaire, examining whether they had previously received botulinum toxin A treatments, as well as the level of discomfort each person experienced during each injection, as rated on a scale of 0 to 5. Patients were also asked whether a difference was noticeable between the left- and right-side injections, and whether the discomfort experienced was less than, equal to, or more than what was anticipated. The injecting physicians also completed an evaluation form in which they commented on any difficulty in accessing the SC plane and whether there was bleeding or bruising immediately following injection; they had space to write in additional comments, at their discretion.

Appointments were made with all patients for return visits taking place 2 weeks postinjection, as well as at 2 and 4 months postinjection. At the time of all follow-up appointments, patients were photographed in a manner identical to that described during the initial visit, and an identical questionnaire was administered at each appointment. Inquiries were made as to whether patients perceived any change in their appearance since injection, what those changes were, and if there was a noticeable difference between the 2 sides of the face. Pa...
Patients were asked to declare whether any bruising or tenderness followed their injections, and they were asked to rate the degree to which they were pleased with the results of the injections, on a scale of 0 to 5, for the left and right side of their faces, individually.

To quantify eyebrow height, all frontal view digital photographs were transferred to Adobe Photoshop (Adobe Systems Inc), where several measurements were taken. First, a horizontal line was drawn through the lateral limbus of bilateral lateral canthi. From this line, eyebrow height was obtained by measuring the distance between the line and a point taken at the superior-most aspect of the eyebrow, positioned directly above the mid-pupillary axis (Figure 2). Measurements were made at 150% magnification, viewing images on an Apple Cinema HD with a 23-in display (Apple Inc) and a screen resolution of 1920 × 1200 pixels.

The intercanthal distance was measured on each individual picture that was used for eyebrow height analysis. The intercanthal measurements were then used to normalize the brow height from each photograph to account for differences in the camera-to-patient distance, which would otherwise compromise the eyebrow height values. This resulted in a ratio of brow height to intercanthal distance that was used for further analysis, hereafter referred to as normalized brow height. For each patient, values of normalized brow height measured during neutral brow positioning were subtracted from those measured during active brow elevation, for both sides receiving the IM and SC injection techniques, yielding a value of normalized brow elevation. This was calculated for pictures taken immediately prior to injection, and at each follow-up visit.

Once this was calculated, mixed-effects linear regression was used to model normalized brow height as a function of treatment (IM vs SC) and visit (initial visit, as well as all 3 follow-up visits). An interaction term between treatment and visit was included to allow for separate comparison of the 2 treatments at each visit. Random intercept terms were included to account for correlation between the left and right sides of the face at each visit and among repeated measurements over time. These analyses were performed by the division of biostatistics at Thomas Jefferson University.

In addition to eyebrow height, statistical analysis was also performed on the degree of discomfort, as reported by each patient, rated on a scale of 0 to 5, for the injections performed on each side of the patient’s face. Similarly, satisfaction scores, as rated by the patients on a scale of 0 to 5 for each side of the face on all follow-up appointments, were analyzed as well. A paired t test was used to compare the discomfort ratings, as well as the satisfaction scores, for IM and SC injection techniques. Statistical analysis was not used for the remaining items on the patient and physician questionnaires owing to the small sample size and limited responses.

### Results

#### Brow Height Measurements

The mean normalized brow elevation for the initial visit was 0.25 (95% CI, 0.20–0.29) for the IM injection, and 0.25 (95% CI, 0.21–0.29) for the SC technique, yielding a mean difference between sides of −0.01 (95% CI, −0.02 to 0.01), which was not statistically significant (P = .53) (Table 1). The mean normalized brow elevation for the first follow-up visit was 0.1 (95% CI, 0.06–0.15) for the IM side and 0.1 (95% CI, 0.06–0.15) for the SC method, yielding a difference of 0.00 (95% CI, −0.02 to 0.01)
between techniques, which was not statistically significant ($P = .85$). The mean normalized brow elevation for the second follow-up visit was 0.19 (95% CI, 0.14–0.24) for the IM technique and 0.18 (95% CI, 0.13–0.23) for the SC injections, yielding a difference of 0.01 (95% CI, −0.01 to 0.03) between injection methods, which was not statistically significant ($P = .19$). Finally, the mean normalized brow elevation for the final follow-up visit was 0.21 (95% CI, 0.15–0.27) for the IM side as well as 0.21 (95% CI, 0.15–0.27) for the SC method, yielding a difference of 0.00 (95% CI, −0.02 to 0.02) between injection methods, which was not statistically significant ($P = .82$).

Initial Patient Questionnaires

Descriptive Statistics

Of the 19 patients who participated in the initial study visit with treatment, 13 (68%) denied any history of receiving botulinum toxin A injections. Overall, 6 patients (32%) described the injections as being less painful than expected, while 10 patients (59%) stated that the injections were equal to their anticipated discomfort level. One patient expressed more pain than anticipated. Among the patients who had received previous treatments, 2 reported less pain than they anticipated, while the remaining 4 described the pain as being equal to what was expected (Table 2).

Discomfort Ratings

Fifteen patients (79%) claimed to feel a noticeable difference in discomfort levels between sides of injection, while 4 (21%) did not. The mean discomfort level, as rated on a scale of 0 to 5, immediately following the completion of both injections, was 1.97 (95% CI, 1.44–2.51) for the IM side and 1.21 (95% CI, 0.81–1.61) for the SC side. The mean difference in rated discomfort was −0.76 (95% CI, −1.53 to 0.0005), with $P = .05$.

Initial Physician Comments

The attending physicians administering injections noted no difficulty in accessing the SC plane to deliver medication. Bleeding was noted in 5 cases. In 3 of these cases, bleeding was bilateral, while in 2 cases, bleeding was noted only on the IM side. Immediate bruising was noted in only 2 cases, both occurring on the IM side.

Follow-up Questionnaire Responses

First Follow-up Visit

Eighteen patients returned for the first follow-up visit, 2 weeks postinjection. Fourteen patients (78%) noted an effect from their injections, while 4 (22%) did not. Effects written in by patients included a reduction in wrinkles, smoother forehead skin, decreased forehead movement, as well as drooping eyelid skin in 1 patient, and a heavier eyelid in another. Eight patients (44%) reported a noticeable difference in appearance between sides, while the 10 remaining patients (56%) did not.

Two patients reported bruising on the side that received the IM injection, while 1 patient reported bruising on the side that received the SC injection. Similarly, 2 patients noted tenderness on the IM side, while 1 declared tenderness on the SC side. The 2 patients with bruising from the IM injection were the same 2 who reported tenderness from this technique.

Second Follow-up Visit

Sixteen patients presented for the second follow-up visit, 2 months postinjection. At this point, 10 patients (63%) noted an effect from treatment, while 6 (38%) did not. Effects noted by the patients were similar to those noted at the initial visit and included decreased forehead wrinkles and lines, tighter and smoother forehead skin, and reduced forehead movement. Six patients (38%) claimed to notice a difference between sides, while 10 (63%) did not.

Final Follow-up Visit

Ten patients returned for the final follow-up visit at 4 months postinjection. At this time, 8 patients (80%) noted an effect from treatment, while 2 (20%) did not. Again, patients specifically noted reduced forehead lines and wrinkles, smoother skin, improved appearance, and decreased eyebrow mobility, as well as 1 case of numbness. Five patients (50%) stated that they could notice a difference between their right and left sides, while the other 50% could not.

Patient Satisfaction Ratings

In addition to the variables mentioned herein, patients were also asked to rate the degree to which they were satisfied with the results seen on each side of their face, on a scale of 0 to 5. For the IM injections, patients rated their satisfaction on follow-up visits 1, 2, and 3 at 2.94 (95% CI, 2.01–3.86), 3.00 (95% CI, 2.04–3.96), and 3.60 (95% CI, 2.43–4.77), respectively. For...
the SC injection technique, satisfaction ratings were 2.95 (95% CI, 2.01-3.89), 2.63 (95% CI, 1.64-3.58), and 2.67 (95% CI, 1.47-3.87) for those same visits. The differences in patient satisfaction were not statistically significant on follow-up visits 1 and 2, although on the final follow-up visit, the difference of 0.93 (95% CI, 0.05-1.8) was statistically significant (P = .04) (Table 3 and Table 4).

**Discussion**

There is variation among health care practitioners in the specific technique of botulinum toxin A injection, with some applying the toxin directly into the body of the muscle being targeted and others injecting into adjacent tissue layers, such as the SC space. Small volumes of high-concentration botulinum toxin A are administered to minimize diffusion of the toxin throughout surrounding tissues. Anecdotal reports of equivalent efficacy using a SC injection exist; however, to our knowledge, there exists no randomized study specifically investigating a difference between the 2 techniques. Theoretical advantages of a SC injection include decreased pain on injection and reduced bruising and tenderness.

In this study we were able to use an EMG probe to determine whether the tip of the injection needle was within the frontalis muscle. Anecdotally, this practice is not used by health care practitioners performing SC injection. We feel that an experienced facial plastic surgeon can gauge the tissue level reached by a needle during needle placement and differentiate between muscle, skin, and the SC space. We had no objective means of ensuring that we were not performing an intradermal injection when targeting the SC space, and we relied on the experience of the attending surgeon performing the injections. We do not intend that an EMG device be used in practice when performing a SC injection, just as it is not currently relied on for an IM injection.

Additional methods of anesthesia include the use of small diameter needles, such as 30 to 32 gauge, slow needle insertion, insertion of the needle tip through a pore, and slow injection. The reconstitution of botulinum toxin A using normal saline containing benzyl alcohol preservative has also been noted to reduce pain associated with treatments. Alternative methods of anesthesia include massaging adjacent skin and the application of vibratory tactile stimulation to the surrounding area, as well as thermal techniques, such as cooling sprays, ice packs, and heating devices.

Several pharmacologic measures have been described to lessen the pain of botulinum toxin A injections, including creams and sprays containing compounds such as lidocaine hydrochloride, prilocaine hydrochloride, tetracaine hydrochloride, and benzocaine, although these practices are time consuming, and contact dermatitis can result. There have also been trials of remedication with analgesics, such as nonsteroidal anti-inflammatory drugs or acetaminophen.

This study represents a randomized, double-blinded investigation, using a split-face, side-by-side comparison of botulinum A toxin injected intramuscularly vs subcutaneously. No statistical difference was noted between the 2 techniques of injection by using the magnitude of active brow elevation as an objective metric. In fact, results from the 2 methods were nearly identical.

In accordance with our hypothesis, patients generally found the SC application of botulinum toxin A to be less painful than the IM technique, rating their discomfort at 1.21 and 1.97, respectively, on a 0 to 5 scale. This difference was significant, although by no means large. Interpretation of this scale is somewhat limited as well, owing to the nature of this subjective question.

As far as issues with bruising and tenderness are concerned, there were more reports of these occurrences with the IM injection; however, most patients experienced neither, and no actual difference could be ascertained. Responses were nearly split down the middle regarding the presence of a noticeable difference in appearance between the

<table>
<thead>
<tr>
<th>Visit</th>
<th>IM</th>
<th>SC</th>
<th>Difference (95% CI)</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Follow-up 1</td>
<td>2.94 (2.01 to 3.86)</td>
<td>2.95 (2.01 to 3.89)</td>
<td>-0.02 (−0.7 to 0.67)</td>
<td>.96</td>
</tr>
<tr>
<td>Follow-up 2</td>
<td>3.00 (2.04 to 3.96)</td>
<td>2.61 (1.64 to 3.58)</td>
<td>0.39 (−0.31 to 1.10)</td>
<td>.27</td>
</tr>
<tr>
<td>Follow-up 3</td>
<td>3.60 (2.43 to 4.77)</td>
<td>2.67 (1.47 to 3.87)</td>
<td>0.93 (0.05 to 1.80)</td>
<td>.04</td>
</tr>
</tbody>
</table>

**Table 4. Questionnaire Responses at Follow-up**

<table>
<thead>
<tr>
<th>Question</th>
<th>Follow-up Patients, No. (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Any effect resulting from injections?</td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>14 (78)</td>
</tr>
<tr>
<td>No</td>
<td>4 (22)</td>
</tr>
<tr>
<td>Noticeable difference between sides?</td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>8 (44)</td>
</tr>
<tr>
<td>No</td>
<td>10 (56)</td>
</tr>
<tr>
<td>Bruising?</td>
<td></td>
</tr>
<tr>
<td>IM</td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>2 (13)</td>
</tr>
<tr>
<td>No</td>
<td>14 (88)</td>
</tr>
<tr>
<td>SC</td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>1 (6)</td>
</tr>
<tr>
<td>No</td>
<td>15 (94)</td>
</tr>
<tr>
<td>Tenderness?</td>
<td></td>
</tr>
<tr>
<td>IM</td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>2 (13)</td>
</tr>
<tr>
<td>No</td>
<td>14 (88)</td>
</tr>
<tr>
<td>SC</td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>1 (6)</td>
</tr>
<tr>
<td>No</td>
<td>15 (94)</td>
</tr>
</tbody>
</table>

Abbreviations: IM, intramuscular; SC, subcutaneous.
2 sides of the patients' faces. When patients were specifically questioned regarding their posttreatment satisfaction as it related to each side of the face, both techniques achieved nearly identical scores when rated on a scale of 0 to 5 at follow-up appointments 1 and 2. The third follow-up appointment did yield a difference in satisfaction scores, with the patients noting a better appearance on the side receiving IM injections. Strictly speaking, this difference was more of a strong trend than a significant difference, as overlap was noted between the 95% CIs. While subjective grading of rhytides and skin appearance was not performed, it was felt that a purely objective analysis of muscular activity as it related to brow height on active elevation was the most appropriate metric for analysis of the efficacy of botulinum toxin A treatments. Considering that the mechanism involved in the reduction of facial lines is the actual weakening of muscular contraction, quantifying the degree of weakness by measuring brow movement should function as a suitable surrogate. Our method of calculating a normalized brow elevation ratio based on the intercanthal distance was adopted from Kim et al,22 who devised a similar method of brow height measurement, with the main difference being that those authors' ratio was calculated by dividing the brow height (as measured from a horizontal line drawn through the medial canthi) by the distance between the lateral limbus of the iris and the medial canthus. We decided to use a completely fixed anatomic landmark for our calculations because we felt that this would more reliably account for any fluctuations in the distance between patient and camera among individual photographs.

Conclusions

The SC administration of botulinum toxin A achieves efficacy equivalent to that attained by IM injection in treating brow lines. Patients report less discomfort on injection of botulinum toxin into the SC plane relative to the IM space. Subcutaneous injection can therefore be used as a technique to mitigate the pain associated with the IM injection of botulinum toxin A without compromising the compound's efficacy.

REFERENCES