Efficacy and Safety of a Novel Botulinum Toxin A for Masseter Reduction: A Randomized, Double-Blind, Placebo-Controlled, Optimal Dose-Finding Study

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BACKGROUND A wide lower face and a square jaw are considered esthetic problems, particularly in Asia.

OBJECTIVE To investigate the optimal dose of a novel botulinum toxin (prabotulinum toxin A) for treating masseteric hypertrophy.

METHODS Ninety subjects with masseteric hypertrophy were randomly divided into 5 groups and treated with placebo (A, normal saline) or prabotulinum toxin A (B: 24, C: 48, D: 72, and E: 96 units). Photography, ultrasonography, and 3-dimensional imaging were performed before and after injection at baseline and at 4, 8, 12, and 16 weeks after treatment. The participants also rated their satisfaction.

RESULTS Masseter thickness significantly reduced in all groups at 12 weeks, compared with that in the placebo group. A dose-dependent reduction in masseter thickness was observed at the resting and maximal clenching positions. Sonography and 3-dimensional imaging revealed a gradual reduction in masseter thickness and volume, respectively, during the first 12 weeks. Despite being slightly effective, a dose of 24 units might be insufficient for resolving square face problems. Patients in Group E reported discomfort during jaw movement.

CONCLUSION Prabotulinum toxin A could effectively improve lower face contour without major complications, with an optimal dose of 48 to 72 units, followed by reinjection after 12 weeks.

A square jaw is considered a common esthetic problem, particularly in Asian populations.[1] Mandibular bone shape and masseteric hypertrophy are the main determinants of lower face contouring. Besides surgical treatments, botulinum toxin injection has been used for slimming the lower face profile.[2] Despite its common use in clinical practice, only few controlled studies with defined objectives quantitatively evaluated the effects of botulinum toxin on lower face contouring.

The relationship between the dose and slimming effects of botulinum toxin remains to be determined. In this study, the optimal dose of botulinum toxin required for treating masseteric hypertrophy, in terms of cost and efficacy, was investigated. Quantitative analyses of the masseteric muscle have been typically performed using ultrasonography or computed tomography.[1,3–5] The current study is the first to report the actual changes in lower face volume via 3-dimensional imaging.

Materials and Methods

Ethical Approval

The study was conducted in accordance with the principles of good clinical practice and regulatory requirements. The Institutional Review Board at the Chung-Ang University Hospital approved all study protocols. Informed consents were obtained from all study participants.

Subjects

According to the results of previous studies, the mean difference in masseter size (test group − control group) was set at 3 months at −3.33 mm with an SD of 2.62 mm.[6,7] Considering a 5% bilateral significance level, 1:1 allocation ratio, and 90% power, the number of participants per each group was calculated as 14 subjects. Considering a possible dropout rate of 20%, 18 subjects/group (90 subjects in total) were recruited. Korean subjects, older than 18 years (mean age = 43.84 ± 8.93 years), diagnosed with bilateral symmetrical masseteric hypertrophy by physical palpation and visual assessment were included in this study. Physical examination and ultrasonography were used to confirm the diagnosis of masseteric hypertrophy based on the thickness of the masseter muscle at maximal clenching (>14 mm for men and >12 mm for women). This criterion was based on the findings of a previous study performed by Lee and colleagues, who measured the thickness of the masseter muscles in Korean adults. According to this previous study, the average thickness of the masseter muscle at maximal clenching was 14.8 ± 1.77 and 12.4 ± 1.47 mm in male subjects and female subjects, respectively.[8] Therefore, masseteric hypertrophy was defined in Korean subjects as a thickness greater than the average thickness in the Korean adult population. Subjects previously treated with botulinum toxin within 3 months or any procedure that might affect lower face contour (e.g., 2-jaw surgery, thread

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insertion, radiofrequency, lifting, and masseter muscle resection) within 12 months were excluded from the study. Subjects with any comorbidities that might affect the results were also excluded. Subjects were randomly divided into 5 groups ($n = 18$ each) and treated with placebo (A, normal saline) or prabotulinum toxin A (B: 24 [12 units/side], C: 48 units, D: 72 units, and E: 96 units). Both subjects and investigators were blinded to treatment.

Materials

Prabotulinum toxin A (PRA, NABOTA, Daewoong Pharmaceutical, Seoul, South Korea) was supplied as a 100-unit freeze-dried powder. The efficacy of PRA, a newly introduced botulinum toxin A, has been shown to be comparable to that of onabotulinum toxin A (BOTOX, Allergarn Inc., Irvine, CA) in the treatment of glabellar frown lines, crow's-feet lines, and upper limb spasticity.[9] Prabotulinum toxin A was synthesized using HI-Pure Technology, a patented technology using strictly refined anaerobic fermentation and purification processes.[1] Using this technology, impurities can be removed as much as possible, as confirmed by size-exclusion high-performance liquid chromatography analysis showing a single 900-kDa peak (>98%). The powder was reconstituted with sterile saline to prepare the assigned concentration according to the study protocol. To ensure the blinding, different volumes of the diluent were added to a vial according to the dosage group so that the practitioner could inject the same volume to the patient regardless of the dosage. The toxin was used immediately after reconstitution. To minimize injection pain, 30- to 33-gauge needles were used.

Intervention

An imaginary line linking the tragus and mouth corner was used to mark a safe injection zone. Before injection, patients were asked to clench their jaws as strongly as possible to define the areas of masseteric prominence. By asking the patients to repeat the clenching action, the practitioner could easily recognize the anterior and posterior margins of the masseter muscle. The needle tip was inserted until it reached the mandible and then retracted 1 to 2 mm. This deep insertion could avoid injection into the superficial facial musculature.[10] The practitioner injected 0.72 mL of the test solution at each side using the 3-point injection technique below the drawn line (Figure 1).

Assessment

Photography, ultrasonography, and 3-dimensional Morpheus imaging (Morpheus Co., Ltd, Gyeonggi-do, Korea) were performed before and after the injection at the baseline and at 4, 8, 12, and 16 weeks after treatment. To reduce measurement errors, all ultrasonographic examinations were performed by the same sonographer and in the same sitting position. The probe was positioned perpendicular to the mandible, along the anterior margin of the masseter muscle, on the imaginary line connecting the lateral commissure of the lip and the intertragic notch. The thickness of the masseter muscle was measured at the maximal clenching and resting positions. The mean value of 3 repeated measurements was calculated. Three-dimensional Morpheus imaging was performed to determine the lower face volume at maximal clenching. In addition, the participants were asked to rate their satisfaction on a scale of 1 (very dissatisfied), 2 (dissatisfied), 3 (somewhat dissatisfied), 4 (neutral), 5 (somewhat satisfied), 6 (satisfied), or 7 (very satisfied). Jaw functional limitation scale (JFLS-8) was used to check discomfort related to injection. Any adverse effects were recorded.

Statistical analyses were performed using SAS Version 9.4 (64-bit; SAS Institute, Cary, NC). Intragroup comparisons before and after treatments were performed using a paired *t*-test or a Wilcoxon signed-rank test. Intergroup comparisons were performed using analysis of covariance, Pearson chi-squared test, or Fisher exact test. Data are presented as the means ± SD. p Values of <0.05 were considered statistically significant.

Results

Reduction in Masseteric Thickness at Maximal Clenching

The mean masseteric muscle thickness at maximal clenching before injection in Groups A, B, C, D, and E was 14.49 ± 1.42, 15.07 ± 1.85, 14.94 ± 1.55, 15.19 ± 1.43, and 15.14 ± 1.29 mm, respectively. The percent changes from the baseline at 4, 8, 12, and 16 weeks after injection were as follows: placebo group (Group A): −4.37 ± 4.42%, −5.31 ± 4.4%, −4.63 ± 4.4%, and −3.93 ± 4.53%; 24-unit group (Group B): −10.42 ± 7.93%, −11.36 ± 9.64%, −12.33 ± 9.85%, and −10.11 ± 8.56%; 48-unit group (Group C): −11.85 ± 8.06%, −15.26 ± 5.35%, −16 ± 6.77%, and −14.03 ± 5.81%; 72-unit group (Group D): −14.3 ± 5.93%, −17.81 ± 5.69%, −20.33 ± 5.46%, and −14.61 ± 5.7%; and 96-unit group (Group E): −15.98 ± 8.21%, −20.78 ± 7.98%, −22.42 ± 7.53%, and −16.13 ± 7.26%, respectively. Overall, muscle atrophy reached its maximum at Week 12 after treatment and declined thereafter in all treatment groups (Figure 2).

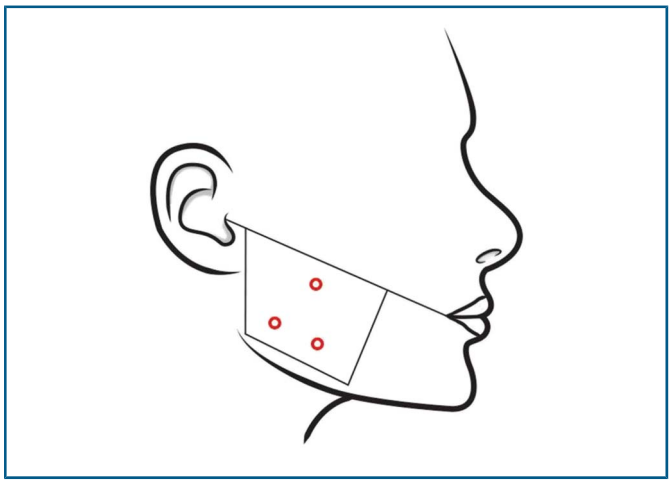

Figure 1: Injection points in the masseteric muscle.

A dose-dependent reduction in masseter thickness was observed at every visit. A significant maximal size reduction was observed at 12 weeks in all treatment groups (Group B: $p = .0114$; the other groups: $p < .0001$ vs the placebo group). Group D showed a greater decrease in thickness (−0.6 mm) at Week 12 than Group C; however, this difference was not statistically significant ($p = .0621$). Group E showed a significant reduction in thickness compared with Group C (−0.91 mm; $p = .0173$).

Reduction in Masseteric Thickness at the Resting Position

The mean masseteric muscle thickness at resting condition before injection in Groups A, B, C, D, and E was 11.07 ± 1.34, 11.64 ± 2.04, 11.34 ± 1.66, 11.44 ± 1.09, and 11.51 ± 1.13 mm, respectively. The percent changes from the baseline at 4, 8, 12, and 16 weeks after injection were as follows: placebo group (Group A): −4.81 ± 5.25%, −5.99 ± 4.77%, −5.18 ± 4.72%, and −5.12 ± 6.47%; Group B: −9.15 ± 8.05%, −12.18 ± 10.31%, −12.9 ± 12.04%, and −11.94 ± 11.19%; Group C: −8.87 ± 9.26%, −12.43 ± 8.5%, −14.67 ± 10.75%, and −13.18 ± 8.23%; Group D: −12.57 ± 8.81%, −15.81 ± 7.12%, −17.55 ± 9.16%, and −15.35 ± 9.14%; and Group E: −11.94 ± 8.67%, −17.17 ± 6.38%, −20.53 ± 8.37%, and −16.29 ± 8.93%, respectively. Similar to the results observed for the maximal clenching position, a maximum masseter thickness reduction was observed at Week 12. However, at 16 weeks, the recovery in thickness was less, and the thickness was lesser than that at the maximal clenching position. A dose-dependent reduction was observed in every visit. The reduction in masseteric muscle thickness at the resting position was greater than that observed during maximal clenching, and this reduction was independent of the dosage and duration of treatment.

Three-Dimensional Imaging Analysis of Lower Face Volume

Compared with the placebo group, PRA-treated groups showed a dose-dependent reduction in lower face volume by −4.67% to 6.63%, −6.28% to 9.47%, −6.33% to 10.72%, and −5.67% to 10.55% at weeks 4, 8, 12, and 16, respectively. All treatment groups showed a significant lower face volume reduction compared with the placebo group (Groups B, C: $p < .01$; Groups D, E: $p < .0001$). The volume of the lower face decreased proportionally with the increase in toxin dosage. The percent changes in lower face volume from the baseline were maximal at Week 12, at which maximal thickness reduction was also observed by ultrasonography. The percent changes in lower face volume in Groups A, B, C, D, and E were −1.81 ± 2.4%, −6.33 ± 3.58%, −8.56 ± 2.99%, −9.73 ± 3%, and −10.72 ± 4.97%, respectively (Figure 3).

Patient Satisfaction

Participants who were satisfied (Grade 6) or very satisfied (Grade 7) were classified as the "satisfied group." The percentages of participants in the satisfied group at Week 4 in Groups A, B, C, D, and E were 11.11%, 27.78%, 50%, 55.56%, and 47.06%, respectively. Subjects in Groups C, D, and E showed significantly higher satisfaction than those in the placebo group. A similar trend was observed at Week 8. At Week 12 after injection, 11.11% (Group A), 44.44% (Group B), 66.67% (Groups C and D), and 77.78% (Group E) of the participants were satisfied with the result, suggesting that a dose of 24 units might exhibit maximal effects at Week 12 (Group B: $p < .05$; Groups C, D, and E: $p < .001$ compared with the placebo group).

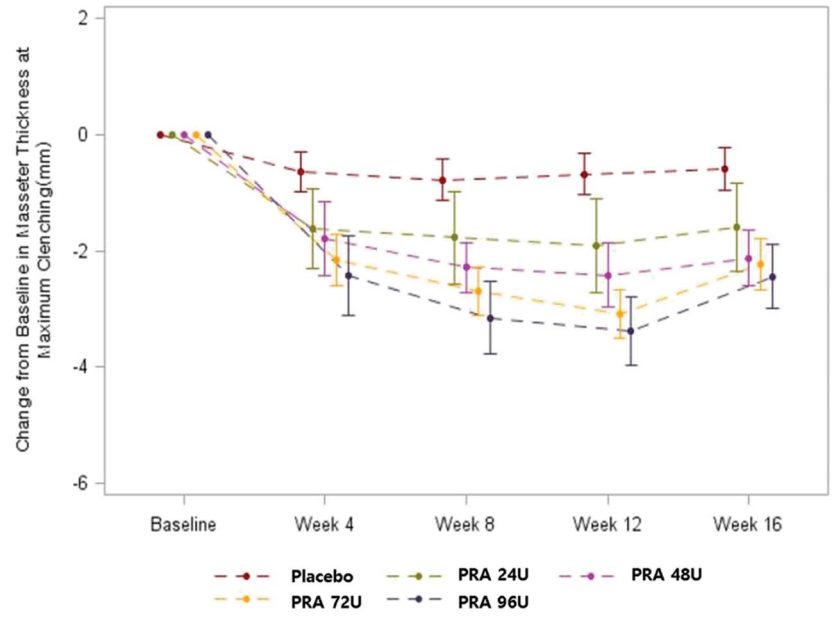

Figure 2: Change from the baseline in masseter thickness during maximal clenching recorded at each visit.

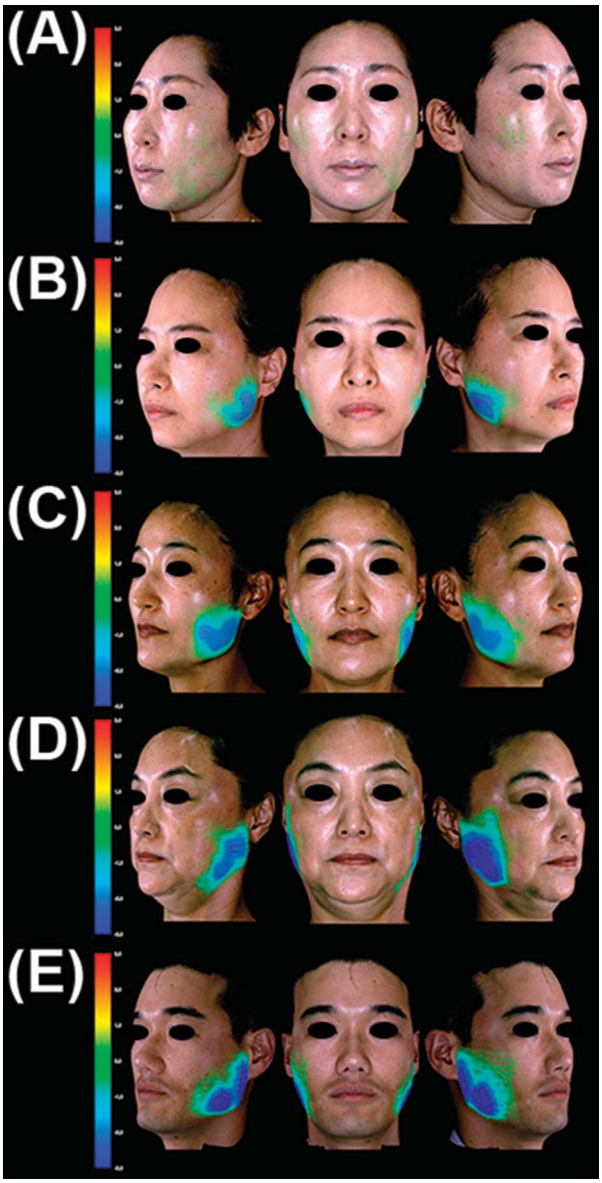

Figure 3: Three-dimensional images of representative patients in each group at Week 12. (A) Placebo (normal saline), (B) 24-unit prabotulinium toxin A (12 units/side), (C) 48-unit prabotulinium toxin A (24 units/side), (D) 72-unit prabotulinium toxin A (36 units/side), and (E) 96-unit prabotulinium toxin A (48 units/side). Program-defined heat map reference ranges from dark blue to red, where dark blue shows the greatest compression.

Safety

The mean JFLS scores before injection in Groups A, B, C, D, and E were 0.15 ± 0.29, 0.13 ± 0.23, 0.08 ± 0.14, 0.18 ± 0.26, and 0.09 ± 0.1, respectively. No significant changes from the baseline were observed in any treatment group at any time point, except for Group E. The mean JFLS scores of Group E at 4, 8, and 12 weeks after injection were 0.34 ± 0.45, 0.3 ± 0.4, and 0.26 ± 0.36, respectively, with significant differences from the baseline (p = .0078, .0078, and 0.043, respectively). The JFLS score decreased over time and returned to the baseline value by Week 16. Two patients, one from the 48-unit group and one from the 96-unit group, reported instant masseter muscle pain that recovered without further complications. The relatively common side effects, such as asymmetric smile and unnatural facial expressions, were not observed in this study. No other adverse drug reactions were observed.

Discussion

Masseter hypertrophy can be treated by either surgical or nonsurgical modalities. In surgical procedures, it is difficult to assess the amount and depth of resection of the masseter muscle; hence, it is challenging to predict the outcome of the procedure.[11] Furthermore, the processes involved are complex and may cause postoperative complications, such as hematoma, facial nerve damage, and masticatory function limitation.[12]

Botulinum toxin is widely used in the cosmetic field for facial wrinkle treatment and facial rejuvenation.[13] Intramuscular injection of botulinum toxin blocks the release of acetylcholine, thereby inducing temporary denervation at the neuromuscular junction.[14] This phenomenon leads to muscular atrophy and decreased bulk, which are reported to last for approximately 6 to 9 months.[7,14] Therefore, botulinum toxin has been used as an alternative to permanent surgery for reducing prominent masseteric bulk.

Although botulinum toxin has relatively few complications and a short downtime, a proper injection method is mandatory to achieve optimal outcomes. Understanding facial anatomy and facial expressions is particularly important to avoid unnaturalness and discomfort after procedure. Sunken cheeks are sometimes observed after the procedure. This deformity might result from placement of the injection points too high or too close to the anterior border of the masseter muscle.[3,14] In addition, unintended injection of botulinum toxin into the zygomaticus major or risorius muscle can result in an asymmetric smile. In contrast to other ethnic groups, who rarely have the risorius muscle,[15] Korean cadaver specimens commonly have the risorius muscle that partially covers the masseter muscle.[16] Considering this anatomical variation, a sufficiently deep insertion of the needle within the safety zone along the lower and posterior margins of the masseter muscle is required to avoid injection into the superficial musculature (Figure 1).

In this randomized, controlled, double-blind study, the authors showed the dose- and time-related efficacy of PRA for the treatment of masseteric hypertrophy. Muscular atrophy reached its maximum at Week 12, after which it showed a steady recovery, which was independent of the injected PRA dose. This finding implied that clinicians might need to consider the optimal timing for "retouching." The authors suggested that waiting for more than 12 weeks to decide whether to perform the retreatment because it is still unclear if the peak efficacy is reached. This finding is in line with the results of previous studies. To and colleagues[17] reported that the maximal masseteric atrophy (30.9%) occurred at 3 months after injection and then decreased to 13.4%. Additionally, Park and colleagues[2] showed that masseter muscle thickness gradually decreased during the

first 3 months after treatment and then showed a slight increase.

Second, reduction in masseter thickness in the resting position was greater than that in the maximal clenching position, independently of the injected dose and time points. This finding indicated that botulinum toxin injection could effectively smoothen the wide angle of the lower face without interrupting essential mastication. The recovery rate of atrophy after Week 12 was slower in the resting position than in the maximal clenching position, suggesting that patient satisfaction with lower face slimming might persist for longer than 16 weeks despite slight recovery because people do not maintain a maximal clenching position in daily life.

Third, a dose-dependent reduction in masseter thickness was observed at both the resting and maximal clenching positions. Masseter thickness decreased more drastically during maximal clenching, and this effect was observed as early as 4 weeks after injection in all PRA-treated groups. Interestingly, a slight reduction in masseter thickness was also observed in the placebo group. Similarly, a previous study carried out by Park and colleagues[18] showed a nonsignificant decrease in masseteric muscle thickness in the control group. The authors presumed that the injected volume of normal saline in the masseter muscle might have teared some of the muscle fibers, thereby hindering complete clenching. However, further studies are needed to clarify the mechanism. At the resting position, only high-dosage groups (D, E) showed a significant thickness reduction within 4 weeks of treatment. Interestingly, a dose of 24 units resulted in a significant thickness reduction at Week 4 during maximal clenching and at Week 8 in the resting position, as well as patient satisfaction at Week 12; however, masseter thickness increased at Week 16. These results suggested that 24 units of PRA might be insufficient for treating square face problems. In the cosmetic field, self-assessment and patient satisfaction are very important parameters for evaluating the success of a treatment. Considering the real-world situation, a dosage higher than 24 units is needed. In this study, the 96-unit group showed a significantly increased JFLS score, which could cause discomfort in jaw function among patients. Considering the cost-effectiveness and safety, a dose of 48 to 72 units of botulinum toxin is recommended for masseteric hypertrophy. For patients who want quicker results, a 72-unit injection might be recommended. A higher dose might be considered in case of severe hypertrophy or subjective discomfort involving tooth grinding and jaw pain.

Our study took advantage of 3-dimensional imaging for the visual analysis of lower face bulkiness. This technique enabled us to evaluate the actual slimming effects of botulinum toxin, in addition to its effect on 2-dimensional thickness. A gradual contouring effect was observed as early as Week 4, which correlated with patient satisfaction.

The present study has some limitations. First, the authors only included Korean subjects; thus, ethnic differences are not considered. Second, this optimal dose-finding study was designed for 16 weeks to compare the maximal efficacy among different groups because the peak improvement occurs around 12 weeks. To check the sustainability and long-term safety, the authors will perform a Phase III study with a 6-month follow-up period. Finally, the authors did not compare the relative efficacy of PRA with that of other botulinum toxins.

Conclusion

In conclusion, PRA could effectively improve lower face contour, without major complications, and downtime. The optimal dose of PRA ranged from 48 to 72 units, and retreatment should ideally be considered after 12 weeks.

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