The Effect of Chemodenervation by Botulinum Neurotoxin on the Degradation of Hyaluronic Acid Fillers: An Experimental Study

İsmail Küçüker, M.D.
Ibrahim Alper Aksakal, M.D.
Ahmet Veysel Polat, M.D.
Murat Sinan Engin, M.D.
Engin Yosma, M.D.
Ahmet Demir, M.D.

Samsun, Turkey

Background: Early degradation is a common complaint for hyaluronic acid fillers. Although the combination of hyaluronic acid fillers with botulinum neurotoxin type A presented improved clinical results, objective measurement of hyaluronic acid volumes has not been previously assessed.
Methods: In this study, the authors have split the calvaria of the rabbit to mimic the glabellar region in humans. In this model, the authors applied hyaluronic acid alone to one side and hyaluronic acid combined with botulinum neurotoxin type A to the contralateral side. Two days and 3 months after the filler injection, magnetic resonance imaging was performed to assess the filler volumes.
Results: Average initial volume of filler only and filler combined with botulinum neurotoxin type A was 0.61 cm^3 on both sides, and there was no difference between initial volumes of the two sides ($p = 0.735$). At the end of 3 months, average degraded volumes of filler-only and filler combined with botulinum neurotoxin sides were 0.33 cm^3 and 0.19 cm^3, respectively, and the degradation difference was significant between the two groups ($p = 0.001$). End volumes for the filler-only and filler combined with botulinum neurotoxin sides were 0.28 cm^3 and 0.42 cm^3, respectively, and end volumes between two sides were also statistically significant ($p < 0.001$).
Conclusion: This study showed that hyaluronic acid filler application in combination with botulinum neurotoxin type A significantly decreases the degradation process and increases the remaining volume of the hyaluronic acid fillers at the end of the paralyzed period. (*Plast. Reconstr. Surg.* 137: 109, 2016.)

According to the 2013 reports of the American Society of Plastic Surgeons, the total number of surgical and nonsurgical procedures is over 11 million, and nearly 9.5 million of these are nonsurgical procedures.[1] Among these procedures, application of botulinum neurotoxin is the most common one, with 3.76 million applications, which is followed by 1.87 million applications of hyaluronic acid fillers. In total, botulinum neurotoxin and hyaluronic acid filler applications cover nearly half of the total applications.[1]

In daily practice, one of the most common complaints for hyaluronic acid fillers is early degradation of the products. Besides the structural properties of the products, trauma such as that resulting from strong muscular contracture also affects the longevity of these fillers. Because of this, combination of these fillers with botulinum neurotoxin type A is common, and improved clinical results have been presented in different clinical studies.[2–10] Although improved clinical results were published in these series, objective measurement of hyaluronic acid volumes was not assessed in any of them when they were applied alone or in combination with botulinum neurotoxin type A.

In this study, we have split the calvaria of the rabbit to mimic the glabellar region in humans. On the rabbit calvarial model, we applied hyaluronic acid alone over the anterior auricular muscle to one side and hyaluronic acid combined with

From the Plastic Surgery and Radiology Departments, Ondokuz Mayis University Faculty of Medicine.
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botulinum neurotoxin type A on the contralateral side. Two days and 3 months after the filler injection, magnetic resonance imaging was performed to assess the initial and end volumes of the fillers on both sides.

MATERIALS AND METHODS

This study was approved by the Institutional Ethics Committee for Animal Studies. Eight male New Zealand White rabbits weighing between 1.5 and 2 kg were used in the study. Rabbits were kept under standard laboratory conditions with free access to food and water. For surgical procedures, rabbits were anesthetized with an anesthetic cocktail consisting of 1.5 ml of ketamine (100 mg/ml), 0.5 ml of acepromazine (10 mg/ml), and 0.5 ml of xylazine (20 mg/ml). Each animal received 0.5 ml/kg of the anesthetic cocktail intramuscularly.

The tonus of the skin overlying the anterior auricular muscle is very similar to the tonus of the skin that overlies the glabellar area. The anterior auricular muscle originates from the frontal bone approximately 2 cm cranial to the medial canthus, and it inserted along the ventromedial border of the ipsilateral ear, approximately 3 cm from the base of the ear. This muscle holds the rabbit's ear in upright position and is vital for the erect stance of the ears.[11] As it is very close to the midline and is symmetric on both sides, this muscle and overlying soft-tissue coverage were used to mimic the corrugator muscles and the glabellar skin in humans.

For the application of botulinum neurotoxin type A, the left periauricular area was shaved over the muscle. Animals received an injection of 2.5 U (0.05 ml) botulinum neurotoxin type A (Botox; Allergan, Inc., Irvine, Calif.) into the left anterior auricular muscles with a single injection as described in a previous study.[11] One week after botulinum neurotoxin type A application, left ears of the rabbits were dropped and ear movements were significantly reduced (Fig. 1).

As muscle was paralyzed, both periauricular areas were shaved over the muscle and 0.5 cc of soft-tissue filler containing 20 mg/ml hyaluronic acid (Restylane; Q-Med, Uppsala, Sweden) was administered over the medial part of the anterior auricular muscle on both sides (Fig. 1). Photographs were obtained for the overall observation of the external surface just after filler application and 3 months later.

Magnetic resonance imaging examination was first performed 2 days after the filler application for determination of the initial volume. Magnetic resonance imaging was performed with a 1.5-T magnetic resonance scanner (Magnetom Symphony; Siemens Medical Systems, Erlangen, Germany) using a head coil. Each examination was obtained by T2-weighted turbo spin echo sequence (repetition time, 5590 msec; echo time, 94 msec; field of view, 133 × 370 mm; slice thickness, 1.5 mm; and matrix, 92 × 256) in the axial plane. The total time required for the magnetic resonance imaging, including preparing the rabbit, was 12 minutes. Before examination, the animals were anesthetized with xylazine (7 mg/kg intramuscularly) and ketamine (40 mg/kg intramuscularly). Magnetic resonance imaging examination was performed with the same parameters with an interval of 3 months. For the analyses, images were transferred to a dedicated offline Leonardo computer workstation (Flexline, San Jose, Calif.). On magnetic resonance imaging scans, injected hyaluronic acid filler volumes presented as hyperintense areas on T2-weighted images. The hyperintense area with the largest diameter was measured in three dimensions and then the filler volume was calculated by using the ellipsoid volume formula ($4/3\ \pi \times$ width axis radius $\times$ length axis radius $\times$ height axis radius) as described in a previous study.[12] The same experienced radiologist performed all image analysis and calculations (Fig. 2). After descriptive statistical analysis, the Mann-Whitney U test was performed for comparison of the data, and values of $p < 0.05$ were accepted as statistically significant.

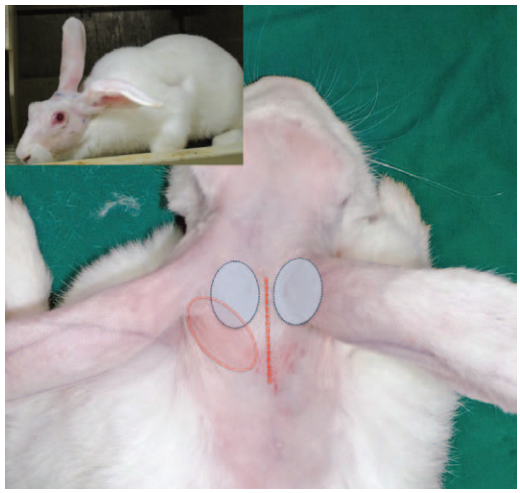

Fig. 1. *Blue ovals* demonstrate filler application sides and *red oval* indicates botulinum neurotoxin type A application side. (*Inset*) The left ear is dropped and its movements are significantly decreased 1 week after chemodenervation with botulinum neurotoxin type A.

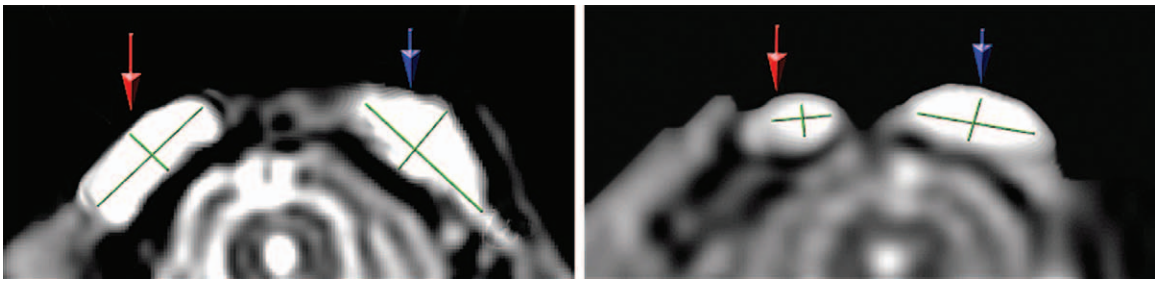

Fig. 2. (*Left*) Magnetic resonance imaging scan obtained 2 days after filler application; filler volumes are similar. (*Right*) Magnetic resonance imaging scan obtained 3 months after filler application; filler volume is higher in the left hemicranium. Fillers combined with botulinum neurotoxin type A degrade less than isolated filler applications.

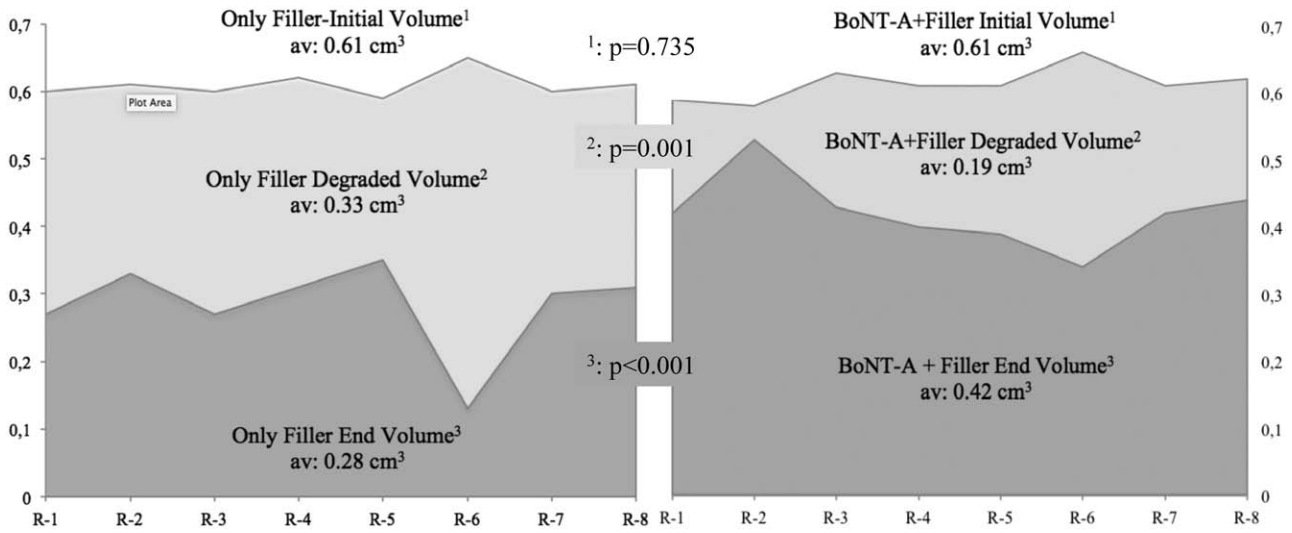

Fig. 3. Initial, degraded, and end volumes of filler-only and botulinum neurotoxin type A combined filler sides in a rabbit model. *BoNT-A*, botulinum neurotoxin type A; *R*, rabbit.

RESULTS

Average initial volumes of filler only and filler combined with botulinum neurotoxin type A were 0.61 cm^3 on both sides, and there was no difference between initial volumes of the two sides ($p = 0.735$). At the end of 3 months, average degraded volumes of filler only and filler combined with botulinum neurotoxin were 0.33 cm^3 and 0.19 cm^3, respectively, and the degradation difference was significant between the two groups ($p = 0.001$). End volumes for the filler-only and the filler combined with botulinum neurotoxin sides were 0.28 cm^3 and 0.42 cm^3, respectively, and end volumes between the two sides were also statistically significant ($p < 0.001$). Initial, degraded, and remaining volumes of the fillers at the third month are summarized in Figure 3. On photographs, external surfaces were similarly filled in the first week, but the filler was prominent on the paralyzed part at the end of 3 months (Fig. 4).

DISCUSSION

Naturally occurring hyaluronic acids are universally present in all living organisms as part of the extracellular matrix. As the structure of hyaluronic acid is uniform among the living species, its immunogenicity is less compared with other fillers.[7,13] Related to this, hyaluronic acid fillers are the most commonly used fillers in the medical industry.[1,7] Native forms cannot be used because of their short half-lives, and are usually degraded in 1 or 2 days.[7] Thus, cross-links are added to the carbon chain to decrease water solubility and thereby decrease the degradation of these fillers.[7] Moreover, the number of cross-links, in addition to the concentration of hyaluronic acid, affects the properties of hyaluronic acid fillers. As the concentration of the hyaluronic acid and the number of cross-links increases, longevity, stability, gel hardness, and ability to resist dilution also increase. Besides intrinsic properties of hyaluronic acid fillers, some external factors such as trauma can also

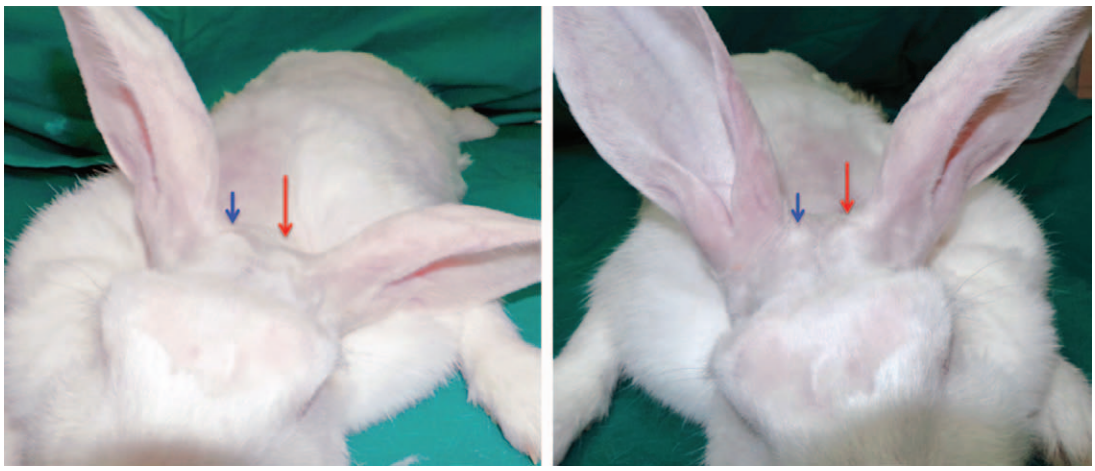

Fig. 4. External surface photographs of rabbit 6. Photographs obtained (*left*) just after and (*right*) 3 months after the filler injection. *Blue arrows*, filler-only side; *red arrows*, filler combined with botulinum neurotoxin type A side.

lead to early degradation of these products.[8] Muscular contraction forms a major trauma on the hyaluronic acid fillers, and degradation occurs faster on areas of contraction.

Muscle paralysis can be managed in two basic ways: mechanical denervation and chemical denervation. In mechanical denervation, motor nerves of the muscles are cut before entering the muscle. Although this technique leads to precise muscle paralysis, as it requires a surgical intervention, mechanical denervation of the facial muscles is not used in routine daily practice. Besides mechanical denervation, facial muscles can also be paralyzed by chemodenervation. Easily obtainable agents such as lidocaine can lead to muscle paralysis by reversible chemodenervation; however, after a short period, muscle movement returns and reapplication is needed. Botulinum neurotoxin type A is a polypeptide that irreversibly inhibits acetylcholine release at the neuromuscular junction, which results in flaccid chemical denervation.[14] After 3 months, the effects of botulinum neurotoxin type A begin no normalize by axonal resprouting.[14] As it causes long-term muscle paralysis with a single administration, it has been used for years for the correction of facial rhytides. Botulinum neurotoxin type A causes irreversible chemodenervation and prevents muscle contraction; it decreases the traumatic degradation of the hyaluronic acid fillers.[8] Because of this, botulinum neurotoxin and hyaluronic acid fillers are commonly combined in clinical practice.[2–10]

In the literature, combination of hyaluronic acid fillers and botulinum neurotoxin type A is commonly studied, and improved clinical results have been reported.[2–10] In 2003, Carruthers et al.[2,3] published two articles related to the combination of hyaluronic acid fillers and botulinum neurotoxin type A. In the first study, they applied hyaluronic acid fillers alone in one group of patients; in the second group, they applied hyaluronic acid fillers in combination with botulinum neurotoxin type A. In this study, they presented improved and longer clinical outcome in the combination group. In the second study, they compared the effectiveness of combination therapy in mild, moderate, and severe glabellar rhytides, and they presented more effective results with combination therapy in moderate and severe glabellar rhytide patients. In 2013, Dubina et al.[5] split the face and applied only filler to one side and a combination of filler and botulinum neurotoxin type A to the other side, and presented better and longer lasting outcomes according to clinical assessments.

In our study, we have used the dorsum of the calvaria of the rabbit to mimic the glabellar region. Like the corrugator muscles, the anterior auricular muscle in rabbit ear is a symmetric and very active muscle. It is vital for the movement and erect stance of rabbits' ears.[11] As the anterior auricular muscle is paralyzed, the movements of the affected ears decrease dramatically. Thus, mechanical trauma related to ear movements can be prevented on one side, and the effect of the combination therapy can be studied.

Although improved clinical results have been published in previous clinical articles, the associated cause of this improved effect is not clearly explained.[2–10] When we reviewed the literature for a relationship between botulinum neurotoxin type A and hyaluronic acid fillers, we could obtain no data showing a possible biochemical correlation. Thus, as also hypothesized in previous articles, mechanical trauma related to ear movements

becomes the most possible cause of the early degradation of the filler on the nonparalyzed side.

We photographed the rabbits to see the differences on external surfaces on the paralyzed and nonparalyzed sides. Although external surfaces seemed similar just after filler application, the external surface of the paralyzed side was more prominent on the photographs obtained 3 months after treatment. In addition to the photographs, we also obtained magnetic resonance imaging scans. Magnetic resonance imaging is the gold standard technique for determining the volumes of these soft-tissue fillers and was also used in previous studies.[12] Although there were clinical studies that presented better clinical outcomes with clinical and photographic assessment protocols, the volume of hyaluronic acid fillers in combination therapy was not studied before. This is the first study that objectively measures the degraded and remaining volumes of the hyaluronic acid fillers with magnetic resonance imaging when they are applied alone or in combination with botulinum neurotoxin type A. In our study, when the combination therapy group was compared with the filler-only group, we showed that chemodenervation with botulinum neurotoxin type A decreases the degradation rate of the hyaluronic acid fillers by 42 percent and increases the remaining volume of the filler by 50 percent. Although we also obtained photographs for a visual comparison, the area is so small and covered with hair; therefore, we could not do an objective comparison on photographs.

CONCLUSION

This study showed that hyaluronic acid filler application in combination with botulinum neurotoxin type A significantly decreases the muscular movements and thereby decreases the degradation process and increases the remaining volume of the hyaluronic acid fillers at the end of the paralyzed period.

İsmail Küçüker, M.D.
Plastic Surgery Department
Ondokuz Mayıs University Faculty of Medicine
Samsun, Turkey
drismailkucuker@yahoo.com.tr

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