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Case Series

Sensitivity of Hyaluronic Acid Fillers to Hyaluronidase: An *in vitro* Analysis

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ABSTRACT

Objective: The purpose of this study was to provide qualitative information on the *in vitro* dissolution of hyaluronic acid (HA) fillers upon exposure to hyaluronidase and to determine whether *in vitro* sensitivity of fillers relates to clinical findings in patients treated with hyaluronidase to manage complications of HA filler placement.

Methods: The authors undertook an *in vitro* study to evaluate how 6 types of HA fillers respond to hyaluronidase exposure. The findings were compared to outcomes in 3 clinical cases in which hyaluronidase was given to manage adverse outcomes of HA injection.

Results: The fillers responded differently to the same dose of hyaluronidase. Fillers with a higher concentration of HA or a greater degree of crosslinking generally were more resistant to enzymatic dissolution. Clinical findings were consistent with *in vitro* results.

Conclusion: The sensitivities of HA fillers to hyaluronidase *in vitro* were consistent with clinical findings.

Keywords: Filler; Hyaluronic acid; Hyaluronidase

INTRODUCTION

Hyaluronic acid (HA) is a glycosaminoglycan disaccharide that occurs naturally in numerous anatomic compartments, including the skin. HA was approved for clinical use by the US Food and Drug Administration (FDA) in 2003, with Restylane (Galderma, Q-Med AB, Uppsala, Sweden) and Juvederm (Allergan, Irvine, CA) being among the first commercially available products [1]. Placement of HA filler has become a popular choice for restoration or augmentation of tissue volume and for skin rejuvenation [1,2]. In 2018, injection of HA was the second most frequently performed nonsurgical technique, with over 800,000 procedures completed [3].

Complications of HA placement

Injection of HA filler can yield untoward aesthetic results, such as in cases of overfilling/overcorrection, placement of filler too superficially or too deeply, misplaced injection, or poor choice of filler product [1,2]. These technical errors can result in subcutaneous nodules or asymmetry. In the periocular region, these complications are amplified by the delicate lymphatic system that affords little liquid drainage, and filler placement too superficially can lead to bluish discoloration known as the Tyndall effect. More serious complications include cutaneous ischemia, secondary to filler injection into the vasculature, and consequent necrosis. Additionally, immune-associated adverse events of filler placement, such as acute or delayed hypersensitivity reactions, are possible [1].

Hyaluronidases

Hyaluronidases are a family of naturally occurring enzymes that depolymerize HA. The US FDA approved hyaluronidase as an adjuvant in multiple settings, including as a means to increase the dispersion and absorption of other injected drugs. In a randomized clinical trial, injected hyaluronidase was shown to readily hydrolyze HA filler placed subcutaneously [4]. Although administration of hyaluronidase to reverse the effects of HA filler is an off-label indication [5], this technique has been recommended as an effective way to manage overcorrection, vascular occlusion, and filler misplacement, as well as late-occurring complications, such as nodules and persistent edema [6].

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Four formulations of hyaluronidase are currently available: a human recombinant agent (Hylenex [Halozyme, San Diego, CA]), an ovine agent (Vitrase [Bausch+Lomb, Rochester, NY]), and 2 bovine agents (Amphadase [Amphastar Pharmaceuticals, Rancho Cucamonga, CA] and Hydase [Akorn, Inc, Lake Forest, IL]). For treatment of complications associated with HA filler placement, hyaluronidase doses ranging from less than 5 units (U) to over 300 U have been applied [2]. Results of an *in vitro* toxicity study suggested that purified ovine hyaluronidase, at varied concentrations and exposure times, did not affect viability of human fibroblast cells [7]. A nonsignificant trend toward decreased viability of human skin was found at high doses only (6.5 U and 14 U of hyaluronidase), but this was potentially attributed to the limited presence of nutrients [7]. Vartanian et al. carried out a prospective study of 8 patients and found that 50 U/mL hyaluronidase (ie, 5-10 U hyaluronidase in a 0.1-0.2 mL volume) is appropriate as an initial dose in a non-emergent situation [4].

There are no established guidelines for administering hyaluronidase to manage complications of HA filler injection, including dose, reconstitution solvent, volume of diluent, timing of injection, or injection technique [2]. In addition, the effectiveness of hyaluronidase-mediated dissolution of filler appears to be dependent on variables such as the type of hyaluronidase, the pH, the manufacturer's formulation, and the dilution. Patient characteristics also can influence effectiveness. For instance, prior bee or wasp stings can predispose a patient to a hypersensitivity reaction to hyaluronidase, and certain medications, including aspirin and antihistamines, can reduce the responsiveness of filler-injected tissues to hyaluronidase [2]. Furthermore, commercially available hyaluronidases are produced in compounding pharmacies in many countries, and the purity, stability, and osmolar equilibrium may be unknown [1].

Filler technologies

In addition to enzyme- and patient-centered variables, the technology of HA fillers may influence its dissolution by enzyme hydrolysis. Despite being an excellent filler material, the naturally occurring form of HA has an impractically short half-life [1]. To address this, manufacturers have modified HA chemistry (eg, with covalent crosslinking) to enhance its stability, resilience, and tissue persistence, without altering the molecule so much that it triggers immune-mediated adverse events [1]. Current commercial preparations of HA gel resorb or biodegrade over 6 to 18 months and have a <1% risk of hypersensitivity [2]. HA formulations differ in terms of monophasic or biphasic consistency, degree of crosslinking, and HA concentration; these variations give rise to different rheologic characteristics, clinical indications, and degradation/resorption times [8].

HA filler products can be classified as monophasic or biphasic, where monophasic products (eg, Juvederm, Belotero [Merz Pharmaceuticals, Frankfurt, Germany]) are cohesive, stable gels, and biphasic products (eg, Restylane) comprise particles suspended in a gel [1,9]. Biphasic formulations are thought to disintegrate more readily in the presence of hyaluronidase [1]. It should be noted that some authors have deemed the monophasic/biphasic categorization scientifically faulty, finding that both Juvederm (deemed monophasic) and Restylane (deemed biphasic) contained both microscopically observable gel particles and extractable HA [10].

A greater degree of covalent crosslinking in HA filler correlates with more resiliency, structural stability, and persistence; extensively crosslinked HA fillers also are more resistant to enzymatic hydrolysis [9]. Additionally, a higher concentration of HA in the filler preparation tends to require a greater dose of hyaluronidase for dissolution (eg, as in Juvederm [24 mg/mL] vs. Restylane [20 mg/mL]) [9].

METHODS

In vitro study design

Six types of HA filler (Macrolane; Juvederm Voluma [with lidocaine]; Juvederm Volite; Teosyal Ultra Deep; Teosyal RHA 1; Restylane) were placed onto blotting paper on a flat surface at room temperature. Each type of filler was deposited in a spherical and a linear shape, yielding 12 test samples, and each sample volume was 0.1 mL. Galenically produced, animal-derived hyaluronidase (30 U) then was applied to each sample with a sterile syringe, and dissolution of the HA sample was observed.

Clinical study design

The study was conducted in accordance with guidelines set forth in the Declaration of Helsinki. Each included patient provided informed consent. In each patient, hyaluronidase was delivered with a 30-gauge needle, 3 to 13 mm in length, positioned perpendicular to the skin [5].

RESULTS

In vitro findings

In response to the same dose of hyaluronidase, the tested HA fillers responded differently by type and according to the deposited filler shape (spherical or linear) (Figure 1). The fillers tended to dissolve more quickly when placed as a linear (rather than a spherical) shape. This difference is attributed to the different surface areas of the shapes. The spherical bolus has a lower surface: volume ratio, and therefore, less surface area exposed to enzyme.

For the Restylane and Macrolane fillers, which are prepared with non-animal stabilized HA (NASHA) technology, dissolution took more time and required additional hyaluronidase to achieve complete liquefaction (Figure 1, Table 1). After the dissolution period, the surface of the HA gel in contact with hyaluronidase was not homogeneous; this suggests that some portions of the filler dissolved faster than others. In contrast, Juvederm Volite filler, produced with Vycross technology, underwent complete and nearly instantaneous dissolution in the presence of 30 U of hyaluronidase. Fillers described as RHA (ie, resilient HA) prepared with "preserved network" crosslinking technology (Teoxane) also dissolved relatively rapidly.

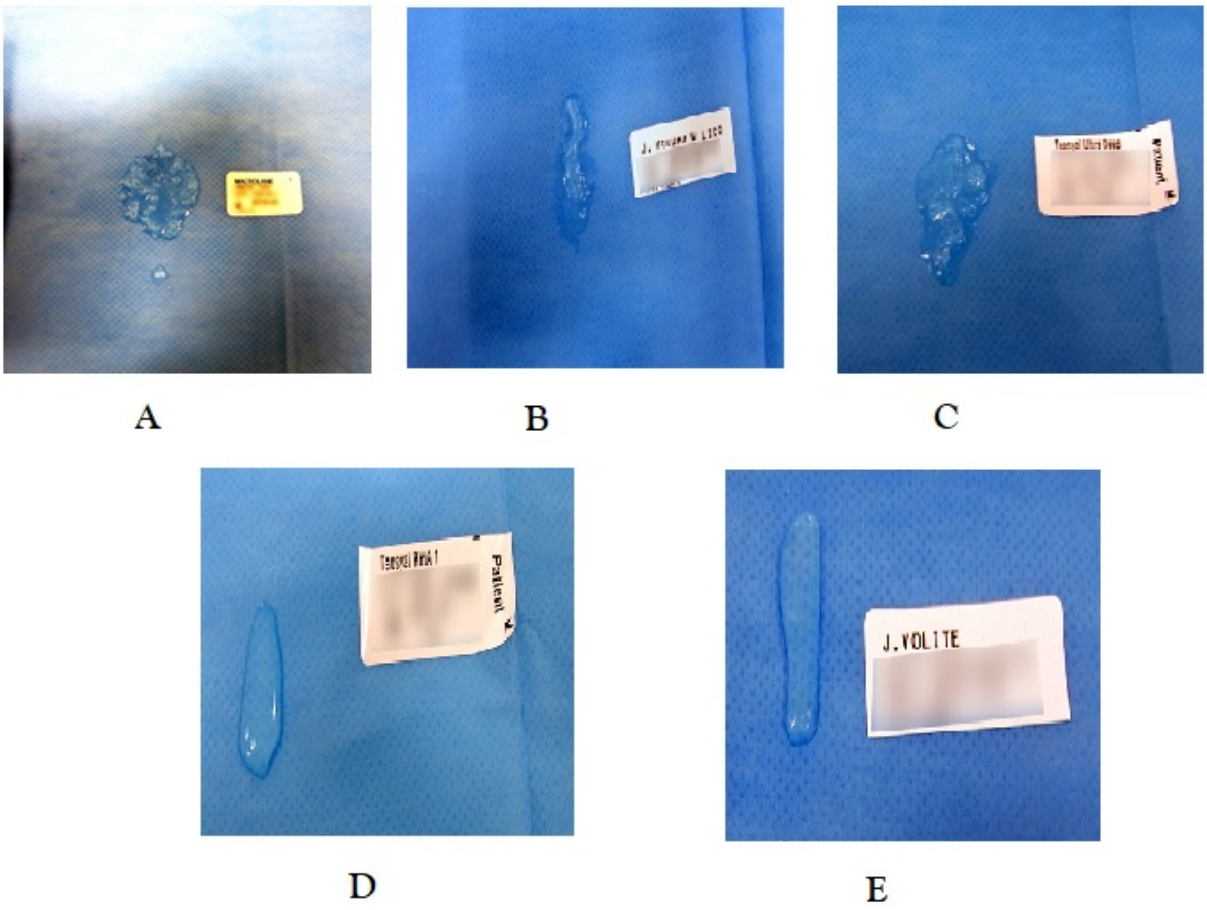


Figure 1: Sensitivity of various fillers to hyaluronidase. A 0.1-mL bolus of filler was placed onto blotting paper at room temperature, and 30 U of hyaluronidase was applied with a sterile syringe. Samples are shown after 10 minutes of digestion. (A) Macrolane (Q-Med), (B) Juvederm Voluma (Allergan), (C) Teosyal Ultra Deep, (Teoxane), (D) Teosyal RHA 1 (Teoxane), (E) Juvederm Volite (Allergan).

Table 1: *In vitro* sensitivities of hyaluronic acid fillers to hyaluronidase.

HA Filler, Product Name and Manufacturer[a]	HA Concentration (mg/mL)	Shape of Filler Bolus	Response of Filler
Macrolane	20	Spherical	Least digested
Q-Med AB		Linear	Least digested
Juvederm Voluma with lidocaine	20	Spherical	Moderately digested
Allergan		Linear	Moderately digested
Teosyal Ultra Deep	25	Spherical	Moderately digested
Teoxane		Linear	Moderately digested
Teosyal RHA 1	15	Spherical	Liquefied rapidly
Teoxane		Linear	Liquefied rapidly
Juvederm Volite	12	Spherical	Liquefied instantly
Allergan		Linear	Liquefied instantly

Restylane	20	Spherical	Least digested
Q-Med		Linear	Least digested

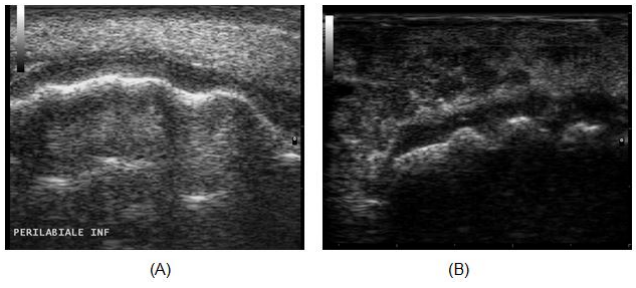
[a]0.1 mL bolus of filler was placed onto blotting paper at room temperature, and 30 U of hyaluronidase was applied with a sterile syringe.

In general, fillers with lower HA concentrations and a lesser extent of crosslinking displayed faster and more homogeneous dissolution. Conversely, the more concentrated and crosslinked fillers exhibited non-homogenous dissolution after the first administration of hyaluronidase. In these samples, a smaller, undissolved bolus of filler persisted and was only digested upon application of additional hyaluronidase (Figure 1, Table 1). This phenomenon could translate to the clinic as a need to inject hyaluronidase in more than 1 session to dissolve filler with a high HA concentration or high degree of crosslinking.

Clinical findings

Three patients who experienced complications after receiving HA filler were evaluated with ultrasonography before and after treatment with hyaluronidase. Other authors have noted that ultrasonography is a viable modality to assess the depth, quantity, and extension of injected HA filler; this information then is used to guide hyaluronidase treatment [5].

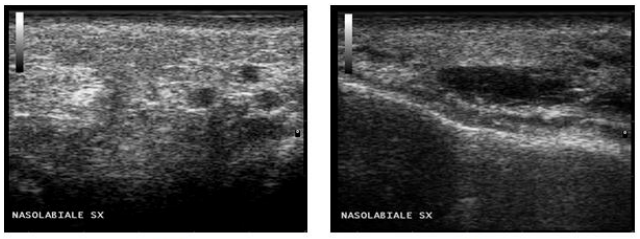
Case 1: This 47-year-old woman underwent injection of Teosyal Kiss (15 mg/mL) in the lips. One week after filler placement, the patient presented with hyschemia, edema, fibrosis, pain, and necrosis. Findings of ultrasonography indicated hypoechoic areas (Figure 2). The patient was treated with 150 U of hyaluronidase as well as antibiotics and steroids. Six days later, the patient was evaluated and was given an additional 250 U of hyaluronidase. Repeat ultrasonography findings performed 3 weeks later indicated resolution of fibrosis and only minor edema persisting around the hypoechoic area.



(A) (B)

Figure 2: (A) Ultrasonographic findings of case 1 before treatment with hyaluronidase and (B) Ultrasonographic findings of case 1 after treatment. The patient received Teosyl Kiss (Teoxane; 15 mg/ml) in the lips.

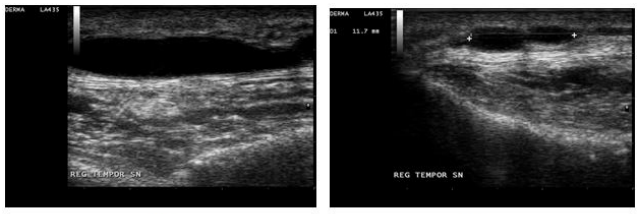
Case 2: This 53-year-old woman received Juvederm Ultra 4 (24 mg/mL) to treat the nasolabial folds (0.3 mL per side) and experienced hyschemia on the left side (Figure 3). She presented 10 days after filler placement. Ultrasonography results demonstrated the presence of deep hypoechoic nodules. The patient received 100 U of hyaluronidase along with oral antibiotics and oral steroids. Follow-up ultrasonography findings 9 weeks after hyaluronidase treatment demonstrated full recovery.



(A) (B)

Figure 3: (A) Ultrasonographic findings of case 2 before treatment with hyaluronidase and (B) Ultrasonographic findings of case 2 after treatment. The patient received Juvederm Ultra 4 (Allergan, 24 mg/mL) in the nasolabial folds.

Case 3: This 30-year-old man received Macrolane (20 mg/mL) treatment and presented with a large anechoic bolus in the temporal area that extended bilaterally (Figure 4). The larger extension was >5 cm in diameter. The patient also had dislocation of the parietal lobe on the left side. He was treated with 250 U of hyaluronidase. By 8 weeks posttreatment, the volume of the bolus had substantially decreased, with the larger size being 2 cm in diameter.



(A) (B)

Figure 4: Ultrasonographic findings of case 3 (A) before treatment with hyaluronidase and (B) after treatment. The patient was treated with Macrolane (Q-Med, 20 mg/mL).

DISCUSSION

Reversibility is an attractive feature that has contributed to the popularity of HA fillers [1]. Our findings suggest that establishment of a single protocol for dissolution of HA filler with hyaluronidase is not feasible. Instead, the practitioner should approach the problem algorithmically, considering the type of filler, the site of injection, and the patient's outcomes when selecting the type and dose of hyaluronidase and the delivery technique. Regarding the filler type, the concentration of HA and the extent of crosslinking appear to be the primary

determinants of sensitivity to hyaluronidase, both *in vitro* and *in vivo*. In general, we recommend delivering an initial hyaluronidase dose and then following up in 1 or more subsequent sessions (e.g. at 3-week intervals) to determine whether additional hyaluronidase is needed [5].

Although there is no standard hyaluronidase dose, several authors have made summary recommendations, based on favorable results of other studies. Cohen et al. found that injection of 10 to 75 U usually is appropriate to hydrolyze 0.2 to 1.5 mL of HA gel [2]. Previously, we noted that 10 to 20 U of hyaluronidase typically is sufficient to treat an affected area of the face and that more than 200 U per treatment should be avoided [5]. DeLorenzi 2013 advised a starting amount of 150 U in patients with no known allergy, increasing to 1500 U in severe cases, such as vascular compromise [1]. When addressing retroseptal/premolar sites, DeLorenzi (2013) recommended 25 to 100 U, followed by gentle massage. To address the Tyndall effect, this author suggested applying 15 to 50 U, with gentle massage. Others have advised treating persistent malar or infraorbital edema or the Tyndall effect (owing to overly superficial HA placement or to post-injection migration of filler) with low-dose hyaluronidase (eg, 25-30 U), potentially over multiple sessions [2].

For non-emergent complications, including nodules or bumps, unsightly overcorrection, or superficial overfilling, multiple authors have noted that low-dose application of hyaluronidase—approximately 3 to 75 U can be sufficient [5,11]. Very low doses, in the range of 1.5 to 3 U, may be advised in areas of thin skin, such as the lower eyelid, and may help avoid immune-mediate adverse outcomes [11] as well as complete reversal of the originally intended aesthetic outcome of filler placement [12].

Hyaluronidase-assisted management of untoward aesthetic outcomes can be carried out months after the initial filler placement, and may be delayed by weeks to allow edema to resolve spontaneously [7]. Uncomplicated nodules owing to excessive or accidental placement of HA may be allowed to self-resolve over time or may be managed with 30 to 75 U hyaluronidase [2].

To manage emergent complications, such as cutaneous ischemia secondary to intra-arterial filler injection, hyaluronidase treatment should occur the first 4 hours after filler placement to avoid or minimize necrosis [5,13]. Cohen et al. noted that, in the context of vascular obstruction and skin necrosis, hyaluronidase injection should be performed with a dose of 30 to 75 U by approximately 4 to 6 hours after filler injection [2]. These authors also suggested that precise delivery of hyaluronidase into the vasculature may not be necessary because the enzyme diffuses readily into the vascular lumen [2].

Previous *in vitro* findings

Several investigators have evaluated the *in vitro* sensitivity of HA fillers to hyaluronidase [9,14-17]. Rao et al. exposed 4 types of HA fillers to varying concentrations of Vitrase and Hylenex *in vitro* [9]. Using visual examination, the authors found that, at 0.1 mL Vitrase to 0.2 mL filler, Restylane was hydrolyzed the most at the end of the 15-minute observation period, followed by Juvederm and then Belotero. Similar results were obtained with application of Hylenex. Responsivity of fillers to hyaluronidases varied in a time- and dose-dependent manner [9].

The authors also found that Belotero was least responsive to either hyaluronidase (Hylenex or Vitrase), followed by Juvederm, Juvederm Voluma, and Restylane [9]. Most of the observed hydrolysis occurred within the first minute and seemed to stabilize by 5 minutes post-exposure, with no appreciable additional change observed from 5 to 15 minutes. The same pattern of responsivity was seen for both hyaluronidase types and for both concentrations of Hylenex (15 U and 30 U to 0.2 mL filler). Restylane was transformed from a gel to a slushy consistency on exposure to Hylenex [9].

Previous *in vivo* findings

Shumate et al. used an *in vivo* animal model to assess the effects of 2 types of hyaluronidases on 3 types of HA-based fillers [8]. The authors concluded that hyaluronidase effects differed by hyaluronidase dose and exposure time, not by filler type. That is, all tested fillers were undetectable by the end of the 6-hour observation period at the highest concentration [8]. These data refuted the hypothesis that different fillers have different *in vivo* responsiveness to hyaluronidase. The investigators suggested that, in the clinical setting, HA fillers are perceived as having distinct susceptibilities to hyaluronidase because of variables that are difficult to fully assess and control, including filler depth, location of enzyme delivery, and filler volume. When Shumate et al. held these variables constant in the animal model, the fillers did not differ substantially in dissolution time [8].

In the current study, we found that filler hydrolysis occurred more quickly *in vitro* when hyaluronidase was applied to a linear bolus with a lower concentration of HA and less extensive crosslinking. Findings in 3 clinical cases supported these results, with additional hyaluronidase sessions needed in the patient who had received filler with higher HA concentration or more crosslinking.

CONCLUSION

Injection of HA filler is a popular aesthetic surgical procedure. One advantage of these fillers is that complications, ranging from excessive augmentation and misplaced filler to vascular infiltration of filler and skin necrosis, can be mitigated or reversed by application of hyaluronidase. However, commercially available HA fillers have distinct technologies and, potentially, dissimilar sensitivities to hyaluronidase exposure. We undertook an *in vitro* study to compare the responses of numerous HA fillers to hyaluronidase, and we compared these findings to 3 clinical cases in which hyaluronidase was given to patients with complications of HA filler placement. We found that sensitivity to hyaluronidase generally was greater in fillers with lower HA concentration and less extensively crosslinked HA. Our *in vitro* and clinical findings were consistent overall, with patients tending to need additional sessions of hyaluronidase treatment when they received HA filler with greater HA concentration or more crosslinking.

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DISCLOSURE

Dr Cavallini and Dr Trocchi are speakers and consultants for Allergan. Dr Papagni has nothing to disclose.

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