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Cross-linked hyaluronic acid filler hydrolysis with hyaluronidase: Different settings to reproduce different clinical scenarios

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Abstract

Skin necrosis is the most severe complication arising from hyaluronic acid (HA) injection. To avoid skin necrosis, hyaluronidase should be injected along the course of the involved artery, to allow blood flow restoration. We evaluated the ability of hyaluronidase to degrade a HA filler in two simulated clinical situations—a compression case and an embolization case—to identify differences in the hyaluronidase injection. In the compression case, a bolus of HA filler was directly soaked in hyaluronidase solution; in the embolization case, a vein harvested from a living patient was filled with the same HA filler and then soaked in hyaluronidase. We then evaluated the quantity of HA remaining after 2 hr. While we found hydrolysis of HA in both cases, in the compression case, we detected almost complete hydrolysis, whereas in the embolization case we observed a reduction of the 60%. Our results support the hypothesis that vessel compression can be resolved with only one injection of hyaluronidase, while in the case of vascular embolization, repeated perivascular injections should be performed owing to the reduction of hyaluronidase activity.

KEYWORDS

embolism, hyaluronic acid, hyaluronidase, impending necrosis, vessel compression

1 | INTRODUCTION

Hyaluronidase is a naturally occurring enzyme that degrades hyaluronic acid (HA), which is one of the four main glycosaminoglycans that constitute the dermal extracellular matrix and regulate its permeability (Buhren et al., 2016). The use of hyaluronidase has changed throughout its commercialization. The current U.S. Food and Drug Administration–approved indications recommend the use of HA for hypodermoclysis, to increase the subcutaneous absorption of beneficial drugs, disperse harmful injected drugs, treat extravasation injury, and improve the absorption of radiopaque agents during subcutaneous urography (Bailey, Fagien, & Rohrich, 2014).

Several hyaluronidase products are available in different countries (Bailey et al., 2014; Buhren et al., 2016; Lee, Grummer, Kriegel, & Marmur, 2010). Hyaluronidase is often used off-label for application in aesthetic dermatology, particularly for treatment of the undesirable effects of HA fillers, such as misplaced injections, overcorrection, unexpected outcomes, the Tyndall effect, granulomas and inflammatory reactions, and vascular occlusion (Hirsch, Brody, & Carruthers, 2007; Landau, 2015).

Due to differences in the physical characteristics of different HA products, commercially available HA fillers demonstrate different sensitivities to degradation by hyaluronidase (Buhren, Schrumpf, Bölke, Kammers and Gerber (2018); Shumate, Chopra, Jones, Messina, & Hee, 2018).

Lambros (2004) and Soparkar, Patrinely, and Tschen (2004) independently described the first case reports on the use of hyaluronidase to treat superficial HA filler accumulation (Lambros, 2004; Soparkar et al., 2004).

Since then, several papers regarding the role of hyaluronidase in degrading HA fillers have been published, focusing especially on the

ability to avoid necrosis (Cohen et al., 2015; DeLorenzi, 2014a; DeLorenzi, 2014b; Sun et al., 2015).

Skin necrosis is the most severe complication that can occur during HA injections (Daines & Williams, 2013; Ozturk et al., 2013). Skin necrosis can be caused by interruption of the vascular supply to the area by two mechanisms: compression of the area around the vessel and obstruction of the vessel (embolization) by the filler material (Chang et al., 2016).

To prevent vascular compromise and avoid an inadvertent intra-arterial or juxta-arterial injection, it is critical that the clinician is aware of the anatomy of the injection site. If vascular compromise occurs, timely intervention is critical to prevent necrosis.

It was demonstrated that both intra-arterial and subcutaneous injection of hyaluronidase could restore the blood flow of HA-embossed vessels (DeLorenzi, 2014a; Kim et al., 2011; Wang, Li, Zhang, Tian, & Wang, 2017).

Thus, in addition to other supportive measures (i.e., acetylsalicylic acid, warm compress, and nitroglycerin), hyaluronidase should be injected along the course of the involved artery to allow flow restoration (Cohen et al., 2015).

In the present study, we evaluated the ability hyaluronidase to degrade a 20 mg/ml HA filler in two different settings reproducing the two different clinical scenarios that can lead to skin necrosis: compression and embolization.

2 | MATERIAL AND METHODS

In this study, we simulated two different clinical scenarios: the "compression case" and the "embolization case." For simulating the compression case, 0.3 ml bolus of a 20 mg/ml HA cross-linked filler (Hyamira BASIC, APHARM s.r.l., Arona, Italy) was directly soaked in a 1 ml solution of hyaluronidase (bovine hyaluronidase concentration: 300 UI/ml) in a test tube (Figure 1). For simulating the embolization case, a vein specimen, collected during a forearm free flap harvesting for tongue reconstruction was filled with 0.25 ml of the same HA filler used for the compression case and soaked in a test tube containing 1 ml of hyaluronidase solution (Figure 2).

In both cases, after 2 hr of soaking, the test tubes were brought to the Pathological Anatomy Department and the remaining quantities of HA were measured.

2.1 | Vein harvesting and "embolization" scenario setting

The vein harvested was a 3 cm cephalic vein collected from the left forearm. After collection, it was washed with a saline solution. Throughout the procedure, a ×3 loupe magnification was used to achieve detailed visualization. The saline-filled vein was examined to exclude the presence of small side branches. Then, a metallic clip was placed on one end of the vein. On the other end, a cannula was inserted and 0.25 ml of a 20-mg/ml cross-linked HA filler was injected

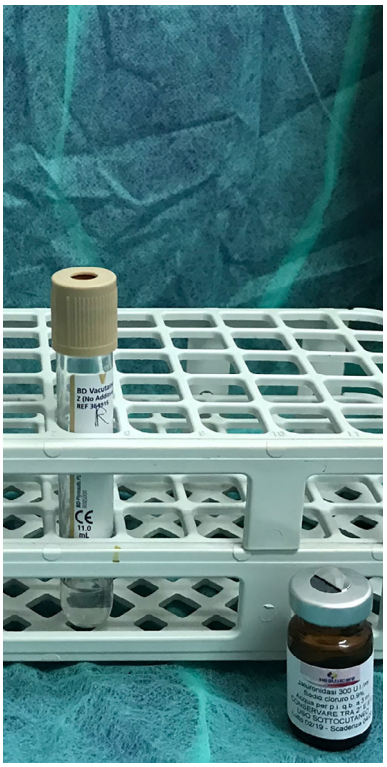

FIGURE 1 A test tube containing 0.3 ml of HA, soaked in a hyaluronidase solution

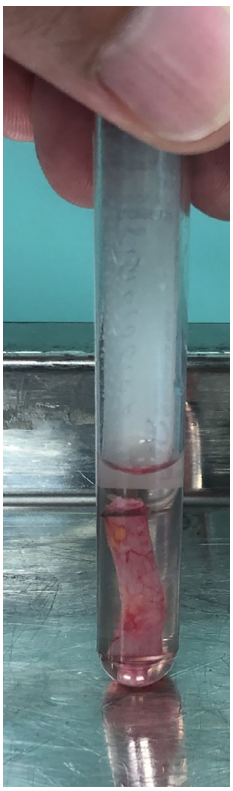

FIGURE 2 The vein specimen, after being washed, filled with HA, and with a metallic clip placed at both ends, soaked in a hyaluronidase solution

(Hyamira BASIC, APHARM s.r.l., Arona, Italy); after filling the vein with HA, a clip was placed on the other end to close the vein (Figure 2).

3 | RESULTS

3.1 | After 2 hr of soaking, we measured the remaining HA in the test tubes

For the compression case, the contents of the test tube were poured into pathology tissue embedding bio-cassettes through a biopsy filter. We used a 3-mm-thick biopsy filter characterized by permeability to water, aqueous solutions, and alcohol; resistance to several chemical reagents, such as solvents, and paraffin; and high color contrast with the specimen. Due to the action of the filter, only the gel component remained in the bio-cassette and was easily visible to the naked eye. We then quantified the gel by aspiration with a piston-stroke pipette. This single-channel pipette is characterized by a controlled volume setting ring, allowing the management of liquid volumes between 0.1 and 10 μl. We measured a remaining HA volume of 10 μl, corresponding to 0.01 cc.

For the embolization case, we removed the vein specimen from the test tube and the metallic clips by both sides. Thereafter, we collected all the HA gel in the vessel lumen in a graduated vial. We detected 0.15 ml of HA filler.

4 | DISCUSSION

HA is a ubiquitous glycosaminoglycan that has been used for facial rejuvenation. Its popularity stems from its good safety profile and ability to achieve reproducible results (McKee et al., 2019). Despite the safety of HA-based applications, side effects can still occur (Daines & Williams, 2013; DeLorenzi, 2014a). Until the early 2000s, the suggested recourses for misplacement of HA fillers were massage, incision and drainage, oral antibiotics, oral steroids, and a tincture of time (Hirsch, Narurkar, & Carruthers, 2006; Sarkar & Hirsch, 2010). By the addition of hyaluronidase, clinicians now have a safe and effective way of minimizing the adverse effects of HA fillers (Bailey et al., 2014; Buhren et al., 2016; Cohen et al., 2015; Lee et al., 2010).

However, the correct method for managing necrosis due to embolism or compression after HA injection is still controversial in the field. Hyaluronidase treatment is always accomplished with subcutaneous diffusion into the tissues affected by ischemia (Wang et al., 2017). Some studies have suggested that it is not necessary to administer hyaluronidase directly into the blood vessels, because its administration by diffusion appears to be effective (Kim et al., 2011; Wang et al., 2017). In 2014, DeLorenzi published a study in which he harvested facial arteries and veins from fresh human cadavers. In his study, the specimens were analyzed after 4 and 24 hours of immersion in hyaluronidase. After the treatment, no HA was detected in both the artery and vein specimens (DeLorenzi, 2014b).

However, he also reported that patients not responding to repeated hyaluronidase injections into ischemic tissues showed an improved cutaneous circulation only after direct hyaluronidase injection into the affected artery (DeLorenzi, 2014b).

Moreover, increasing evidence suggests that different HA filler brands have different resistance to hyaluronidase. In 2011, Kim et al. used a rabbit ear model to demonstrate that hyaluronidase effectively prevents skin necrosis if it is injected within 4 hr after the vascular occlusion of an artery, using the Restylane EMV (Emervel Classic, HA 20.0 mg/ml, lidocaine; Galderma; manufactured by Q-Med AB, Uppsala, Sweden) as filler (Kim et al., 2011).

Recently, Wang et al. (2017) used the same rabbit ear model to show that a subcutaneous injection of hyaluronidase is more effective than an intra-arterial injection. Moreover, the authors showed that hyaluronidase effectively degrades EMV within 1 hr.

Thereafter, Menzinger, Kaya, Saurat, & Kaya (2016) reported that hyaluronidase effectively and dose-dependently degrades EMV in a murine model in vivo.

Regarding another brand of HA filler, Allergan JUV (Juvéderm Ultra 3, HA 24.0 mg/ml, lidocaine; Pharm-Allergan, Irvine, CA), the results of its interaction with hyaluronidase are more inconsistent. Whereas Sall et al. and Jones et al. reported a strong resistance to degradation against bovine or ovine hyaluronidase (Jones, Tezel, & Borrell, 2010; Sall & Ferard, 2007), Rao et al. as well as Juhász et al. demonstrated that ovine or recombinant human hyaluronidase effectively degrades JUV (Juhasz, Levin, & Marmur, 2017; Rao, Chi, & Woodward, 2014).

These controversial results could be related to differences in the origin of the hyaluronidase used (bovine, ovine, and recombinant human), the dosage, duration of incubation, and other experimental conditions. The current hypothesis is that higher HA content and cross-linking-techniques correlate to higher resistance to hyaluronidase (Shumate et al., 2018).

JUV and EMV have a different structure: the strong degree of crosslinking of the monophasic JUV may limit access of hyaluronidase to the HA substrate, whereas the biphasic nature of EMV and its distinct particles offer a greater interaction surface (Sall & Ferard, 2007).

In a recent study, Buhren et al. (2018) found that the filler with the highest content of HA (JUV, 24 mg/ml) was also the most resistant to degradation, compared to that of fillers with lower concentrations (Belotero Balance Lidocaine, HA 22.5 mg/ml, with lidocaine, Merz Pharmaceuticals GmbH, Frankfurt, Germany, manufactured by ANTEIS SA (Geneva, Switzerland); and Emervel Classic, HA 20.0 mg/ml, lidocaine, Galderma, manufactured by Q-Med AB (Uppsala, Sweden).

Although many studies evaluated HA sensitivity to hyaluronidase, a direct comparison of the same filler in different clinical scenarios has not been performed yet.

In this study, we tried to simulate two different clinical scenarios associated with skin necrosis: perivascular compression and intravascular embolization.

It has been clearly established that, in the case of HA accumulation, the injection of hyaluronidase next to the filler can be performed in a safe and easy way (Moraes Ferraz, Sandkvist, & Lundgren, 2018). When perivascular compression occurs, HA accumulation can be felt by clinical palpation, and hyaluronidase can be easily and directly injected next to the accumulation (Chang et al., 2016; Wang et al.,

2017). To simulate this clinical scenario, we directly soaked an HA filler bolus in hyaluronidase solution in a test tube. On the other hand, if vascular embolization occurs, the administration of an intra-arterial injection of hyaluronidase is very complex. For this reason, pulsed perivascular injection of hyaluronidase has been suggested (DeLorenzi, 2017). To simulate this clinical scenario, we soaked a vein specimen, prefilled with the same HA filler used for the compression, in a hyaluronidase solution. After 2 hr, we performed semiquantitative evaluation of the remaining HA presence. In the compression scenario, we detected 10 μl of remaining HA, a very small amount compared to the starting 0.3 ml. In the embolization scenario, we found 0.15 ml of HA remaining, from a starting volume of 0.25 ml.

Our results are in contrast to those reported by DeLorenzi (DeLorenzi, 2014b), who observed complete hydrolysis of an HA bolus pre-filled in the arteries and veins collected from cadaveric specimens.

However, the two studies have important differences. First, we used a vein collected from a living patient, while DeLorenzi collected it from cadavers. Thus, our choice better mimics actual clinical practice. Second, the capability of hyaluronidase to diffuse through a vein wall may differ between living patients and cadavers, although hyaluronidase has been shown to increase vessel permeability. On the other hand, we only tested hyaluronidase activity using a vein instead of an artery, and this may represent a limitation of the present study.

Although arteries and veins both function as conduits and are lined by a continuous, nonfenestrated endothelium, they differ in fundamental ways. Arteries have thick walls and pulsate. Veins have thin walls and do not pulsate. Veins have valves; arteries do not. Endothelial junctions in arteries are tighter compared with those in veins, reducing their permeability (Aird, 2007). Moreover, when veins are grafted into arterial circulation, they acquire arterial-like properties, including a thickened wall and, in animal models, reduced permeability (Kwei et al., 2004).

For the aforementioned reasons, in the present study we did not evaluate hyaluronidase activity in an artery. However, based on our knowledge of the vessel's anatomy and physiology, we predict that hyaluronidase would have lesser efficacy in hydrolysing HA trough an arterial wall than through a vein.

In 2017, DeLorenzi proposed a new protocol, called "new high dose pulsed hyaluronidase protocol for hyaluronic acid filler vascular events" (HDPH). The protocol involves the administration of relatively high doses of hyaluronidase into the ischemic tissue, repeated every hour until resolution (DeLorenzi, 2017). Repeated injections of hyaluronidase are mandatory because this drug loses its effect after a few hours (Kim et al., 2018).

Our results support this idea, as the direct contact of hyaluronidase with HA accumulation can lead to better hydrolysis than when the hyaluronidase has to diffuse through a vessel wall.

5 | CONCLUSION

In the present study, we analyzed the hyaluronidase capability of reversing an "impending necrosis" in two clinical scenarios: compression and embolization. Based on these results, we support the hypothesis that vessel compression caused by a well-marked area of HA accumulation can be resolved with only one injection of hyaluronidase if it is properly injected next to the HA accumulation and with the right posology. On the other hand, in case of vascular embolization, repeated perivascular injections have to be performed, as proposed in the HDPH protocol, due to the reduction of hyaluronidase activity diffusing trough a vessel wall (DeLorenzi, 2017).

CONFLICT OF INTEREST

The authors have no potential conflicts of interest with respect to the research, authorship, and publication of this article.

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