Cosmetic Medicine

Review Article

The Role of Hyaluronidase in the Treatment of Complications From Hyaluronic Acid Dermal Fillers

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Abstract

Hyaluronidases, a family of enzymes that are able to degrade hyaluronic acid (HA), are employed in medicine to increase drug diffusion and reverse the effects of HA filler injections. Hyaluronidases are able to dissolve subcutaneous nodules or to correct excessive quantities of injected filler. Knowledge of the use, methods of application, and adverse effects of hyaluronidases is essential for the aesthetic practitioner. Therefore, we performed an extensive review of the available literature from 1928 to 2011 and compared the different enzymes available, recording each author's indications regarding usage and side effects.

Keywords

noninvasive plastic surgery, fillers, facial skin necrosis, cosmetic medicine, hyaluronidase, hyaluronic acid, dermal

Hyaluronidases are enzymes that degrade hyaluronic acid (HA), a fundamental constituent of the extracellular matrix that can be injected subcutaneously for aesthetic purposes. These enzymes are widely employed in aesthetic medicine, due to their role in preventing complications from inappropriate injection of HA, eliminating HA nodules, or correcting unsightly HA overfilling. Hyaluronidases have been employed for several years as a spreading agent to promote the diffusion of several substances injected subcutaneously,[1] to remove the cumulus-corona-oocyte complex formed during intracytoplasmic sperm injection,[2] to prevent tissue damage after extravasation of several substances,[3] for edema reduction,[4] and for treatment of vitreous hemorrhage.[5]

It is essential for the aesthetic practitioner to have a sound knowledge of the indications, modalities of application, and adverse effects of hyaluronidases. However, although the indication, usage, effects, and complications of these enzymes have been reported for several applications, an extensive review of this topic in aesthetic medicine is lacking.

HYALURONIDASE: ACTION AND CLASSIFICATION

Hyaluronic acid is a glycosaminoglycan (GAG), the main component of the extracellular matrix that links protein filaments, collagen fibers, and connective tissue cells.[5] It is formed by a polymer of disaccharides composed of D-glucuronic acid and D-N-acetylglucosamine, linked with β-1,4 and β-1,3 glycosidic bonds. Hyaluronidases are endoglycosidases that depolymerize HA, producing multiple effects in tissues: they decrease the normal high viscosity of HA, lessening its lubricating quality and acting as a "spreading factor" facilitating the diffusion of several substances injected subcutaneously, like dyes and antiviral vaccines.[6]

Meyer[7] previously discovered the action of these proteins and classified the hyaluronidases into 3 groups[3]: mammalian hyaluronidase (testis tube), leech/hookworm hyaluronidase, and microbial hyaluronidase. Each group differs with regard to its mechanism of action (Figure 1):

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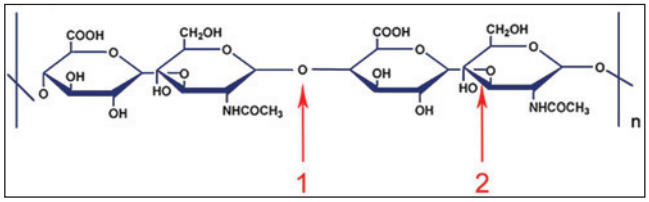

Figure 1. Hyaluronic acid molecular structure and action site of the 3 groups of hyaluronidases. The molecule is cleaved by mammalian hyaluronidases (1), bacterial hyaluronidases (1), and leeches hyaluronidases (2).

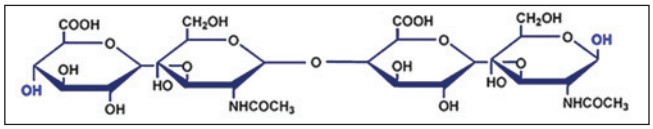

Figure 2. Product of mammalian hyaluronidases.

- Mammalian glycosidases are endo-β-N-acetlyhexosaminidases that degrade the β-1,4 glycosidic linkages of HA (Figure 2), producing tetrasaccarides. These enzymes act on HA, chondroitin, chondroitin-4,6-sulfate, and dermatan sulfate and can be found in mammalian spermatozoa and lysosomes, as well as in the venom of snakes, reptiles, and hymenoptera.[8]
- The second class comprises endo-β-D-glucuronidases. These hyaluronidases degrade the β-1,3 glycosidic bond, resulting in tetra- and hexasaccharides (Figure 3). These enzymes, in contrast to mammalian glycosidases, degrade only HA and remain inert toward other GAGs.[9] These enzymes can be found in the salivary glands of leeches and hookworms.
- Microbial hyaluronidases are classified as hyaluronate lyases. They differ from the other hyaluronidases because they do not use hydrolysis, but there is a β-elimination reaction at β-1,4 glycosidic linkages, producing an unsaturated disaccharide (Figure 4). These enzymes have been isolated from several microorganisms, including, for example, strains of *Clostridium, Micrococcus, Streptococcus*, and *Streptomyces*.[8]

Hyaluronidases may also be divided in 2 groups according to pH-dependent activity:

- *Acid active hyaluronidases*. This group of enzymes expresses their activity between pH 3 and 4.
- *Neutral active hyaluronidases, active between pH 5 and 8*. Hyaluronidases from snake venom and bee venom belong to this group.[10]

Hyaluronidases for medical use were initially derived from crude extracts of ovine or bovine testicular tissue (called bovine testicular hyaluronidase [BTH]). The

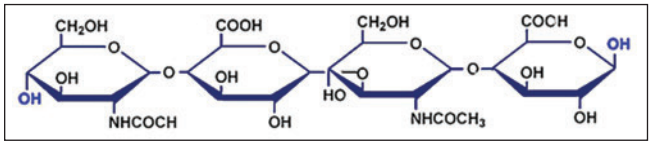

Figure 3. Product of leeches hyaluronidases.

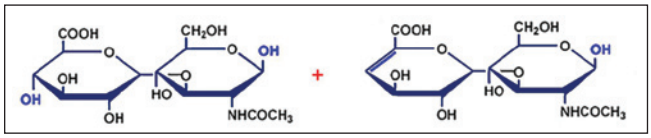

Figure 4. Product of microbial hyaluronidases.

enzyme obtained with this procedure is impure and immunogenic, containing several contaminating substances such as proteases, immunoglobulin, and vasoactive factors. An alternative, hyaluronate lyase from *Streptococcus agalactiae*, may be employed. This formulation is purer than BTH and has a higher specificity. Moreover, BTH produces several oligosaccharide fragments of hyaluronan that could have a role in allergic reactions and act as growth factors, promoting possible metastasis.[11] Employing the bacterial lyase could avoid these complications.

Now, a newer formulation is available, called Hylenex (Halozyme Therapeutics, San Diego, California), a human recombinant hyaluronidase that is considered less immunogenic and safer.[5] After intravenous administration, hyaluronidases undergo elimination through the kidneys with a known clearance ($t_{1/2}$ = 2.1 ± 0.2 min), where $t_{1/2}$ is half-time. Nevertheless, the mechanism of inactivation inside the dermis and other tissues is still unknown, mainly depending on HA synthesis. The hyaluronidase effects in this context persist for approximately 48 hours.[12]

APPLICATIONS

Hyaluronidases are employed for medical use to increase tissue absorption of several drugs. This enzyme is able to promote the drug's diffusion into the extracellular matrix and to increase blood vessel permeability. In effect, the subministration of these enzymes could prevent tissue damage after extravasation of several substances (eg, parental nutrition solution, electrolyte infusions, antibiotics, aminophylline, mannitol, and chemotherapeutic agents), reducing their concentration.[3]

Other authors have observed that the combination of hyaluronidase and urokinase decreases the mortality rate after myocardial infarction in an animal model.[13] Recombinant hyaluronidase is currently employed for intracytoplasmic sperm injection to remove the cumulus-corona-oocyte complex, composed of granulosa cells inside a matrix of oligosaccharide chains cross-linked by hyaluronan binding proteins and proteoglycans.[2] However, the most important application of hyaluronidases is hypodermoclysis (ie, the capacity of increasing absorption and dispersion of the injected drug). These enzymes have been

widely employed as spreading agents, along with chemotherapeutics, local anesthetics, and contrast media.

In chemotherapeutics, hyaluronidases are injected along with the chemotherapeutic agent, thus improving the penetration of the drug inside the malignant tissue (eg, brain tumors).[1] In ophthalmic surgery, hyaluronidases are employed for retrobulbar anesthesia,[14] whereas in pain therapy, hyaluronidases are administered along with local anesthetics in caudal blocks, directly at the site of pain (eg, joints, tendons) or through an intrathecal route.[15] In radiography, hyaluronidases are added to the contrast media to improve reabsorption. Nevertheless, although the combination of hyaluronidases with local anesthetics does not affect the activity of the enzymes, iodinated contrast media have been demonstrated to reduce the activity of hyaluronidases.[16]

Off-label applications of hyaluronidases include edema reduction and lysis of epidural adhesions to treat chronic painful conditions like radiating pain and lower back pain.[4] Moreover, hyaluronidases are utilized for edema reduction in the case of paraphimosis, intestinal intussusception, supraglottal airway edema, transplanted organ rejection, and treatment of vitreous hemorrhage.[5]

APPLICATIONS IN PLASTIC SURGERY AND DERMATOLOGY

In plastic surgery and dermatology, hyaluronidases are commonly employed to improve the effects of local anesthetics and to dissolve the injected HA. Baring and Marshall[17] described their own technique for infiltration in open septorhinoplasty. Probably the most important application of hyaluronidase enzymes for the plastic surgeon and dermatologist is the correction of HA filler injection. This procedure can be performed for aesthetic purposes and/or functional aims.

In the first case, hyaluronidases can be employed for eliminating nodules or bumps, for treating an overcorrection by HA filler injection and/or excessive superficial infiltrations. In all these cases, the dosage of hyaluronidases usually ranges from 3 to 75 units.[18] For nodules, hyaluronidases may be successfully employed. With inflammatory and painful nodules, independent of the usage of hyaluronidases, Narins et al[19] recommend initiating use of an antibiotic for 2 weeks (eg, clarithromycin 500 mg/d covers a wide range of infections). In selected cases, especially when large amounts of HA filler should be eliminated, an incision for drainage of the HA is suggested, along with enzyme injection. When hyaluronidases are not effective in dissolving the nodules (eg, in the case of inflammatory nodules or long-lasting fillers), steroids could be employed after careful evaluation. In these cases, steroids should be administered only after antibiotic therapy has been initiated. This treatment is not appropriate for early onset inflammatory nodules, before antibiotic treatment, and before having attempted a treatment with hyaluronidase.

Hyaluronidases may be effectively utilized to correct overinjection of HA filler. In these patients, the modalities of hyaluronidase injection depend on the localization and quantity of the previous HA filler injection (Table 1). Even low doses of ovine hyaluronidase (< 3 units) may be effective in reversing excessive augmentations (eg, in lower lids).[18] However, when a long-lasting filler has been employed and shows resistance to hyaluronidases, excision may be considered.[19]

Table 1. Dosage of Hyaluronidases by Region of Application

Region	Hyaluronidase (Units)
Nasal and perioral skin	15-30[20,21]
Periorbital area	30[21]
Infraorbital area	10-15[18]
Lower lid	1.5[22]

Hyaluronidase may also be employed for complications deriving from HA filler injection, like intra-arterial injection or utilization of excessive HA. In both cases, the injected HA may cause cutaneous ischemia by compression or embolization of the subdermal plexus. In these patients, the skin discolors within a few hours, while necrosis and ulcers may be visible within 24 hours. Hyaluronidases may be effectively applied in the first 4 hours. Injection of the enzymes in the following hours does not seem to be effective in reducing skin necrosis.[23]

Other authors also advise employing these enzymes 6 hours after HA filler injection with 75 IU of hyaluronidase.[20] In the first hours, at the first sign of blanching, a topical treatment with nitroglycerin paste is recommended, along with hyaluronidases.[24] Late application of hyaluronidases (more than 24 hours after HA injection) has not proved effective in avoiding skin necrosis. Nevertheless, their usage may reduce the size of the necrotic area and improve the healing process.

INFILTRATION TECHNIQUE

The affected area should be investigated with ultrasonography before any treatment is employed, in order to assess the depth, quantity, and extension of the previously injected HA. The areas to be treated should be marked on the overlying skin, and infiltration by hyaluronidases should be extremely accurate and limited to the affected area. The practitioner should adjust the quantity to be injected to the type of hyaluronic acid (eg, filler with higher concentrations of hyaluronic acid will require higher quantities of hyaluronidases) and the number and extent of affected areas.

In our experience, a few units of enzyme (10-20 units) are adequate to treat each affected area. In any case, the injection of more than 200 units of hyaluronidase per treatment should be avoided. Infiltration does not usually require dilution of the enzymes with local anesthetics. A 30-gauge needle, ranging from 3 to 13 mm in length, may be effectively

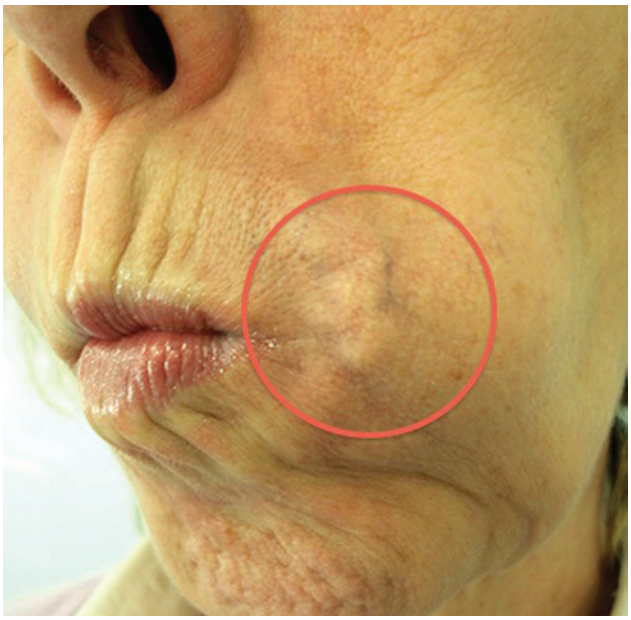

Figure 5. A 41-year-old woman treated with 2 mL of cross-linked hyaluronic acid. Inflammatory nodules were observed 2 months after the treatment.

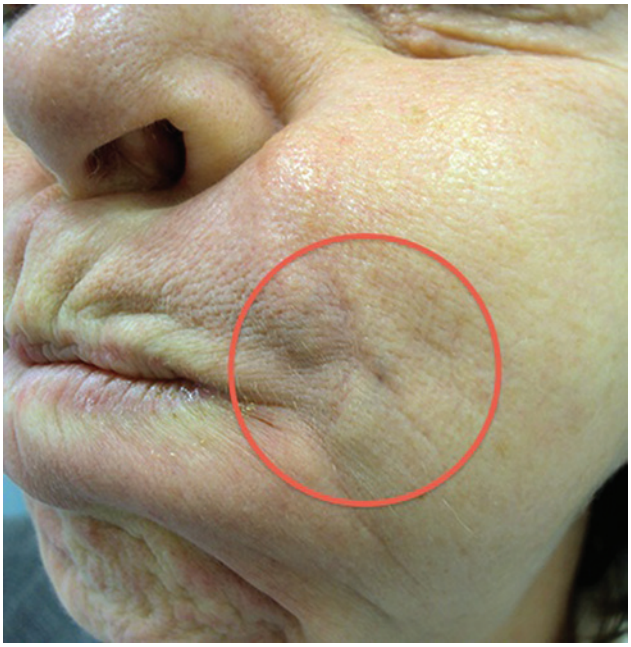

Figure 6. Hyaluronidases were then employed, injecting 600 units in 3 sessions, performed every 7 days. The results 3 months after the treatment with hyaluronidases are shown.

employed. The injection should be performed perpendicular to the skin. Multiple treatments may be necessary. The treatments should be planned every 3 weeks, although shorter times may be prescribed for high hyaluronate density fillers (Figures 5 and 6).

Some examples of hyaluronidases are listed in Table 2. Availability and indications for each product are specific to each country (see the section on Technical Notes).

DRUG INTERACTIONS

Knowledge of the mechanisms involved in the pharmacokinetics of hyaluronidase is limited. The clearance of hyaluronidase in the serum occurs with a half-time of 2.1 ± 0.2 minutes by inactivation in the kidneys and liver.[5] The most common interactions occur with furosemide, benzodiazepines, phenytoin, dopamine, and α-adrenergic agonists. Hyaluronidase antagonists include anti-inflammatory agents (eg, indomethacin, dexamethasone, and salicylates), numerous plant-based drugs (eg, flavonoids and antioxidants), antihistamines, mast cell stabilizers, heparin, vitamin C, and dicumarene.[3,12] The preliminary patient interview should aim to investigate the consumption of the above-mentioned substances that could mimic tissue resistance to hyaluronidases.[5] Radiographic contrast media have been advocated for reduction of hyaluronidase activity, which, conversely, is slightly increased by corticosteroids.[16] According to Szepfalusi et al,[25] hyaluronidase may represent a potent antigen in coadministration with chemotherapeutics and dexamethasone, triggering IgE synthesis.

COMPLICATIONS

Allergic reactions are the only complications reported after employing hyaluronidases. Different allergic reactions have been described, depending on the region of application. Local injection site reactions are the most common. According to the literature, the incidence of local allergic reactions ranges from 0.05% to 0.69%.[14,26,27] These data have been calculated under peribulbar anesthesia, and the main symptoms include edema, erythema, pain, and itching. Although the incidence of mild allergic reactions could be higher because they may have been unrecognized,[27] urticaria and angioedema have been reported in less than 0.1% of cases.[5] In cases where high dosages of hyaluronidases (200 000 IU) were administered, allergic complications could rise to 31.3%[20] (Table 3).

Immediate and delayed allergic reactions to hyaluronidases have been described as type I (mediated by IgE) and type IV (mediated by T cells) hypersensitivity.[25,28] Moreover, these enzymes have been demonstrated to increase capillary permeability,[29] potentially worsening the above-mentioned reactions. An allergy may be diagnosed by prick or intradermal test,[16,26] and serum immunoglobulin E (IgE) antibodies highly specific to hyaluronidases may be observed after exposure.[15] The allergic history of the patient is unrelated to the reactions to hyaluronidase.[25] Such reactions include local complications (local edema, tumor, mild pain),[14] itching sensation, generalized maculopapular rash,[30] and urticaria,[15] although the reported symptoms are rare, mild, and benign in most cases.

Concerning the reactions mediated by IgE, a study in pediatric oncological populations sensitized by intravenous hyaluronidase treatment or by cross-reactivity with

Table 2. Example of the Most Common Commercially Available Hyaluronidases

Product Name	Produced by	Origin	Description
Vitrase	ISTA Pharmaceuticals	Purified ovine testicular hyaluronidase	Each vial of 6200 USP units contains 5 mg lactose, 1.92 mg potassium phosphate dibasic, and 1.22 mg potassium phosphate monobasic.
Amphadase	Amphastar	Purified bovine testicular hyaluronidase	Each vial contains 150 USP units of hyaluronidase per milliliter with 8.5 mg sodium chloride, 1 mg edetate disodium, 0.4 mg calcium chloride, monobasic sodium phosphate buffer, and not more than 0.1 mg thimerosal (mercury derivative).
Hylenex	Halozyme therapeutics	Purified recombinant human hyaluronidase	Each vial contains 150 USP units/mL, nonpreserved.

Table 3. Incidence of Allergic Reactions to Hyaluronidases

Author	Incidence, %	Injection Site	Units	Timing	Local Symptoms	Systemic Symptoms	Treatment
Leibovitch et al[26]	0.13	Peribulbar	100-300	12-72 hours after injection	Proptosis, periorbital erythema, swelling, extraocular muscle function restriction, periorbital pain or itchiness, conjunctival chemosis	None	Systemic antibiotics and steroids
Szepfalusi et al[25]	31.25	Intravenous	200 000	Immediate	—	Anaphylactic reactions: burning on the skin, urticaria, macular exanthema, dyspnea, blood pressure drop	—
Kempeneers et al[14]	0.05	Retrobulbar	—	—	Angioedema	—	—
Eberhart et al[27]	0.69	Peribulbar	112.5	24 hours after injection	Angioedema, itching	None	—

—, not available.

other IgE antibodies showed that anaphylactic reactions are the most severe, and immediate allergic reactions were observed after intravenous administrations of chemotherapeutics.[25] Additionally, some authors suggested that the coadministration of hyaluronidase, chemotherapeutics, and dexamethasone might trigger IgE synthesis.[25]

Even in pain therapy,[30] where hyaluronidases are administered along with local anesthetic in caudal blocks or directly at the site of pain (eg, joints, tendons) or through an intrathecal route, anaphylactic reactions have been observed.[15] A number of allergic reactions to hyaluronidases have been described in ophthalmic surgery, where these enzymes are employed for retrobulbar anesthesia with lidocaine or bupivacaine.[14] A case of delayed allergic reaction with secondary visual loss was also reported.[31]

The subcutaneous injection of hyaluronidases produces, in most cases, a mild local allergic reaction. Generalized allergic reactions occur more commonly after administration of systemic high doses of enzyme (Table 4). A careful patient interview may help in individuating previous enzyme administration with possible sensitization.

TECHNICAL NOTES

A hyaluronidase prescription should take into account 2 issues: (1) drug availability and (2) off-label indications for aesthetic purposes. The prescription for hyaluronidases in the United States is regulated, like every drug, by the US Food and Drug Administration (FDA) Center for Drug Evaluation and Research (CDER), which reviews new drug applications supplied by a pharmaceutical company. When the drug meets the requirements for safety and effectiveness, the dosage, route, and indications are established.[41]

Several categories of hyaluronidases have been approved for clinical use, from animal or recombinant production. These enzymes have been approved for 3 purposes: (1) as an adjuvant to increase the absorption and dispersion of other injected drugs, (2) to produce hypodermoclysis, and (3) as an adjunct in subcutaneous urography for improving reabsorption of radiopaque agents.

Although the promotion of any drug for indications not approved by the FDA is not allowed, off-label prescription is not formally prohibited and is delegated to the practitioner's choice, if considered safe and effective.

In the European Union, marketing approval is released after testing of the product's quality, efficacy, and safety. Authorization is granted by centralized or national procedures. Analogously to the United States, off-label promotion is not allowed by Article 87 of Directive 2001/83/EC. Nevertheless, off-label prescriptions are allowed, provided the patients' best interests and autonomy are respected. Hyaluronidases are labeled in the European Union for the following indications: (1) as adjuvant

Table 4. Review of Allergic Reactions to Hyaluronidases Reported in the Literature

No.	Study	Years	Units	Timings	Local/Systemic	Symptoms
1	Kim et al[15]	55/F	1500 U: epidural	1 hour	Systemic	Generalized urticaria
2	Ahluwalia et al[31]	77/F	—	28 hours	Local, systemic	Swelling, nausea and vomiting, extraocular muscle function restriction
3	Leibovitch et al[26]	75/F	100-300 U	12 hours	Local	Proptosis, erythema, Swelling, extraocular muscle function restriction, periorbital pain, itchiness
4		87/F		24 hours		
5		70/M		24 hours		
6		85/F		48 hours		
7		75/F		72 hours		
8	Kim et al[30]	68/F	1500 U: epidural	Immediate	Systemic	Itching sensation and a generalized maculopapular rash
9		35/F	1500 U: tendon	12 hours	Local	Swelling, mild pain
10		55/F	1500 U: anserinus bursa	24 hours	Local	Swelling
11	Eberhart et al[27]	—	112.5 U: peribulbar	24 hours	Local	Angioedema, itching
12						
13						
14	Ebo et al[32]	47/F	1500 U subcutaneous (scar)	30 minutes	Local, systemic	Angioedema, anaphylactic reaction
15		50/F	1500 U subcutaneous (infiltration of the femoral cutaneous nerve)	20 minutes	Systemic	Generalized maculopapular rash, angioedema of the larynx
16	Szepfalusi et al[25]	4/M	200 000 U intravenous	Immediate	Systemic	Urticaria, tachycardia, shock
17		5/F				Tachycardia, exanthema, dyspnea
18		8/M				Itching, exanthema, dyspnea
19		14/M				Total body urticaria
20		11/F				Dyspnea, exanthema, shock
21	Varma and Metcalfe[33]	79/F	1500 U: peribulbar	48 hours	Local	Periorbital swelling, pain, redness
22	Musa et al[34]	50/F	1500 U: sub-Tenon's anesthesia	36 hours	Local	Pain, decreased vision, proptosis, swelling, extraocular muscle function restriction
23	Kirby et al[35]	67/F	15 000 U: peribulbar	Immediate	Local, systemic	Chemosis, periorbital edema, hypertension, sweating, nausea, incontinence
24	Delaere et al[36]	84/F	150 U: peribulbar	24 hours	Systemic	Nausea, vomiting
25		74/F				Nausea, vomiting
26		31/M				Itchy neck and throat, swallowing difficulties
27	Escolano et al[28]	71/M	—	Immediate	Systemic	Generalized rash
28	Quhill et al[37]	70/F	150 U: peribulbar	5 days	Local	Proptosis, pain, decrease of visual acuity, extraocular muscle function restriction
29	Youssef et al[38]	—	—, peribulbar	24-48 hours	Local	Periorbital swelling, decrease of visual acuity
30		—				
31		—				
32	Agrawal et al[39]	41/F	30 U: sub-Tenon's anesthesia	5 minutes	Local	Periorbital erythema, edema
33	Minning[29]	7/F	—	30 minutes	Local	Hemorrhage
34	Kempeneers et al[14]	73/F	Peribulbar	12 hours	Local	Pseudotumor
35	Taylor and Pollowitz[40]	54/F	—	Immediate	Local	Edema

—, not available.

therapy in subcutaneous drug administration, (2) to increase penetration of a local anesthetic, (3) to promote the reabsorption of contrast medium in urology, and (4) to promote reabsorption of subcutaneous hematomas.

Similarly, in Italy, drug prescriptions are regulated by law number 94/98, which allows prescriptions for those molecules approved by the European Union or already present in drugs regularly marketed. The administration of hyaluronidase for the treatment of HA injection is therefore considered off-label, informed consent of the patient is required, and the related prescriptions (specifying the indication) must be reported in a medical record. In addition, the pharmacist must send a copy of the off-label prescription to the local health office and then to the Public Health Department.

The production of hyaluronidases has been abandoned in Italy for marketing issues (eg, Jalovis 5 fl 250 IU by Coli Srl [Pomezia, Italy] was available until November 9, 1990; Jaluran fl 3 fl 300 IU by Pfizer Italia Srl [Borgo San Michele, Italy] was available until May 30, 2003; and Jaluran Ipod 3 fl 300 IU by Bioindustria Srl [Novi Ligure, Italy] was available until July 29, 1986). In these cases, the drug may be obtained legally as a galenic formulation.

CONCLUSIONS

Hyaluronidases should be considered an effective instrument for the practitioner employing HA fillers, both for correcting the achieved results and for avoiding severe and disfiguring complications. Proper knowledge of their use and prompt recognition of complication signs after filler injection are essential for everyday practice.

Disclosures

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