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Expert Opinion

Hyaluronidase: a review of approved formulations, indications and off-label use in chronic pain management

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Importance of the field: Hyaluronidase for injection is an adjuvant that increases the absorption and dispersion of other injected drugs or fluids (hypodermoclysis); and improves absorption of radiopaque agents in subcutaneous urography. Ovine hyaluronidase is approved for the treatment of vitreous hemorrhages.

Areas covered in this review: We review approved indications for injectable hyaluronidase and off-label uses as well as safety, efficacy and dosing information. We compare formulations made using animal tissue extracts versus the novel human recombinant type. Emphasis is on the human recombinant form and off-label uses in patients with chronic pain.

What the reader will gain: Hyaluronidase reduces the obstacle that the interstitial matrix presents to fluid and drug transfer. It is a mucolytic enzyme derived from mammalian tissue or synthesized *in vitro* in pure form (rHuPH20) using recombinant technology. Hyaluronidase is used off-label in chronic pain management to facilitate removal of epidural adhesions with mechanical and/or hydrostatic forces and to treat edema.

Take home message: The recently introduced rHuPH20 formulation obviates any risk of allergic reaction or prion-related illnesses. Reduction of edema by hyaluronidase and facilitation of epidural adhesioloysis may be beneficial in treating certain chronic painful conditions.

Keywords: drug absorption, edema, epidural adhesiolysis, hyaluronic acid, hyaluronidase, hypodermoclysis, mucolytic, spreading factor, urography, vitreous hemorrhage

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1. Introduction

Hyaluronidase has been legally marketed in the USA since 1948. The approved uses are based on the ability of hyaluronidase to increase the spread and dispersion of other injected drugs. Those uses were preceded by demonstration of the existence of factors in the mammalian testicle that modify the permeability of connective tissue. Subsequent work demonstrated that a factor extracted from testicular tissue enhances the spread of solutions of dyes and toxins injected intracutaneously into experimental animals. The factor was called 'spreading factor' and was identified to be hyaluronidase. [1]. Spreading factor is also present in, for example, venoms, toxins, bacteria and spermatozoa. This review will focus on animal-derived hyaluronidase approved by the FDA for human use and a human form produced using recombinant technology. Hyaluronidase transiently degrades hyaluronic acid (HA), a glycosaminoglycan found extensively in the interstitial matrix and basement membrane. HA, a main component of interstitial gel, inhibits the flow of substances through the tissue.

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Table 1. Hyaluronidase formulations.

	pH	Osm	Source	Absorption dose	Hypodermoclysis dose	Subcutaneous urography
Vitrase	6.7	290 – 310	Lyophilized, Ovine	50 – 300 U *150 U	150 U for 1000 ml solution	75 U per scapula
Amphadase	6.8	295 – 355	Bovine	30 – 300 U *150 U	150 U for 1000 ml solution	75 U per scapula
Hydase	6.9	275 – 305	Bovine	50 – 300 U *150 U	150 U for 1000 ml solution	75 U per scapula
Hylenex	7.4	290 – 350	Human	50 – 300 U *150 U	150 U for 1000 ml solution	75 U per scapula

*Usual dose for adults.
All from the US FDA. When added to local anesthetic for ophthalmologic blocks, different doses are used. Peribulbar/retrobulbar doses as small as 3.75 – 7.5 IU can be effective [20,21].

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The subcutaneous route is sometimes preferred for fluid resuscitation and for drug delivery. Therapy by this route is limited by the opposing effects of the extracellular matrix which restricts subcutaneous spread and hence tissue surface area available for absorption. The surface area for absorption can be greatly increased by co-administration of hyaluronidase to enzymatically degrade hyaluronic acid in the extracellular matrix. This enzymatic action allows more ready diffusion of drug to the target area or diffusion of fluid to blood vessels. Consequently, the adverse local effects drugs may be reduced on tissues, or fluid absorbed more rapidly due to increased contact with blood vessels. Hyaluronidase enhances the infusion rates and penetration of molecules up to 200 nm in diameter up to 20-fold [2].

Prior to the introduction of a formulation derived from purified recombinant hyaluronidase, rHuPH20, in 2005 all approved formulations of hyaluronidase were derived from crude extracts of ovine or bovine testicular tissue. The animal extracts were impure (less than 1% enzymatic activity per mg total protein) and immunogenic [2]. The extracts frequently are contaminated with proteases, immunoglobulin and factors that increase capilliary permeability and can give rise to IgE-mediated allergic reactions upon repeat administration [3-5]. The latest hyaluronidase formulation to receive FDA approval, rHuPH20 (Hylenex), was introduced onto the market in an attempt to increase the safety profile and make the drug more tolerable [6]. It contains 447 amino acids with an approximate molecular weight of 61,000 Daltons. rHuPH20 is manufactured using Chinese hamster ovary (CHO) cells for the expression of the enzyme. CHO cell proteins are removed by a series of purification steps but the trace levels remain in the final product and pose the potential for allergic reactions [6].

2. Pharmacodynamics of hyaluronidase

The intended pharmacological action of hyaluronidase is to modify the interstitial matrix by enzymatically depolymerizing hyaluronic acid. Hyaluronic acid, a high-molecular-weight glycosaminoglycan, is a major component of extracellular matrix of vertebrates that connects protein filaments, collagen fibers and connective tissue cells [7,8]. Hyaluronidase splits the glucosaminidic bond between C1 of the N-acetylglucosamine moity and C4 of a glucuronic acid in hyaluonic acid. The activity of marketed formulations of hyluronidase is determined by measuring *in vitro* the ability of hyaluronidase to depolymerize hyaluronic acid [9]. The assay is a standard United States Pharmacopeia (USP) assay. Activity is expressed in units. A unit is defined as the amount of enzyme that liberates one micromole of N-acetylglucosamine from HA per minute at 37°C and pH 4.0. One international unit (IU) = one USP unit = one National Formulary (NF) unit [10].

The activity of hyaluronidase is pH-dependent. Hyaluronidase made from bovine testes is 70% in the active form at a pH of 4, and retains activity at a pH of 7.5. Thus testicular type works at a larger range of pH's when compared with the type found in plasma which is only active at a pH below 5.3 [11].

As noted previously, marketed formulations of hyaluronidase are derived from crude extracts of ovine or bovine testicular tissue or are synthesized using recombinant technology. The FDA considers each hyaluronidase product to be distinct, that is no hyaluronidase product is therapeutically equivalent to any other approved hyaluronidase product [9]. A summary of different hyaluronidase formulations is shown in **Table 1**. The usual effective dose is 150 U for all formulas for use as spreading factor.

3. Pharmacokinetics and metabolism of hyaluronidase

Knowledge of mechanisms involved in the disappearance of injected hyaluronidase is limited [11]. The clearance of hyaluronidase in the serum occurs with a $t_{½}$ of 2.1 ± 0.2 min, and is followed by inactivation in the kidneys and liver [11]. The mechanism of inactivation of hyaluronidase after introduction into the dermis remains a matter of speculation. Subcutaneous effects are short lived due to the antagonistic effects of HA synthesis [12]. In adult humans, the effects on the interstitial matrix are completely reversed within 48 h [11].

4. Clinical efficacy

Requirement for approval of any drug by the FDA prior to 1962 was that a drug was safe. The requirement thereafter is that a product is both safe and effective. The FDA contracted

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with the National Academy of Science/National Research Council to make an initial evaluation of the effectiveness of over 3400 products that had been evaluated only for safety between 1938 and 1962. This review established the clinical effectiveness of products containing hyaluronidase for three indications: as an adjuvant added to solutions to increase the absorption and dispersion of other injected drugs; for hypodermoclysis; and as an adjunct in subcutaneous urography for improving resorption of radio-opaque agents. The finding was based on *in vitro* enzymatic activity of the products [9]. The FDA ruled that functional assay tests used to determine the number of hyaluronidase USP units may be used to establish the effectiveness of new hyaluronidase products intended for uses approved for products already legally marketed [9].

In addition to the three approved indications given above, ovine hyaluronidase is approved for the treatment of vitreous hemorrhage.

4.1 Off label for edema reduction and adhesiolysis

Lysis of epidural adhesions is an interventional technique used to treat certain chronic painful conditions such as radicular pain and/or lower back pain [13]. The goal is to remove barriers such as adhesions in the epidural cavity thought to contribute to the pain process and prevent the delivery of pain relieving drugs to the target site. Hyaluronidase is used to facilitate removal of adhesions.

Another off-label use of hyaluronidase is to reduce edema in various clinical conditions such as paraphimosis, intestonal intussusception, supraglottic airway edema and transplanted organ rejection [14-17].

It is possible that the effects of hyaluronidase on edema might play a role in the beneficial effects of hyaluronidase when it is used to treat epidural adhesions. This is because edema is thought to be part of the pathological process producing radiculopathy and lower back pain [13]. Published data is not conclusive but clinical observations plus results of studies under review for publication favorably support the role of hyaluronidase in the benefits patients obtained by epidural adhesiolysis.

5. Safety and tolerability

Allergic reactions are the main concern when hyaluronidase is administered [10]. This is especially true of the animal-derived formulations for reasons previously mentioned. Local injection site reactions are the most frequent adverse event reported following subcutaneous injection of hyaluronidase. However, allergic reactions such as urticaria and angioedema have been reported in < 0.1% of patients who received hyalurondase injections.

No reports of adverse effects or immune reactions were found in studies conducted on rats and monkeys using intravenous, ocular and dermal administration of rHuPH20 [8]. In rats up to 12,000 units were injected. At 10,500 units, minor renal tubule pathology (dilation) was noted. The drug appears to be well tolerated based on the results from these studies.

Theoretically, the risk of allergic reaction is increased by repeat injections but no reports were found that documented if the risk was a clinical problem with any of the hyaluronidase formulations. Concerns have been expressed about prion illness related to the use of the crude animal products. No cases have been confirmed. Because of its origin, rHuPH20 presents no risk for prion illness [6].

5.1 Drug interactions

According to the Physicians' Desk Reference, hualuronidase should not be used with furosemide, benzodiazepines, phenytoin, dopamine or alpha adrenergic agonists because of physical or chemical incompatabilities. Hyaluronidase is antagonized by anti-inflammatory agents including indomethacin, dexamethasone,and salicylates as well as many plant-based drugs like flavonoids and antioxidants [11,18]. Antihistamines, mast cell stabilizers, heparin, vitamin C and dicumarene oppose the action of hyaluronidase as well [5,6]. Patients taking salicylates, cortisone, ACTH, estrogens or antihistamines may require larger amounts of hyaluronidase for equivalent dispersing effect, since these drugs apparently render tissue resistant to the action of hyaluronidase [10].

Schulze, *et al.* [19] examined the effects of various drugs used during epidural adhesiolysis on hyaluronidase activity. They found that iodinated radiographic contrast media, 10% NaCl and the absence of corticosteroids reduce hyaluronidase activity. Local anesthetics had no demonstrable effect and 0.9% NaCl resulted in higher hyaluronidase activity.

6. Conclusion

Hyaluronidase has been successfully used as a spreading factor for over 60 years. Studies have shown increased absorption and dispersion of drugs used in combination with hyaluronidase without an increase in incidence or severity of side effects of the co-administered drugs compared with when the drugs are administered alone. All injectable formulations generally have identical indications, activities and dosing.

As hyaluronic acid is found virtually everywhere in the human body, the applications of this drug to facilitate the absorption of other drugs are vast and many off-label uses for hyaluronidase exist. Facilitation of epidural adhesiolysis and reduction of edema by hyaluronidase may be useful in treating radiating pain and lower back pain.

7. Expert opinion

Substantial data verify the safety and efficacy of hyaluronidase for the approved indications. Recent introduction of rHuPH20 is consistent with a principle established long ago of isolating active ingredients from crude preparations and using active ingredients in pure form. When this is done, the possibility must be considered that other substances

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in the crude preparation containing the active ingredient might favorably influence the intended effect of the active ingredient. We found no data testing this possibility for hyaluronidase. Use of enzymatic activity as a measure of efficacy establishes equalivancy of hyaluronidase formulations *in vitro* but does not explore positive or negative influences that may be in crude extracts. Possible influences could be on hyaluronidase activity or via other actions of substances in the extract.

Appropriate dose of hyaluronidase for epidural adhesiolysis needs to be investigated. The dose of crude extract used usually is 1500 IU. Recently, physicians at our institution have been using 150 – 300 units of the rHPH20 formulation. Noteworthy is that dose–response studies were not done to establish that 1500 IU of the crude extract formulation is the optimal dose for adhesiolysis.

That hyaluronidase helps reduce edema may contribute to the benefits of using it in the management of lower back pain and radiating pain.

Declaration of interest

The authors state no conflict of interest and have received no payment in preparation of this manuscript.

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