

Hyaluronidase in Ophthalmology

Steven M. Silverstein, MD, FACS^{a*}

Scott Greenbaum, MD, FACS^b

Robert Stern, MD^c

^a*Silverstein Eye Centers, 4240 Blue Ridge Blvd, Suite 1000, Kansas City, Missouri 64133; Clinical Professor, Kansas City University of Medicine and Biosciences, Adjunct Clinical Professor, University of Missouri School of Medicine, Kansas City, Missouri, E-mail: ssilverstein@silversteineyecenters.com*

^b*Greenbaum Eye Associates, 68-60 Austin St, Forest Hills, New York 11375, E-mail: thecannula@aol.com; Assistant Clinical Professor, Department of Ophthalmology, New York University School of Medicine, New York, New York*

^c*230 W. 125th St, New York, New York, 10027; Professor, Department of Basic Biomedical Sciences, Touro/Harlem College of Osteopathic Medicine, New York, New York, E-mail: robert.stern@ucsf.edu or robert.stern@touro.edu*

* *Corresponding Author*

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ABSTRACT

Purpose

The application of hyaluronidase in ophthalmology dates back 60 years, when it was first included in retrobulbar blocks. Now it is used routinely in retrobulbar, peribulbar, and sub-Tenon's blocks. Until recently, the only sources of hyaluronidase were animal derived, either pharmaceutically manufactured or compounded. Animal-derived products have been associated with low purity, variable potency, and uncertain safety. Given these concerns, use of recombinant human hyaluronidase merits consideration to help improve the safety and quality of local anesthetic blocks. In order for practitioners to make informed therapeutic decisions regarding the use of hyaluronidase in ophthalmology, the safety and efficacy of available products must first be evaluated.

Design

We analyzed multiple clinical samples from national and regional compounding pharmacies for purity and potency, and compared them to recombinant human hyaluronidase. In addition, a literature search, including the Food and Drug Administration Adverse Event Reporting System (FDA AERS) database, was performed to further evaluate adverse event and safety data.

Results

The laboratory analyses demonstrated that animal-derived hyaluronidase, specifically compounded products, has variable purity and potency, whereas recombinant human hyaluronidase possesses high purity and consistency. The literature supported routine inclusion of hyaluronidase in anesthetic blocks to help reduce the potential for complications. Although compounded animal-derived preparations have been used extensively, their varying levels of impurities and their potential for allergic reactions may create unnecessary risk.

Conclusions

Hyaluronidase use in local anesthetic blocks for ophthalmic surgery is beneficial. The high purity, favorable efficacy, and established safety profile of recombinant human hyaluronidase suggest it may be a suitable alternative for clinical use compared with compounded animal-derived products.

INTRODUCTION

Hyaluronidase is an enzyme which degrades hyaluronan (HA) and has diverse clinical applications resulting from its ability to facilitate the dispersion and/or absorption of an array of medications and fluids. It provides rapid penetrance of anesthetic agents, particularly to locations difficult to access. In ophthalmology, hyaluronidase is used most often as an adjunct to local anesthesia for retrobulbar, peribulbar, or sub-Tenon's blocks. The use of hyaluronidase in ophthalmology began as early as 1949, when Atkinson added it to retrobulbar and lid blocks.¹

Hyaluronidase products approved by the United States Food and Drug Administration (FDA) have included those derived from bovine (AMPHADASE,[®] Amphastar Pharmaceuticals, Inc, Rancho Cucamonga, CA) and ovine (Vitrase,[®] ISTA Pharmaceuticals, Inc., Irvine, CA) sources, along with a recombinant human product (Hylenex,[®] Halozyme Therapeutics, San Diego, CA; rHuPH20). Compounded formulations of animal-derived hyaluronidase also have been used in ophthalmology. When selecting products appropriate for use in clinical practice, it is important for clinicians to be aware of the differences among these various sources.

Notably, many case reports of adverse events have been published in the literature regarding the use of animal-derived hyaluronidase with local anesthetic ophthalmic blocks.² Obtained via a Freedom of Information Act request to the FDA, 98 cases of adverse events associated with animal-derived hyaluronidase used in an ophthalmology setting were reported between January 1998 and August 2011.³

Symptomatology reported in these cases

included an increase in intraocular pressure, optic disc hemorrhage, exophthalmos, blindness, eye pain, orbital and eyelid edema, reduced visual acuity, hypersensitivity reaction, swelling, tenderness, and inflammation. These adverse events were often associated with disability, hospitalization, and/or the need for intervention. The main commonality among all cases was the use of animal-derived hyaluronidase.³

In this article, we review the history, mechanism of action (MOA), and clinical uses of hyaluronidase in ophthalmology. We identify and test some of the current sources of hyaluronidase for their associated product characteristics, including purity and concentration, in addition to highlighting the importance of proper product selection and compounding of hyaluronidase. Finally, we measure and compare these objective parameters from both animal derived and human recombinant hyaluronidase, and examine the use of rHuPH20 and its role in therapy.

OVERVIEW OF HYALURONIDASE

Hyaluronidase has been used to facilitate the dispersion and/or absorption of fluids or medications for more than 70 years.⁴ It rapidly hydrolyzes HA, which is a glycosaminoglycan found in the extracellular matrix of most types of connective tissue (eg, skin, joint cartilage).⁵ Connective tissue consists of many macromolecules, which serve as a barrier to bulk fluid flow through the interstitial matrix.⁶ HA is a mega-dalton molecule with a half-life of 15 to 20 hours in the skin.⁵ Interestingly, the half-life of HA in the circulation ranges between 2 and 5 minutes.⁷ When HA is broken down by hyaluronidase, it causes a transient increase in the permeability of the connective tissue. This increased permeability lasts for approximately 24 to 48 hours when a single subcutaneous (SC) 150-U dose of rHuPH20 is administered,⁸ after which there is no histologic change in collagen or sign of inflammation in animal models after 28 days.⁵

Hyaluronidase is used in ophthalmology as a component of retrobulbar, peribulbar, and sub-Tenon's blocks, which

will be discussed in greater detail. Use of hyaluronidase in subcutaneous rehydration therapy (SCRT) has been shown to be safe and effective for mild-to-moderate dehydration in the adult, pediatric, geriatric, and palliative/chronic care populations.⁹ In a similar fashion to SCRT, hyaluronidase can be given subcutaneously to help administer urographic contrast media when intravenous

(IV) administration is not feasible, such as in younger patients.¹⁰ Additionally, hyaluronidase has been shown to be successful in facilitating the subcutaneous administration of various medications, and can be used to treat extravasated fluids, drugs, electrolytes, and nutritional and diagnostic substances.¹⁰

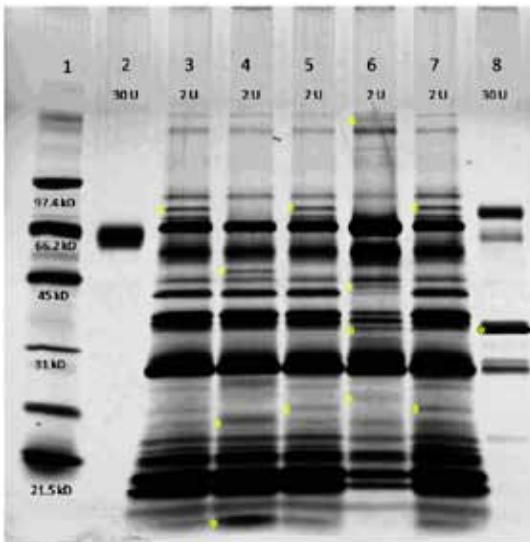
CURRENT USES OF HYALURONIDASE IN OPHTHALMOLOGY

Hyaluronidase is most frequently used in combination with anesthetics for ophthalmologic surgery (eg, retrobulbar block, peribulbar block, sub-Tenon's block, and van Lint block). Rationale for the inclusion of hyaluronidase in combination with local anesthesia techniques includes smaller increases in intraocular pressure (IOP),¹¹ less distortion of the surgical site,^{12,13} decreased incidence of postoperative strabismus, and potential for limiting local anesthetic myotoxicity because of quicker spread.^{14,15} In some studies, inclusion of hyaluronidase increases globe and lid akinesia, which may improve the safety of the procedure.¹⁶

Ovine hyaluronidase also has been investigated for the clearance of vitreous hemorrhage, where its efficacy and safety have not been established. Two phase 3 clinical trials demonstrated that a single intravitreal injection of ovine hyaluronidase was not associated with any serious safety issues and hyaluronidase had some therapeutic utility in the management of vitreous hemorrhage.^{17,18} It is important to note that the FDA Advisory Committee stated in 2003 that insufficient evidence exists to support the efficacy of ovine hyaluronidase in the treatment of vitreous hemorrhage.¹⁹ However, the panel also noted that the benefits could outweigh the risks in some patient subgroups.

We identified clinical trials which examined the application of hyaluronidase for local ophthalmic anesthetic blocks since 1985. In addition, we made a Freedom of Information Act inquiry in order to obtain adverse event records

Figure 1. Characterization by sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) of a recombinant human hyaluronidase (lane 2; 30 Units), five compounded animal-derived hyaluronidase preparations (lanes 3 to 7, with compounded hyaluronidase #1 in lane 3 to compounded hyaluronidase #5 in lane 7; 2 Units each), and an animal-derived hyaluronidase product (lane 8; 30 Units). The animal-derived hyaluronidase product was concentrated approximately 34-fold using a Microcon[®] centrifugal filter device with a 3 kDa cut-off. Molecular weight (MW) protein standards (with nominal MW) are shown in lane 1. Unlike animal-derived hyaluronidase, the recombinant human hyaluronidase (rHuPH20) is formulated with a protein excipient (ie, human serum albumin-used for stabilization). As such, the rHuPH20 bulk drug substance (provided by Halozyme Therapeutics, Inc., San Diego, CA) was used for this analysis. Unique bands in each preparation are denoted with an *



associated with hyaluronidase from 1998 to 2011.

Retrobulbar Block

Although retrobulbar blocks are used less commonly than peribulbar blocks²⁰ because of potential anatomic risk factors associated with administration and the need for an additional facial nerve block to prevent blinking, they may still be preferred for certain procedures that would benefit from lower volumes of local anesthetic (ie, 3-5 mL).²¹ Nine randomized, prospective, controlled studies evaluating hyaluronidase added to retrobulbar blocks in more than 1,300 patients were reviewed.^{16,22-29} A variety of anesthetic mixtures and hyaluronidase doses (ranging from 0.75 to 200 IU/mL) were used. The end points included akinesia, induction time, need for supplementary block, and volume of local anesthetic. Addition of hyaluronidase to retrobulbar blocks generally resulted in improved akinesia and was well tolerated. Fewer complications, such as a lower tendency for prolapse, were observed in patients who received hyaluronidase.^{25,27} Overall, a dose between 3.75 and 75 IU/mL hyaluronidase in retrobulbar blocks appears sufficient to provide a beneficial effect on akinesia.

Van Lint Blocks

Because retrobulbar blocks do not provide lid akinesia, they are often combined with van Lint blocks. This method of facial nerve block was the first to be reported and is considered to be the classic technique.^{30,31} Typically, 5 to 10 mL of anesthetic (often the same mixture as used for the retrobulbar block) is used, although some suggest that volumes as low as 2 mL are sufficient.^{30,31}

Peribulbar Block

The peribulbar block technique was developed to minimize the risk of injury to structures within the intraconal space. It is performed by injection into the extraconal space using larger volumes of local anaesthetic (eg, up to 12 mL).²¹ The larger volume is necessary for its spread into the entire corpus adiposum of the orbit and eyelids to block the orbicularis muscle.

Fourteen randomized, prospective, controlled studies evaluating hyaluronidase in peribulbar blocks in nearly 1,800 patients were reviewed and analyzed.^{11,13,22,32-39} These studies involved a variety of local anesthetic combinations and hyaluronidase doses (ranging from 3.75 to 300 IU/mL). The end points included akinesia, induction time, need for supplementary block, and volume of local anesthetic. Evidence of improvement in peribulbar block with hyaluronidase is equivocal. Approximately half of the studies show a benefit to including hyaluronidase, whereas the rest demonstrate equivalence across a range of hyaluronidase doses.

Hyaluronidase was well tolerated. In some of these studies, fewer complications such as increased IOP were observed in patients who received hyaluronidase.¹¹ Although hyaluronidase does not consistently improve peribulbar block efficacy, it is beneficial for its effects on facilitating the spread of larger volumes of anesthetic and reducing complications.

Sub-Tenon's Block

A sub-Tenon's block (STB) administered using either a needle or cannula²¹ provides high-quality anesthesia of the whole globe using relatively small volumes (eg, 1.25-5 mL).^{21,40} The addition of hyaluronidase in STB has been studied in varying combinations across six randomized, prospective, controlled studies encompassing more than 550 patients.⁴¹⁻⁴⁶ Testing the addition of hyaluronidase to STB has been conducted using assorted local anesthesia combinations and doses of hyaluronidase (ranging from 15-150 IU/mL). End points included akinesia, induction time, volume of local anesthetic, and quality of the block. Hyaluronidase addition to STB generally resulted in improved akinesia and was well tolerated. Although there was a wide range of hyaluronidase doses used and a lack of dose-response data, the studies demonstrate that doses between 15 and 150 IU/mL can provide benefit when used in STB.

SOURCES OF HYALURONIDASE

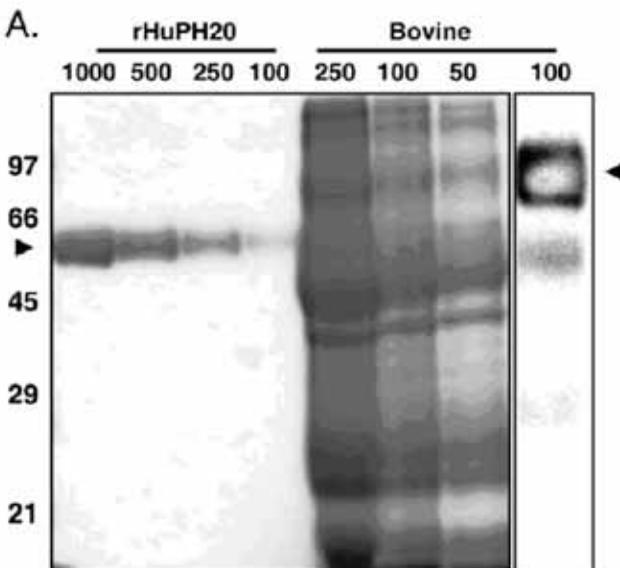
Animal Derived

There are FDA-approved animal-derived products in addition to animal-derived active pharmaceutical ingredients (API), which are produced mainly for commercial and research purposes, and may be compounded for human use. Approved products with hyaluronidase of bovine origin include Amphadase and Hydase.^{47,48} Vitrase is an approved product using an ovine source of hyaluronidase.⁴⁹ In addition to animal-

derived sources, there is recombinant human hyaluronidase, rHuPH20, which was approved by the FDA in 2005.⁸

Reliable sources of hyaluronidase have changed within the past decade, and there continues to be an issue of supply shortage.^{50,51} Discontinuation of Hydase production was effective April 2009. Amphadase was noted to be on long-term backorder until late 2011 because of a depleted supply of raw material. The Vitrase 6,200-unit single-dose vial was discontinued, although Vitrase is still available in 200-U/mL vials.⁵⁰

Figure 2. Characterization by sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) of recombinant human hyaluronidase (rHuPH20). rHuPH20 1000, 500, 250, and 100 Units per lane loaded compared with 250, 100, and 50 Units bovine testes hyaluronidase. The last column shows a Western blot analysis of bovine hyaluronidase preparations with the 1D6 mouse anti-ram hyaluronidase monoclonal antibody (Babraham Bioscience Technologies, Cambridge, UK). Only one 75-kDa band (minor band in the smear of proteins of the bovine preparation), identified by an arrow on the figure, cross-reacted strongly with the monoclonal antibody, confirming that the hyaluronidase enzyme is a minor component of the animal-derived hyaluronidase preparation. The lower band corresponds to excess protein (due to overloading of the bovine hyaluronidase preparation) that did not react with the antibody. Reproduced from reference 5, with permission.



Hylenex was recalled after glass particles were found in the vials during a routine inspection in 2010, but production and distribution have since been resumed.

Sources and Preparation

Animal-derived hyaluronidase, whether extracted from bovine or ovine testes, is generally purified through a series of multiple precipitation, fractionation, and filtration steps.⁵² After 3 to 6 weeks of processing, 1 ton of raw material yields approximately kilogram quantities of hyaluronidase. A purified hyaluronidase API extracted from an animal source usually contains less than 1% of the enzyme per milligram of total protein.⁵ The extract is often contaminated with proteases, immunoglobulin, and other elements, which can increase capillary permeability⁵³ or potentially cause IgE-mediated hypersensitivity reactions when used.⁵⁴⁻⁵⁶

Hypersensitivity reactions and anaphylaxis have been observed following systemic administration of hyaluronidase.⁵⁷ The extraction and purification processes may be less rigorous when manu-

Figure 3. Reactions to different doses of thimerosal-free hyaluronidase. Low, medium, and high doses were equivalent to 10, 20, and 30 U of drug, respectively. Reproduced from reference 83, with permission.



facturers produce hyaluronidase as an API rather than via FDA current Good Manufacturing Processes (cGMP) for approved products.

Intermittent product shortages compel clinicians to use compounded hyaluronidase products in an effort to maintain the efficacy of local anesthetic blocks. Use of compounded hyaluronidase has become a more common alternative because of the supply issue, in addition to reports of an increased incidence of postoperative complications such as permanent diplopia and ptosis without the use of hyaluronidase.⁵⁸ Sources of animal testes-derived hyaluronidase as API are plentiful as evidenced by the range of national and global websites from which compounding pharmacies can obtain the material they require.⁵⁹⁻⁶¹ There is concern that some compounding pharmacies order hyaluronidase as an API from less reputable sources or websites, because it is unknown whether the manufacturer is regulated by any quality-assurance measures. Standards such as validated sterilization procedures, potency and purity assays, contamination checks, and proper storage and handling may not be regulated in certain countries. As a result, a significant concern has arisen regarding animal-derived hyaluronidase not

produced in a cGMP setting, as little information may be available about the identity of the source, quality, and safety due to impurities found in many of these products.

The compounding process to produce animal-derived hyaluronidase solution is routine. To generate hyaluronidase 150 IU/mL for injection, the following materials are needed: hyaluronidase 15,000 units, sodium chloride 850 mg, edentate disodium 100 mg, calcium chloride dihydrate 53 mg, thimerosal 10 mg,

anhydrous sodium phosphate monobasic 170 mg, sterile water for injection 100 mL, and sodium hydroxide 1% solution in a quantity sufficient to give the solution a pH between 6.4 and 7.4.^{62,63} In an aseptic environment and using an aseptic technique, the compounds are dissolved in sterile water for injection and then sodium hydroxide is added drop by drop to obtain the desired pH.

In terms of quality control for compounded hyaluronidase, the solution must be checked to ensure that it is not discolored and does not contain a precipitate.^{62,63} In addition, it should be monitored for sterility and pyrogenicity. If large quantities are being prepared, the solution should be tested for potency. The product must be packaged in tight, light-resistant containers and stored in the refrigerator. When stored in this manner, the compounded hyaluronidase can be given up to a 6-month expiration date. Sterile filtration should be performed immediately before use to help ensure safety of the product.

Should compounded hyaluronidase be used, it is important as a clinician to be certain that the compounding pharmacy and its products meet or exceed the United States Pharmacopeia or National Formulary

monograph, American Society of Hospital Pharmacists, Board of Pharmacy, National Association of Boards of Pharmacy Good Compounding Practices, and FDA guidelines.^{51,64} Of note, FDA regulations on compounding pharmacies are not as stringent as are those for pharmaceutical products. There is no official mandated requirement for adverse event reporting associated with compounded products, nor is there a method to nationally or locally disseminate such information.⁶⁵ The main regulatory body for compounding pharmacies is the state board of pharmacy, and only two state boards of pharmacy require adverse event reporting (ie, North Carolina and Missouri).

Experience with Animal-Derived Product

The FDA has been made aware of more than 55 quality problems associated with compounded pharmaceutical products since 1990.⁶⁶ Many of these situations resulted in product recalls. In 2001, the FDA Division of Prescription Drug Compliance and Surveillance collected 29 products produced by 12 different compounding pharmacies for analysis of quality, purity, and potency. Ten of these products failed in one or more of the quality assurance parameters, which is a failure rate of 34%. In this survey, 2 of the 29 products tested were preparations of hyaluronidase (from different compounding pharmacies), one of which failed a quality

Table 1. Hyaluronidase activity (in U/mL), total protein concentration (mg/mL), and hyaluronidase activity per milligram total protein (U/mg) for a recombinant human hyaluronidase (rHuPH20), five compounded animal-derived hyaluronidases and an animal-derived hyaluronidase product.

Product	Hyaluronidase activity (U/mL) ^a	Total protein (mg/mL) ^b	Hyaluronidase activity per milligram total protein (U/mg) ^c
rHuPH20 bulk drug substance ^d	117,000	0.97	120,000
Compounded animal-derived hyaluronidase #1	220	0.34	650
Compounded animal-derived hyaluronidase #2	188	0.33	570
Compounded animal-derived hyaluronidase #3	186	0.30	620
Compounded animal-derived hyaluronidase #4	162	0.23	700
Compounded animal-derived hyaluronidase #5	210	0.42	500
Animal-derived hyaluronidase product	202	0.011	18,000

^aHyaluronidase activity in U/mL was determined using a modified version of the United States Pharmacopeia turbidimetric assay.

^bFor the five compounded animal-derived hyaluronidase preparations, total protein was based on the mean of two values obtained from a bicinchoninic acid assay (Thermo Scientific, Waltham, MA) and a Bradford assay (Thermo Scientific, Waltham, MA). The percent difference between the two assays was no more than 30% for any of the compounded formulations. For the animal-derived hyaluronidase product, total protein was based on a Bradford result only. For the rHuPH20 bulk drug substance, total protein was determined by absorbance at $\lambda = 280$ nm.

^cHyaluronidase activity in U/mg was calculated based on hyaluronidase activity in U/mL and protein concentration in mg/mL (values reported in this table).

^dUnlike animal-derived hyaluronidase, the recombinant human hyaluronidase (rHuPH20) is formulated with a protein excipient (ie, human serum albumin-used for stabilization). As such, the rHuPH20 bulk drug substance was used for this analysis (data in this row are the mean values from 16 lots of rHuPH20 bulk drug substance).

test for potency because it was less potent than labeled.

In our own investigation, one of the authors ordered compounded hyaluronidase from five pharmacies and also procured an animal-derived hyaluronidase product. These preparations, along with rHuPH20 (rHuPH20 bulk drug substance provided by Halozyme Therapeutics, Inc.) were evaluated for activity per milligram total protein and purity by sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE). Table 1 shows that the five compounded animal-derived hyaluronidase preparations have a low specific activity ranging from 500 to 700 Units per milligram total protein. In comparison, the pharmaceutically-prepared, animal-derived hyaluronidase had an activity of 18,000 U/mg total protein, and rHuPH20 had an activity value of 120,000 U/mg total protein. These three levels of activity can be explained by comparing the different levels of impurities, as evidenced by SDS-PAGE analysis (Figure 1). The compounded preparations were of very low purity (<1% of the specific activity of rHuPH20), as illustrated by the presence of a smear of protein species in the SDS-PAGE, whereas the animal-derived hyaluronidase product was modestly pure (approximately 15% of the specific activity of rHuPH20), as depicted by the presence of several distinct protein species in the SDS-PAGE. rHuPH20 demonstrated high purity, as evidenced by the presence of a single protein band corresponding to human hyaluronidase (approximately 99% purity). Based on the differences in activity per mg total protein, it is probable to conclude that the purity of compounded hyaluronidase is about 140- to 200-fold lower than that of rHuPH20. Impurities also vary among the five compounded animal-derived hyaluronidase products, as illustrated by the observation of at least three different protein migration patterns among the five compounded preparations. Figure 2 further characterizes by SDS-PAGE and Western blot analysis the difference in purity between rHuPH20 and an animal-derived hyaluronidase.⁵ It is

important to note that the presence of protein impurities and thimerosal (preservative found in Amphadase and compounded products) may add to the potential for adverse events or toxicity.⁶⁷

In addition to potency and purity concerns, there also have been reports of safety issues when using animal-derived hyaluronidase. Fourteen publications have reported hypersensitivity reactions to retrobulbar or peribulbar blocks associated with use of animal-derived hyaluronidase (100-300 IU/mL).^{2,68-81} The most common reactions included periorbital edema and erythema, conjunctival chemosis, itch or pain, proptosis, angioedema, and restriction of eye movements (ranging from mild to total ophthalmoplegia).⁶⁸ The reactions occurred almost immediately in some patients and less commonly, up to a few days afterward. Many of the patients had undergone an earlier procedure which included the administration of hyaluronidase, which suggests that the reactions may be a result of sensitization to the animal-derived product. The cornea and anterior chamber remained clear, although loss of vision occurred in some cases as the result of compression of the optic nerve or increase in intraocular pressure. The authors concluded that hyaluronidase should be used in ophthalmologic procedures, albeit carefully, with special care to monitor for previous experience and adverse reactions. These should be treated promptly to avoid loss of vision in severe cases.

It is evident that animal-derived hyaluronidase has several limitations, whether it is commercially manufactured or compounded. Although compounded animal-derived preparations have been used extensively, their varying levels of impurities and their potential for allergic reactions may create unnecessary risk. This risk makes it important to research thoroughly the source of hyaluronidase and its manufacturer and/or compounding pharmacy before ordering it for clinical use. In addition, antigenicity of animal-derived compounded products is of concern because they may cause a hypersen-

sitivity reaction in some patients.

HUMAN RECOMBINANT HYALURONIDASE

rHuPH20 provides a highly purified and highly potent product as demonstrated by the results shown in Table 1 and Figures 1 and 2.⁵ The purity of rHuPH20 is the result of a modern recombinant protein biotechnology manufacturing process that uses Chinese hamster ovary cells to express the human recombinant genetically engineered enzyme, which is then purified through a series of orthogonal column chromatography and other processing steps to remove non-hyaluronidase impurities and potential pathogens.⁸²

In addition, rHuPH20 may provide a preferable safety profile. In a study of 100 adult volunteers, participants were injected with 15 IU (0.1 mL) rHuPH20 intradermally in one arm and 0.1 mL 0.9% saline in the other arm.⁸² The statistical evaluation was designed to assure that the upper bound of two-sided confidence interval of the allergic response rate is less than 10%. Positive allergic reaction was defined as wheal with pseudopods within 5 minutes of injection, which persisted for at least 20 minutes, and was accompanied by localized itching. No positive allergic reactions were observed with either injection, and the incidence of wheal was comparable between both groups at 5 minutes (rHuPH20, 78%, saline, 84%).

Five minutes after injection, localized itching was observed in 8% of saline injections compared with a 2% incidence for rHuPH20 injections. Thirty minutes after injection, erythema, wheal, and localized itching were comparable between the two groups, but there was a higher incidence of ecchymosis and discomfort with the saline injection. In another study of intradermal administration of hyaluronidase, 25% of patients experienced hypersensitivity reactions (ie, site redness and mild pruritus) which appeared to be dose-dependent when injected with compounded hyaluronidase (Figure 3).⁸³ Eleven adverse events were reported, but were judged to be unrelated to hyaluronidase. In a report of 162 subjects

dosed with 3 U (0.02 mL) of Amphadase, 5% (8/162) of subjects had a positive allergic reaction. This dose is five times less than the dose tested in the rHuPH20 allergenicity study.⁸² When allergic sensitivity to Vitrase was studied, there were no positive allergic reactions reported (N=65) with a dose of 3 U (0.03 mL), which is approximately 3.3 times less than the rHuPH20 dose tested.

The safety profile results from other clinical studies performed using rHuPH20 consistently demonstrate a favorable outcome. In pediatric SCRT studies, all adverse events were considered to be mild to moderate in severity and hyaluronidase was safe and well tolerated.^{84,85} Similarly, in adult SCRT studies, adverse events were mild to moderate and hyaluronidase was well tolerated.^{86,87} These findings of safety and tolerability are also consistent with hyaluronidase use to facilitate the administration of antibiotics⁸⁸ and pain medication.^{89,90} rHuPH20 has been demonstrated to be well tolerated, with a favorable safety profile across various populations and clinical settings.

At the time of publication, one report has been presented regarding the use of rHuPH20 in ophthalmology.⁹¹ Greenbaum included 150 IU of rHuPH20 in a sub-Tenon's block consisting of 1.25 mL of a 50/50 mixture of 4% lidocaine and 0.75% bupivacaine and administered this formulation to 10 patients undergoing cataract surgery. No complications occurred during the administration of local anesthesia. Currently, Dr Greenbaum is investigating the use of rHuPH20 in paravulbar blocks. Preliminary observations from this pilot study have suggested that rHuPH20 is safe and well tolerated for cataract surgery and may improve the efficacy of the paravulbar block.

DISCUSSION AND CONCLUSIONS

Hyaluronidase has a clear role in ophthalmology, having been shown to improve the quality, efficacy, and safety of local anesthetic blocks. Overall, studies demonstrate improved akinesia or time to onset of block when adding hyaluronidase to retrobulbar, peribulbar, or sub-Tenon's blocks, along

with fewer complications or adverse effects. In addition, hyaluronidase use allows for lower volumes of anesthetic to be employed, which helps minimize elevations in IOP during surgical procedures.

The literature discussing the use of hyaluronidase in local anesthetic blocks demonstrates a high degree of variability in methodology and dosages. However, this variation suggests that hyaluronidase can be used safely and successfully.

Given the value of hyaluronidase use in ophthalmology, it is important to note that the source of hyaluronidase merits careful consideration. Animal-derived hyaluronidase preparations (specifically compounded formulations) can be associated with hypersensitivity reactions or toxicities, in addition to being characterized by significantly lower purity and potency, than human recombinant hyaluronidase. Of notable concern with compounded animal-derived hyaluronidase is the potential lack of information regarding impurities and the unknown compliance with quality-assurance measures.

rHuPH20 was shown to be highly pure and highly potent (approximately 140- to 200-fold increase compared with compounded animal-derived hyaluronidase; approximately 5.6-fold increase compared with manufactured animal-derived hyaluronidase). It has demonstrated a favorable safety profile and is associated with no reports of serious hypersensitivity reactions. Although animal-derived hyaluronidase products have been used in ophthalmology for nearly 60 years, the improved safety, purity, and consistency of human-derived rHuPH20 deserves consideration for its routine use in local anesthetic blocks.

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CONFLICT-OF-INTEREST STATEMENT

SMS: Advisor to Halozyme Therapeutics; consultant fee, Halozyme Therapeutics; SG: Consultant fee, Halozyme Therapeutics;

patent, royalty, Alcon Surgical, Greenbaum Anesthesia Cannula; RS: Nothing to disclose
Role of study sponsor: Study sponsor, Halozyme Therapeutics, provided support for the publication and performed the analysis for the compounded hyaluronidase products. Halozyme Therapeutics had no role in study design; collection or interpretation of data; writing of the manuscript; decision to submit the manuscript for publication.

REFERENCES

1. Atkinson WS. Use of hyaluronidase with local anesthesia in ophthalmology: preliminary report. *Arch Ophthalmol.* 1949; 42:628-633.
2. Leibovitch I, Tamblin D, Casson R, Selva D. Allergic reaction to hyaluronidase: a rare cause of orbital inflammation after cataract surgery. Graefe's *Arch Clin Exp Ophthalmol.* 2006; 244:944-949.
3. Center for Drug Evaluation and Research. *Hyaluronidase Adverse Event Data (AERS)*; August 5, 2011.
4. Chalk E, Duthie ES. A mucolytic enzyme in testis extracts [letter]. *Nature.* 1939; 144:977-978.
5. Bookbinder LH, Hofer A, Haller MF, et al. A recombinant human enzyme for enhanced interstitial transport of therapeutics. *J Control Rel.* 2006; 114:230-241.
6. Day TD. The permeability of interstitial connective tissue and the nature of the interfibrillary substance. *J Physiol.* 1952; 117:1-8.
7. Fraser JR, Laurent TC, Laurent UB. Hyaluronan: its nature, distribution, functions, and turnover. *J Intern Med.* 1997; 242:27-33.
8. Hylenex recombinant (hyaluronidase human injection) [package insert]. San Diego, CA: *Halozyme Therapeutics*; August 2011.
9. Spandorfer PR. Subcutaneous rehydration: updating a traditional technique. *Pediatr Emerg Care.* 2011; 27:230-236.
10. United States Department of Veterans Affairs. Hyaluronidase monograph (Amphadase, Hydase, Vitrase, Hylenex) final. <http://www.pbm.va.gov/DrugMonograph.aspx>. Accessed August 31, 2011.
11. Dempsey GA, Barrett PJ, Kirby IJ. Hyaluronidase and peribulbar block. *Br J Anaesth.* 1997; 78:671-674.
12. Canavan KS, Dark A, Garrioch MA. Sub-Tenon's administration of local anaesthetic: a review of the technique. *Br J Anaesth.* 2003; 90:787-793.
13. Prosser DP, Rodney GE, Mian T, Jones HM, Khan MY. Re-evaluation of hyaluronidase in peribulbar anaesthesia. *Br J Ophthalmol.* 1996; 80:827-830.
14. Brown SM, Coats DK, Collins ML, Underdahl JP. Second cluster of strabismus cases after periorbital anesthesia without hyaluronidase. *J Cataract Refract Surg.* 2001; 27:1872-1875.

15. Hamada S, Devys JM, Xuan TH, et al. Role of hyaluronidase in diplopia after peribulbar anesthesia for cataract surgery. *Ophthalmology*. 2005; 112:879-882.
16. Remy M, Pinter F, Nentwich MM, Kampik A, Schöfeld CL. Efficacy and safety of hyaluronidase 75 IU as an adjuvant to mepivacaine for retrobulbar anesthesia in cataract surgery. *J Cataract Refract Surg*. 2008; 34:1966-1969.
17. Kuppermann BD, Thomas EL, de Smet MD, Grilone LR, for the Vitrase for Vitreous Hemorrhage Study Groups. Safety results of two phase III trials of an intravitreal injection of highly purified ovine hyaluronidase (Vitrane®) for the management of vitreous hemorrhage. *Am J Ophthalmol*. 2005; 140:585-597.
18. Kuppermann BD, Thomas EL, de Smet MD, Grilone LR, for the Vitrase for Vitreous Hemorrhage Study Groups. Pooled efficacy results from two multinational randomized controlled clinical trials of a single intravitreal injection of highly purified ovine hyaluronidase (Vitrane®) for the management of vitreous hemorrhage. *Am J Ophthalmol*. 2005; 140:573-584.
19. US Department of Health and Human Services Food and Drug Administration Center for Drug Evaluation and Research (CDER). Dermatology and Ophthalmic Drugs Advisory Committee. 2003. <http://www.fda.gov/ohrms/dockets/ac/03/transcripts/3929T1.pdf>. Accessed October 11, 2011.
20. Leaming DV. Practice styles and preferences of ASCRS members--2003 survey. *J Cataract Refract Surg*. 2004; 30:892-900.
21. Nouvellon E, Cuvillon P, Ripart J, Viel EJ. Anaesthesia for cataract surgery. *Drugs Aging*. 2010; 27:21-38.
22. Kallio H, Paloheimo M, Maunuksele EL. Hyaluronidase as an adjuvant in bupivacaine-lidocaine mixture for retrobulbar/peribulbar block. *Anesth Analg*. 2000; 91:934-937.
23. DeBroff BM, Hamilton RC, Loken RG, Gimbel HV, Ferensowicz M. Retrobulbar anesthesia with 7.5 vs. 0.75 IU/mL of hyaluronidase. *Can J Ophthalmol*. 1995; 30:262-264.
24. Johansen J, Kjeldgård M, Corydon L. Retrobulbar anaesthesia: a clinical evaluation of four different anaesthetic mixtures. *Acta Ophthalmol*. 1993; 71:787-790.
25. Krohn J, Seland JH, Hovding G, Bertelsen T, Aasved H, Haugen OH. Retrobulbar anesthesia with and without hyaluronidase in extracapsular cataract surgery: a prospective, randomized, double-blind study. *Acta Ophthalmol*. 1993; 71:791-795.
26. Abelson MB, Mandel E, Paradis A, George M. The effect of hyaluronidase on akinesia during cataract surgery. *Ophthalmic Surg*. 1989; 20:325-326.
27. Thomson I. Addition of hyaluronidase to lignocaine with adrenaline for retrobulbar anaesthesia in the surgery of senile cataract. *Br J Ophthalmol*. 1988; 72:700-702.
28. Nicoll JMV, Treuren B, Acharya A, Ahlen K, James M. Retrobulbar anesthesia: the role of hyaluronidase. *Anesth Analg*. 1986; 65:1324-1328.
29. Mindel JS. Value of hyaluronidase in ocular surgical akinesia. *Am J Ophthalmol*. 1978; 85:643-646.
30. Jankovic D, ed. Regional Nerve Blocks and Infiltration Therapy. Textbook and Color Atlas. 3rd ed. <http://www.scribd.com/doc/39463186/Regional-Nerve-Blocks-and-Infiltration-Therapy-Textbook-and-Color-Atlas-3rd-Ed?query=van+hint>. Accessed August 31, 2011.
31. Greenbaum S. Anesthesia for eye surgery. In: Tasman W, Jaeger EA, eds. *Duane's Ophthalmology*. On CD-ROM, 2006 ed. <http://www.oculist.net/downat0502/prof/ebook/duanes/index.html>. Accessed October 17, 2011.
32. Pacella E, Collini S, Pacella F, Piraino DC, Santamaria V, DeBlasi RA. Levobupivacaine vs. racemic bupivacaine in peribulbar anaesthesia: a randomized double blind study in ophthalmic surgery. *Eur Rev Med Pharmacol Sci*. 2010; 14:539-544.
33. Mantovani C, Bryant AE, Nicholson G. Efficacy of varying concentrations of hyaluronidase in peribulbar anaesthesia. *Br J Anaesth*. 2001; 86:876-878.
34. van den Berg AA, Montoya-Pelaez LF. Comparison of lignocaine 2% with adrenaline, bupivacaine 0.5% with or without hyaluronidase and a mixture of bupivacaine, lignocaine and hyaluronidase for peribulbar block analgesia. *Acta Anaesthesiol Scand*. 2001; 45:961-966.
35. Brydon CW, Basler M, Kerr WJ. An evaluation of two concentrations of hyaluronidase for supplementation of peribulbar anaesthesia. *Anaesthesia*. 1995; 50:998-1000.
36. Crawford M, Kerr WJ. The effect of hyaluronidase on peribulbar block. *Anaesthesia*. 1994; 49:907-908.
37. Mather C, Smith JH, Bloom PA. The efficacy of 0.75% bupivacaine with pH adjustment and hyaluronidase for peribulbar blockade: the incidence of prolonged ptosis. *Eur J Ophthalmol*. 1994; 4:13-18.
38. Roberts JE, MacLeod BA, Hollands RH. Improved peribulbar anaesthesia with alkalinization and hyaluronidase. *Can J Anaesth*. 1993; 40:835-838.
39. Sarvela J, Nikki P. Hyaluronidase improves regional ophthalmic anaesthesia with etidocaine. *Can J Anaesth*. 1992; 39:920-924.
40. Greenbaum S. The first use of recombinant human hyaluronidase in cataract surgery anesthesia: a pilot study [poster 263]. Presented at the annual meeting of the American Academy of Ophthalmology; November 10-13, 2007; New Orleans, LA.
41. Schulenburg HE, Sri-Chandana C, Lyons G, Columb MO, McLure HA. Hyaluronidase reduces local anaesthetic volumes for sub-Tenon's anaesthesia. *Br J Anaesth*. 2007; 99:717-720.
42. Aslam S, Sarker SJ, Tran-Dang M, et al. Effect of hyaluronidase on ocular motility and eyelid function in sub-Tenon's anaesthesia: randomised controlled trial. *Eye*. 2006; 20:579-582.

43. Alwitary A, Chaudhary S, Gopee K, Butler TKH, Holden R. Effect of hyaluronidase on ocular motility in sub-Tenon's anesthesia: randomized controlled trial. *J Cataract Refract Surg.* 2002; 28:1420-1423.44. Moharib MM, Mitra S, Rizvi SG. Effect of alkalinization and/or hyaluronidase adjuvancy on a local anesthetic mixture for sub-Tenon's ophthalmic block. *Acta Anaesthesiol Scand.* 2002; 46:599-602.
45. Rowley SA, Hale JE, Finlay RD. Sun-Tenon's local anaesthesia: the effect of hyaluronidase. *Br J Ophthalmol.* 2000; 84:435-436.
46. Guise P, Laurent S. Sub-Tenon's block: the effect of hyaluronidase on speed of onset and block quality. *Anaesth Intensive Care.* 1999; 27:179-181.
47. Hydase (hyaluronidase injection) [package insert]. San Diego, CA: PrimaPharm, Inc.; 2005.
48. Amphadase (hyaluronidase injection USP) [package insert]. Rancho Cucamonga, CA: Amphastar Pharmaceuticals, Inc.; November 2005.
49. Vitrase (hyaluronidase injection) ovine, 200 USP units/mL [package insert]. Irvine, CA: ISTA Pharmaceuticals; 2010.
50. American Society of Health-System Pharmacists. Hyaluronidase Injection. June 17, 2011. <http://www.ashp.org/DrugShortages/Current/bulletin.aspx?id=580>. Accessed June 21, 2011.
51. Hagan JC III, Hill WE. Use of a compounding pharmacy hyaluronidase for ophthalmic injection anesthesia [letter]. *J Cataract Refract Surg.* 2001; 27:1712-1714.
52. BBI Enzymes. Hyaluronidase/Mucopolysaccharidase. http://www.bbienzymes.com/content_202_. Accessed June 21, 2011.
53. Benditt EP, Schiller S, Mathews MB, Dorfman A. Evidence that hyaluronidase is not the factor in testicular extract causing increased vascular permeability. *Proc Soc Exp Biol Med.* 1951; 77:643-645.
54. Britton RC, Habif DV. Recent advances in surgery. *Surgery.* 1953; 33(6):917-942.
55. Kind LS, Roffler S. Adverse reactions to hyaluronidase. *Proc Soc Exp Biol Med.* 1961; 106:734-735.
56. Schwartzman J. Hyaluronidase in pediatrics. *NY State J Med.* 1951; 215-221.
57. Lee HK, Choi EJ, Lee PB, Nahm FS. Anaphylactic shock caused by the epidurally-administered hyaluronidase. *Korean J Pain.* 2011 Dec; 24(4):221-225.
58. Jehan FS, Hagan JC III, Whittaker TJ, Subramanian M. Diplopia and ptosis following injection of local anesthesia without hyaluronidase. *J Cataract Refract Surg.* 2001; 27:1876-1879.
59. DIYSite.com. Hyaluronidase. <http://www.diytrade.com/china/4/products/4561969/Hyaluronidase.html>. Accessed August 31, 2011.
60. EMD Chemicals. http://www.emdchemicals.com/is-bin/INTERSHOP.enfinity/WFS/Merck-US-Site/en_CA. Accessed June 20, 2011.
61. Sigma-Aldrich. Hyaluronidase. <http://www.sigmaaldrich.com/technical-service-home/product-catalog.html>. Accessed August 31, 2011.
62. Allen LV Jr. Hyaluronidase 150 u/mL injection. *U.S. Pharmacist.* 2001; 26:68-71.
63. Formulations. *Int J Pharm Comp.* 2001; 5:299-309
64. US Food and Drug Administration. Drugs. Application of federal law to practice of pharmacy compounding. <http://www.fda.gov/Drugs/GuidanceComplianceRegulatoryInformation/PharmacyCompounding/ucm155666.htm>. June 18, 2009. Accessed August 31, 2011.
65. Committee on Health, Education, Labor, and Pensions. Federal and State Role in Pharmacy Compounding and Reconstitution: Exploring the Right Mix to Protect Patients. October 23, 2003. <http://ftp.resource.org/gpo.gov/hearings/108s/90129.txt>. Accessed August 8, 2011.
66. US Food and Drug Administration. Drugs. Report: limited FDA survey of compounded drug products. <http://www.fda.gov/Drugs/GuidanceComplianceRegulatoryInformation/PharmacyCompounding/ucm155725.htm>. June 18, 2009. Accessed August 31, 2011.
67. Jumper JM, McCauley MB, Equi RA, Duncan KG, Duncan J, Schwartz DM. Corneal toxicity of intraocular hyaluronidase. *J Ocul Pharmacol Ther.* 2002; 18:89-97.
68. Delaere L, Zeyen T, Foets B, Van Calster J, Stalmans I. Allergic reaction to hyaluronidase after retrobulbar anaesthesia: a case series and review. *Int Ophthalmol.* 2009; 29:521-528.
69. Eberhart AH, Weiler CR, Erie JC. Angioedema related to the use of hyaluronidase in cataract surgery. *Am J Ophthalmol.* 2004; 138:142-143.
70. Kempeneers A, Dralands L, Ceuppens J. Hyaluronidase induced orbital pseudotumor as complication of retrobulbar anesthesia. *Bull Soc Belge Ophthalmol.* 1992; 243:159-166.
71. Kim TW, Lee JH, Yoon KB, Yoon DM. Allergic reactions to hyaluronidase in pain management—a report of three cases. *Korean J Anesthesiol.* 2011; 60:57-59.
72. Kirby B, Butt A, Morrison AM, Beck MH. Type I allergic reaction to hyaluronidase during ophthalmic surgery. *Contact Dermatitis.* 2001; 44:52.
73. Minning CA Jr. Hyaluronidase allergy simulating expulsive choroidal hemorrhage. *Arch Ophthalmol.* 1994; 112:585-586.
74. Quhill F, Bowling B, Packard RB. Hyaluronidase allergy after peribulbar anesthesia with orbital inflammation. *J Cataract Refract Surg.* 2004; 30:916-917.
75. Taylor IS, Pollowitz JA. A little-known phenomenon: allergic reaction to hyaluronidase. *Ophthalmology.* 1984; 91:1003.
76. Varma D, Metcalfe TW. Orbital cellulitis after peribulbar anaesthesia for cataract surgery. *Eye (Lond).* 2003; 17:105-106.
77. Youssef Z, Pennefather PM, Watts MT. Orbital cellulitis vs allergic reaction to hyaluronidase as the cause of periorbital oedema. *Eye (Lond).* 2005; 19:691-692.
78. Ahluwalia HS, Lukaris A, Lane CM. Delayed allergic reaction to hyaluronidase: a rare sequel to cataract surgery [letter]. *Eye.* 2003; 17:263-266.

79. Escolano F, Parés N, Gonzalez I, Castillo J. Allergic reaction to hyaluronidase in cataract surgery [letter]. *Eur J Anaesthesiol*. 2005; 22:729-730.
80. Musa F, Srinivasan S, King CM, Kamal A. Raised intraocular pressure and orbital inflammation: a rare IgE-mediated allergic reaction to sub-Tenon's hyaluronidase [letter]. *J Cataract Refract Surg*. 2006; 32:177-178.
81. Nicholson G, Hall GM. Allergic reaction to hyaluronidase after a peribulbar injection [letter]. *Anaesthesia*. 2003; 58:814-815.
82. Yocum RC, Kennard D, Heiner LS. Assessment and implication of the allergic sensitivity to a single dose of recombinant human hyaluronidase injection: a double-blind, placebo-controlled clinical trial. *J Infus Nurs*. 2007; 30:293-299.
83. Vartanian AJ, Frankel AS, Rubin MG. Injected hyaluronidase reduces Restalyne-mediated cutaneous augmentation. *Arch Facial Plast Surg*. 2005; 7:231-237.
84. Allen CH, Etwiler LS, Miller MK, et al, for the INcreased Flow Utilizing Subcutaneously-Enabled Pediatric Rehydration Study Collaborative Research Group. Recombinant human hyaluronidase-enabled subcutaneous pediatric rehydration. *Pediatrics*. 2009; 124:e858-e867.
85. Spandorfer PR, Friend K, Harb G. Recombinant human hyaluronidase-facilitated subcutaneous vs intravenous rehydration therapy in children. Presented at the 2010 Pediatric Academic Societies' Annual Meeting; May 1-4, 2010; Vancouver, Canada.
86. Dychter S, Ebel D, Mead TR, Yocum RC. Comparison of the tolerability of recombinant human hyaluronidase + normal saline and recombinant human hyaluronidase + lactated ringer's solution administered subcutaneously: a phase IV double-blind, randomized pilot study in health volunteers. *Curr Ther Res*. 2009; 70:421-438.
87. Thomas JR, Yocum RC, Haller MF, von Gunten CF. Assessing the role of human recombinant hyaluronidase in gravity-driven subcutaneous hydration: the INFUSE-LR study. *J Palliat Med*. 2007; 10:1312-1320.
88. Harb G, Lebel F, Battikha J, Thackara JW. Safety and pharmacokinetics of subcutaneous ceftriaxone administered with or without recombinant human hyaluronidase (rHuPH20) versus intravenous ceftriaxone administration in adult volunteers. *Curr Med Res Opin*. 2010; 26:279-288.
89. Thomas JR, Wallace MS, Yocum RC, Vaughn DE, Haller MF, Flament J. The INFUSE-Morphine study: use of recombinant human hyaluronidase (rHuPH20) to enhance the absorption of subcutaneously administered morphine in patients with advanced illness. *J Pain Symptom Manage*. 2009; 38:663-672.
90. Thomas JR, Yocum RC, Haller MF, Flament J. The INFUSE-Morphine IIB study: use of recombinant human hyaluronidase (rHuPH20) to enhance the absorption of subcutaneous morphine in healthy volunteers. *J Pain Symptom Manage*. 2009; 38:673-682.
91. Greenbaum S. The first use of recombinant human hyaluronidase in cataract surgery anesthesia: a pilot study [poster 263]. Presented at the annual meeting of the American Academy of Ophthalmology; November 10-13, 2007; New Orleans, LA.