CENTER FOR DRUG EVALUATION AND RESEARCH

APPLICATION NUMBER:

21-640

PHARMACOLOGY REVIEW

PHARMACOLOGY/TOXICOLOGY COVER SHEET

- NDA number: **NDA 21-640**
  Review number: 000
  Sequence number/date/type of submission: 000/August 4, 2003/Commercial
  Information to sponsor: Yes ( ) No (X)
  Sponsor and/or agent: ISTA Pharmaceuticals, Inc., 15279 Alton Parkway, Suite 100, Irvine, CA 92618 (Tel: 949-788-5303; Fax: 949-727-0833)
  Manufacturer for drug substance: Biozyme Laboratories Limited, Unit 6, Gilchrist-Thomas Estate, Blaenavon, Gwent NP4 9RL, South Wales, UK

Reviewer name: Zhou Chen, Ph.D.
Division name: Anti-Inflammatory, Analgesic, and Ophthalmic Drug Products
HFD #: HFD-550
Review completion date: September 5, 2003

Drug:

Trade name: **Vitrase**
Generic name (list alphabetically):
Code name:
Chemical name: Not provided
Chemical structure: Unknown
Molecular formula/molecular weight:

Relevant INDs/NDAs/DMFs: NDA 6-343 and NDA 21-414

Drug class: Protein enzyme

Indication: As an adjuvant to increase the absorption and dispersion of other injected drugs; for hypodermoclysis; and as an adjunct in subcutaneous urography for improving resorption of radiopaque agents

Clinical formulation: USP units/vial

Ingredients	Amount/4 ml
Hyaluronidase (ovine)	USP unit
Lactose monohydrate NF/EP	5 mg
Potassium phosphate, monobasic, NF/EP	1 22 mg
Potassium phosphate, dibasic, USP/EP	1.92 mg
Water for injection USP/EP	qs

Route of administration: Subcutaneous injection

Executive Summary

I. Recommendations

A. Recommendation on Approvability

Approval is recommended for this NDA application from a nonclinical perspective.

B. Recommendation for Nonclinical Studies

No nonclinical studies were submitted. The nonclinical pharmacology and toxicology information in this NDA is based on DESI notice (Federal Register Vol 35, No 185, p14800-14801) for hyaluronidase (Wydase, NDA 6-343). Additional nonclinical safety data related to intravitreal use of the product were provided in NDA 21-414.

The sponsor requests a categorical exclusion from the requirement (21CFR314.50(d)(2)) for any additional pharmacology/toxicology studies based upon the data presented in NDA 6-343 (Wydase) and NDA 21-414 (Vitrase). The exclusion is granted. No extra nonclinical studies are necessary.

C. Recommendations on Labeling

The labeling for the Carcinogenesis, Mutagenesis, Impairment of Fertility section and the Pregnancy section are identical with the labeling for Wydase, with the exception that the name Wydase is replaced with Vitrase. No modification is recommended.

II. Summary of Nonclinical Findings

A. Brief Overview of Nonclinical Findings

No nonclinical studies were submitted. For nonclinical findings, please reference NDA 21-414.

B. Pharmacologic Activity

No non-clinical studies were submitted. For pharmacologic activity information, please reference NDA 21-414.

C. Nonclinical Safety Issues Relevant to Clinical Use

There are no nonclinical safety issues relevant to clinical use.

III. Administrative

A. Reviewer signature: /S/

B. Supervisor signature: Concurrence - /S/

Non-Concurrence - /S/
(see memo attached)

C. cc: list:
NDA 21-640/Division File
NDA 21-640/Original NDA
HFD-550/CSO/Gorski
HFD-550/MO/Harris
HFD-550/TL Pharm/Yang
HFD-550/Pharm/ChenZh

This is a representation of an electronic record that was signed electronically and this page is the manifestation of the electronic signature.

/s/

Zhou Chen
11/4/03 11:14:15 AM
PHARMACOLOGIST

Josie,

Josie Yang
11/4/03 11:34:56 AM
PHARMACOLOGIST

51 page(s) have been removed because it contains trade secret and/or confidential information that is not disclosable.

PHARMACOLOGY/TOXICOLOGY COVER SHEET

NDA number: **NDA 21-414**
Review number: 000
Sequence number/date/type of submission: 000/January 3, 2002/Commercial
Information to sponsor: Yes (X) No ( )
Sponsor and/or agent: ISTA Pharmaceuticals, Inc., 15279 Alton Parkway, Suite 100, Irvine, CA 92618 (Tel: 949-788-5303; Fax: 949-788-6013)
Manufacturer for drug substance: Biozyme Laboratories Limited, Unit 6, Gilchrist-Thomas Estate, Blaenavon, Gwent NP4 9RL, South Wales, UK

Reviewer name: Zhou Chen, Ph.D.
Division name: Anti-Inflammatory, Analgesic, and Ophthalmic Drug Products
HFD #: HFD-550
Review completion date: February 15, 2003

Drug:

Trade name: **Vitrase**
Generic name (list alphabetically): Ovine hyaluronidase, Ovine testicular hyaluronidase
Code name: HY06A
Chemical name: Not provided
Chemical structure: Unknown
Molecular formula/molecular weight: Ovine hualuronidase occurs in two natural forms, α- and β-hyaluronidase. The drug substance contains — other proteins as impurities,

Relevant INDs/NDAs/DMFs: INDs 49,939 ,

Drug class: Protein enzyme

Indication: For the treatment of vitreous hemorrhage to improve reduced visual acuity and to facilitate the physician's ability to diagnose the underlying retinal pathology

Clinical formulation: international units)/vial

Ingredients	Amount/4 ml
Hyaluronidase (ovine)	USP unit
Lactose monohydrate NF/EP	5 mg
Potassium phosphate, monobasic, NF/EP	1.22 mg
Potassium phosphate, dibasic, USP/EP	1.92 mg
Water for injection USP/EP	qs

Route of administration: Intravitreal injection

Proposed use: USP unit (55 iu)/0.05 ml, intravitreal injection, single dose

Executive Summary

I. Recommendations

A. Recommendation on Approvability

B. Recommendation for Nonclinical Studies

No recommendation is necessary.

C. Recommendations on Labeling

II. Summary of Nonclinical Findings

A. Brief Overview of Nonclinical Findings

In studies with the rabbit vitreous clot model, hyaluronidase was effective in clot clearance, optic nerve and retinal clarity, and red reflex in a dose-dependent manner. In toxicity studies, temporary inflammatory reactions were observed following intravitreal injection of hyaluronidase. It is likely that inflammatory response to Vitrase may facilitate clot clearance.

B. Pharmacologic Activity

The activity of Vitrase is to alter the structure of the vitreous matrix to increase the natural clearance mechanisms and promote more rapid clot removal. By breaking down the high molecular weight hyaluronan in the vitreous, Vitrase allows less restricted diffusion of chemotactic factors, a consequent increased influx of phagocytic cells, and greater access to the clot for these cells. The removal of high molecular weight hyaluronan can also increase the natural flow of vitreous fluid and may reduce the intrinsic stability of the clot. The response to the smaller fragments derived from the hyaluronan and the potential for a limited inflammation from the xenobiotic protein assist in recruiting an optimum number of phagocytic cells to bring about rapid clearance of the hemorrhage.

C. Nonclinical Safety Issues Relevant to Clinical Use

Temporary ocular inflammatory responses following intravitreal injection of Vitrase were observed in both rabbit and monkey studies, which were expected treatment reactions. No toxicologically significant side effects were noted in animal studies.

III. Administrative

A. Reviewer signature: _____

B. Supervisor signature: Concurrence - _____

Non-Concurrence - _____
(see memo attached)

C. cc: list:
NDA 21-414/Division File
NDA 21-414/Original NDA
HFD-550/CSO/Gorski
HFD-550/MO/Harris
HFD-550/TL Pharm/Yang
HFD-550/Pharm/ChenZh

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PHARMACOLOGY/TOXICOLOGY REVIEW

I. PHARMACOLOGY:

Studies reviewed:

VIT-0312011: Immunohistochemical characterization of cells in rabbit vitreous following treatment with Vitrase. Vol. 1-4, Page 001
TOX-11-08961X: Efficacy of current and "single use" formulations on the clearing of injected homologous blood in rabbits. Vol. 1-4, Page 038
TOX-06-08961X: Efficacy of various doses and different raw material lots on the clearing of injected hemorrhage in rabbits. Vol. 1-4, Page 116
TOX-10-08961X: Efficacy of three doses of hyaluronidase on vitreous blood clot in rabbits. Vol. 1-4, Page 170
TOX-13-08961X: Efficacy of a repeat injection of hyaluronidase on the clearing of injected homologous blood in rabbits. Vol. 1-4, Page 224
VIT 03-08-98: Efficacy of clearance of induced vitreous hemorrhage in comparing biozyme lots 130B, 224B, 222B, Fraction II (pure hyaluronidase), and no treatment in rabbits. Vol. 1-4, Page 239

PT-VIT-0925001: Efficacy of ISTA Pharmaceuticals Vitrase (from Biozyme Lot 224C) alone or in combination with [redacted] in clearing injected blood in rabbits. Vol. 1-4, Page 364
PT-VIT-1110001: Efficacy comparison of [redacted] 224A, 233A and 222A against biozyme Lot 224C in clearing injected blood in rabbits. Vol. 1-4, Page 428

Studies NOT reviewed:

[redacted]

Primary pharmacodynamics and mechanism of action:

VIT-0312011: Immunohistochemical characterization of cells in rabbit vitreous following treatment with Vitrase. Vol. 1-4, Page 001

The purpose of this study was to characterize a population of cells that appear to migrate into the posterior chamber of the rabbit eye after intravitreal injection of Vitrase. Eighteen Dutch belted rabbits were used in this study. Vitrase (Lot #: 224C) was intravitreally injected into 16 rabbits (50 µl, 75 iu, both eyes). Two treated animals were randomly selected on days 0, 1, 2, 4, 7, 10, 14 and 28 for indirect ophthalmoscopic and slit lamp biomicroscopic examinations. The animals were then sacrificed and the ocular tissues were collected for immunohistochemical examinations. Two control animals received either a sham injection into the vitreous or an intravitreal injection with 0.9% saline in both eyes. These animals were immediately sacrificed.

Eyes injected with Vitrase developed either vitreous haze, fluffy white precipitation (FWP) or cells in the vitreous or a combination of these effects. These effects were clinical indicators of cellular infiltrates, which were confirmed by microscopic examination. These cells

were observed in the body of the vitreous and immediately adjacent to the retina. Infiltrates detected by light microscopy were present on day 2, and appeared more numerous by day 4 through day 14. On day 28, the cellular infiltrates in the posterior chamber were diminished to the day 4 level.

Immunohistochemical analysis of the cell infiltrates identified several cell types commonly found during inflammation. Activated macrophages were identified in the vitreous on day 2 and all subsequent time points. T- and B-lymphocytes were identified in the vitreous on day 4 and all subsequent time points. Activated macrophages and T-lymphocytes were most numerous on days 7, 10 and 14. On day 28, cell infiltrates appeared to return to the levels observed on day 4. The presence of macrophages suggested that the initial inflammatory response following Vitrase injection might be cellular in nature. The presence of B-lymphocytes suggested the possibility that a humoral response might be elicited.

Drug activity related to proposed indication:

TOX-11-08961X: Efficacy of current and "single use" formulations on the clearing of injected homologous blood in rabbits. Vol. 1-4, Page 038

The purpose of this study was to compare the effects on clot clearance using the current drug formulation (CF, the same as the clinical formulation) and single use formulation (SUF, with 26.6-fold higher lactose concentration) in the rabbit model. Twenty female New Zealand Red rabbits were used in this study. The animals were intravitreally injected with autologous blood (50 µl) in both eyes. Following a 14-day incubation period to allow clot formation, the animals were examined by indirect ophthalmoscopy and subsequently, the animals were injected with 50 µl/75 iu of test article in 1 eye (10 eyes per formulation). The contralateral eye was treated with either control article (saline, 10 eyes), CF vehicle (5 eyes) or SUF vehicle (5 eyes). The animals were checked using indirect ophthalmoscopy bi-weekly for 10 weeks.

The results showed that the CF and SUF hyaluronidase products were equally significantly effective in clot clearance, optic nerve and retinal clarity, and red reflex compared to the saline and vehicle controls. The results suggested that the differences in the vehicle components (the lactose concentrations) have no effects in this model.

TOX-06-08961X: Efficacy of various doses and different raw material lots on the clearing of injected hemorrhage in rabbits. Vol. 1-4, Page 116

The purpose of this study was to compare the effects on clot clearance using hyaluronidase with various active ingredient manufacturing lots (224C, 200C and 222B) and doses in the rabbit model. Thirty female New Zealand Red rabbits were used in this study. The animals were intravitreally injected with autologous blood (50 µl) in both eyes. Following a 14-day incubation period to allow the clot formation, the animals were examined by indirect ophthalmoscopy to determine the status of the retinal obstruction by the clot. Subsequently, 25 animals (5/group) were injected with 50 µl of test article [7.5 (Lot 224C only), 55 (Lot 224C only) or 75 (all 3 lots) iu/eye] in both eyes. The remaining 5 animals were retained as untreated controls. The animals were examined using indirect ophthalmoscopy bi-weekly for 6 months.

The results showed that all manufacturing lots (224C, 200C and 222B) were equally effective in clot clearance. An improvement in optic nerve and retinal clarity, red reflex scores, and clot clearance (mean % clot surface area and clot density) was noted at all doses relative to the untreated controls. Significant effects on clot clearance were achieved at 75 iu. There was a trend for higher doses to have greater improvement in these parameters.

TOX-10-08961X: Efficacy of three doses of hyaluronidase on vitreous blood clot in rabbits. Vol. 1-4, Page 170

The purpose of this study was to compare the effects on clot clearance with intravitreal injection of 75, 55 and 7.5 iu/eye of hyaluronidase (Lot #: 224B) in the rabbit model. Twenty-four New Zealand Red rabbits were used in this study. The animals were intravitreally injected with autologous blood (50 µl) in both eyes. Following a 14-day incubation period to allow the clot formation, the animals were examined by indirect ophthalmoscopy. Subsequently, the animals (8/group) were injected with 50 µl of test article (7.5, 55 or 75 iu) in 1 eye. The contralateral eye was treated with control article (saline, 4 eyes/group) or received no treatment (4 eyes/group). The animals were examined using indirect ophthalmoscopy bi-weekly for 10 weeks.

The results showed a clear dose-response in the treatment effects. Hyaluronidase at 55 and 75 iu/eye was effective in the improvement of clot surface area, optic nerve and retinal clarity, and red reflex compared to the saline control and low dose groups. In almost all parameters, the results obtained from eyes receiving 7.5 iu hyaluronidase were not different than the saline or no treatment controls.

TOX-13-08961X: Efficacy of a repeat injection of hyaluronidase on the clearing of injected homologous blood in rabbits. Vol. 1-4, Page 224

The purpose of this study was to select eyes that could be classified as non-responders or poor responders to a single 75 iu Vitrase injection, and to determine the effects of a second 75 iu injection of Vitrase on the clearance of intravitreal blood in the rabbit efficacy model. Sixty New Zealand Red rabbits were used in this study. Fourteen days after intravitreal injection of autologous blood to each eye of the rabbits, hyaluronidase (75 iu, Lot 224B) was intravitreally injected into the rabbit eyes. The treatment effects were evaluated 28 days following hyaluronidase injection using indirect ophthalmoscopy. The eyes with clot surface area $\geq$ 90% were identified as non-responders (15 eyes). The eyes with clot surface area between 70% and 89% were poor responders (36 eyes). The eyes were then randomized into treatment group and non-treatment group. The eyes in the treatment group received the second intravitreal injection of hyaluronidase 75 iu. Ocular examinations for red reflex, optic clarity, retinal clarity, and clot surface area were conducted in all eyes bi-weekly for 6 weeks.

The sponsor indicated that examination of the clot surface area showed a marked treatment effect, particularly in the non-responder group. However, 2 of the eyes receiving the second treatment developed retinal detachments and hemorrhage.

PT-VIT-0925001: Efficacy of ISTA Pharmaceuticals Vitrase (from Biozyme Lot 224C) alone or in combination with [redacted] in clearing injected blood in rabbits. Vol. 1-4, Page 364

[redacted]

PT-VIT-1110001: Efficacy comparison of [redacted] 224A, 233A and 222A against biozyme Lot 224C in clearing injected blood in rabbits. Vol. 1-4, Page 428

The purpose of this study was to compare the efficacy of [redacted] from 3 biozyme hyaluronidase lots (Lots 224A, 233A and 222A). Biozyme Lot 224C, the finished drug product, was used as positive control in this study. Forty New Zealand Red rabbits (10/group) were used in this study. The animals were intravitreally injected with autologous blood (50 µl) in both eyes. Following a 14-day incubation period to allow the clot formation, the animals were examined by indirect ophthalmoscopy. Subsequently, the animals were injected with 50 µl of test article (75 iu) in 1 eye. The contralateral eye was treated with control article (saline). The animals were examined using indirect ophthalmoscopy bi-weekly for 10 weeks.

All test articles were equally effective on reducing clot surface area throughout the study. Other parameters (clot density, red reflex, optic nerve clarity and retinal clarity) were also significantly improved compared to the contralateral controls. The test articles appeared to work in a similar manner. On the other hand, an unacceptable level of toxicity was associated with the [redacted] intermediates. Retinal detachment and/or retinal hypopigmentation were noted in all 3 [redacted] groups but not in the Lot 224C group or eyes treated with saline. Histopathological examinations on the eyes with retinal detachment showed loss of cell layer organization and thinning of the retina. The relationship between the adverse events and clot regression was unclear.

Pharmacology summary and conclusions:

Following intravitreal injection of Vitrase in rabbits, macrophages, T-lymphocytes and B-lymphocytes, which are commonly related to inflammatory response, were identified as infiltrating cells. It is likely that inflammatory response to Vitrase may facilitate clot clearance by recruiting phagocytic immune cells.

In studies with the rabbit vitreous clot model, hyaluronidase was effective in clot clearance, optic nerve, and retinal clarity, and red reflex in a dose-dependent manner. The differences in the lot numbers and vehicle components (the lactose concentrations) showed no effects in this rabbit model. For eyes that were classified as poor-responders or non-responders, a second intravitreal injection of hyaluronidase showed a marked treatment effect. However, retinal detachments and hemorrhage were found. [redacted] a major component of Vitrase, had no effects on intravitreal clot clearance even at very high doses, up to [redacted]

In some treated eyes, especially in the eyes treated with intermediates, retinal detachment and cataract were noted. The relationship between the adverse events and clot regression was not clear.

II. SAFETY PHARMACOLOGY:

None submitted.

III. PHARMACOKINETICS/TOXICOKINETICS:

Studies reviewed:

— 45643: Pharmacokinetics and tissue distribution of ^{125}I-ACS Hyaluronidase in male Dutch belted rabbits after a single intravitreal injection. Vol. 1-5, Page 001
PT-VIT-0606012: Probe pharmacokinetic study of intravitreal hyaluronidase. Vol. 1-5, Page 123

Pharmacokinetics and tissue distribution of ^{125}I-ACS Hyaluronidase in male Dutch belted rabbits after a single intravitreal injection

Key study findings:

Following a single intravitreal administration of ^{125}I-hyaluronidase into rabbits, high levels of radioactivity were found in vitreous humor, sclera and retina. The systemic exposure to the radioactivity was very low. Quantifiable levels of radioactivity were observed up to 240 hr post dose in plasma and blood.

Study No: — 45643
Vol/Page: Vol.1-5, Page 001
Conducting laboratory and location: [redacted]

Date of study initiation: March 21, 2000
GLP compliance: Yes
QA report: Yes (X) No ( )
Species/strain: Male Dutch belted rabbits
N: 3/group.
Age/weight: 4-4.5 months old, 1.5-2.1 kg
Route: Intravitreal injection
Dosage: 50 µl/11 µg (136 U or 110 iu)/8.20-8.29 µCi, single dose, both eyes
Drug: ^{125}I-Hyaluronidase (0.5 mCi/10.53 µg/ml, Lot#: N441239)
Hyaluronidase (Lot#: 222C)
Methods: liquid scintillation spectroscopy
LOQ: ≤ double background values

Study design:

Group	Test article	N	Euthanasia/necropsy time after dosing (hr)
1	Untreated	1	Background control
2	^{125}I-Hyaluronidase	3	Immediately
	^{125}I-Hyaluronidase	3	12
	^{125}I-Hyaluronidase	3	24
	^{125}I-Hyaluronidase	3	48
	^{125}I-Hyaluronidase	3	72
	^{125}I-Hyaluronidase	3	96
	^{125}I-Hyaluronidase	3	144
	^{125}I-Hyaluronidase	3	240
	^{125}I-Hyaluronidase	3	336

The purpose of this study was to determine the concentrations and distribution of radioactivity levels in ocular tissues, blood and plasma, and selected systemic tissues (brain, heart, liver, lungs, kidneys) following a single intravitreal injection of hyaluronidase in male pigmented rabbits. The animals (3 animals/time point) were euthanized at 0 (immediately following dosing), 12, 24, 48, 72, 96, 144, 240 and 336 hr post dose. Aqueous humor, vitreous, lens, retina, iris, conjunctiva, sclera, optic nerve head and cornea samples were collected. Blood samples and brain, heart, lungs, kidneys and liver samples were also collected from each animal. Radioactivity was measured by the liquid scintillation spectroscopy.

Results:

The study results are summarized in the table below. The highest levels of radioactivity were found in vitreous humor, sclera and retina. The systemic exposure to the radioactivity was very low. The long half-life of the drug in the ocular tissues suggested a lack of specific mechanism for the clearance of the relatively large hyaluronidase molecule. Quantifiable levels of radioactivity were observed up to 240 hr post dose in plasma and blood. The long elimination half-life in plasma is considered to be related to slow clearance from the site of administration.

PK parameters in rabbits treated with hyaluronidase

Tissue	Tmax (hr)	Cmax (µg-eq/g)	T1/2 (hr)	AUC_{0-t} (µg-eq hr/g)	$AUC_{0-\infty}$ (µg-eq hr/g)
Blood	24			0 866	
Plasma	24		49 1	1 35	1 38
Left aqueous humor	48		67 4	58.4	59.5
Right aqueous humor	48		42.6	72.9	73 3
Left cornea	48		73.8	98 0	104
Right cornea	48		52.1	102	104
Left conjunctiva	0		119	21 8	23.5
Right conjunctiva	12			22.9	
Left iris	48		191	81 3	107
Right iris	12		79.1	86.7	93.2
Left lens	24			28.0	
Right lens	12			29.3	
Left optic nerve head	12		188	31.4	41.4
Right optic nerve head	12			45.7	
Left retina	12		101	202	216
Right retina	12		75.2	236	245
Left sclera	0		112	140	151
Right sclera	0		69.9	153	159
Left vitreous humor	0		85.2	521	542
Right vitreous humor	0		59.3	604	617
Brain	12			0.002	
Heart	24			0.290	
Kidneys	24		152	1.35	1.72
Liver	24			0.416	
Lungs	12			0 322	

PT-VIT-0606012: Probe pharmacokinetic study of intravitreal hyaluronidase

Key study findings: After intravitreal injection, Vitrase moved out of the vitreous (with a t1/2 of 3 days) into the aqueous humor where it decayed into a lower molecular form and was cleared in about 2 weeks. Proteins in the vitreous also moved into the aqueous humor. Intravitreal injection of the Vitrase also caused the appearance of the _____ gelatinases.

Study No: PT-VIT-0606012
Vol/Page: Vol.1-5, Page 123

Conducting laboratory and location [redacted]

Date of study initiation: May 19, 1997
GLP compliance: No
QA report: Yes ( ) No (X)
Species/strain: Dutch belted rabbits
N: 2/sex/group/time-point
Age/weight: 8-10 weeks old, 2 kg
Route: Intravitreal injection
Dosage: 100 iu/40 µl, single dose
Drug: Biotinylated Vitrase (Lot#:130B), left eye
Vehicle: BSS, right eye
Methods: SDS-PAGE, Western blot analysis

Study design:

Group	Treatment	Group	Treatment	# of eyes/group	Termination time (days after dosing)
1	BSS	2	Biotin-hyaluronidase	4	0
3	BSS	4	Biotin-hyaluronidase	4	1
5	BSS	6	Biotin-hyaluronidase	4	2
7	BSS	8	Biotin-hyaluronidase	4	3
9	BSS	10	Biotin-hyaluronidase	4	4
11	BSS	12	Biotin-hyaluronidase	4	6
13	BSS	14	Biotin-hyaluronidase	4	7
15	BSS	16	Biotin-hyaluronidase	4	10
17	BSS	18	Biotin-hyaluronidase	4	14
total				72	

The purpose of this study was to determine the distribution and decay of Vitrase in ocular tissues following a single dose injection into the vitreous of rabbits. The animals were euthanized at 0 (immediately following dosing), 1, 2, 3, 4, 6, 7, 10 and 14 days post dose (4 animals/time point). Vitreous, aqueous humor, retina, iris, and retinal pigment epithelium (RPE) samples were collected. [redacted] Western blot analysis and [redacted] were used in enzyme evaluation.

Results:

Soluble vitreous: The total amount of biotinylated Vitrase showed a steady decay in soluble vitreous fraction with a t1/2 value of 2.85 days (68.4 hr). The total protein content did not appear to be directly affected by Vitrase injection. Gelatin [redacted] revealed that Vitrase treatment led to the appearance [redacted] gelatinase activity one day after injection and it remained for a week.

Insoluble vitreous (refers to the material that was removed by centrifugation from the vitreous and then made soluble by [redacted] extraction): No hyaluronidase activity was recovered in this fraction. However, some biotinylated proteins (from Vitrase preparation) were recovered. The protein profiles of the recovered material demonstrated that Vitrase led to a fast (at time 0) and long lasting (throughout the 2 weeks of the study) reduction in the amount of protein accompanied with a dramatic increase in the gelatinase activity in the 1st week.

Retina, RPE and iris: No significant observations were noted in these fractions for total protein, gelatinase activity, or protein profiles. Trace amount of hyaluronidase activity could be detected for 2-3 days post injection.

Aqueous humor: Hyaluronidase activity appeared very quickly in aqueous humor (at time 0) and decayed at a much slower rate than in vitreous humor (could be detected through 2 weeks post injection). The enzyme activity was associated with an immediate increase in the total protein without an alteration in the overall protein profile. For the components of the Vitrase preparation, high molecular weight forms decayed fast and lower molecular weight forms accumulated, indicating the degradation of the high molecular weight forms into more stable, lower molecular weight forms. An increase in gelatinase activities was also noted where additional activities of [redacted] appeared 1 day after injection and remained for more than 1 week.

In summary, after intravitreal injection, Vitrase moved out of the vitreous (with a t1/2 of 3 days) into the aqueous humor where it decayed into a lower molecular weight form and was cleared in about 2 weeks. Proteins in the vitreous also moved into the aqueous humor and the amounts of proteins associated with the insoluble collagen fibrils in the vitreous decreased. Intravitreal injection of the Vitrase also caused the appearance of the [redacted] gelatinases.

PK/TK summary and conclusions:

Following a single intravitreal administration of ^{125}I-hyaluronidase in rabbits, high levels of radioactivity were found in vitreous humor, sclera and retina. The systemic exposure to the radioactivity was very low (plasma Cmax = [redacted]). Quantifiable levels of radioactivity were observed up to 240 hr post dose in plasma and blood.

In the second PK study, after intravitreal injection, Vitrase moved out of the vitreous (with a t1/2 of 3 days) into the aqueous humor where it decayed into lower molecular forms and was cleared in about 2 weeks. Proteins in the vitreous also moved into the aqueous humor.

IV. GENERAL TOXICOLOGY:

None submitted.

V. GENETIC TOXICOLOGY:

None submitted.

VI. CARCINOGENICITY:

None submitted.

VII. REPRODUCTIVE AND DEVELOPMENTAL TOXICOLOGY:

None submitted.

VIII. SPECIAL TOXICOLOGY STUDIES:

OCULAR TOXICITY STUDIES

Studies reviewed:

P0798018: Comparison of intravitreal injections of biozyme hyaluronidase Lot 130B vs Lot 224C in rabbits. Vol. 6, Page 001

P0299028: Comparison of intravitreal injections of current versus single use formulations with negative controls in rabbits. Vol. 6, Page 109

TOX-07-08961X: Probe study: Dose escalation of Vitrase in rabbits. Vol. 6, Page 194

56380: A pilot intravitreal injection toxicity study of hyaluronidase for intravitreal ophthalmic injection in the cynomolgus monkey. Vol. 9, Page 001

56359: An intravitreal injection toxicity study of hyaluronidase for intravitreal ophthalmic injection (with a 90-day observation period) in the cynomolgus monkey. Vol. 9, Page 080

TOX-08-08961X: Safety of multiple injections of Vitrase in rabbits. Vol. 9, Page 407

VIT 07-22-96: Probe study: determining the toxicology of "old" vs "new" ACS hyaluronidase. Vol. 9, Page 443

152-002: Comparative ocular irritation and toxicity study of Vitrase and heat-stressed Vitrase given by intravitreal injection to Dutch Belted rabbits. Supplement 1, Page 027

152-003: Comparative ocular irritation and toxicity study of Vitrase given by intravitreal injection to Dutch belted rabbits. Supplement 2, Page 001

95C-01869-00: probe study: safety of intravitreal hyaluronidase injections. Vol. 7, Page 003

VIT 02-17-97: Probe study: Determining the toxicology of various commercial Hyaluronidase preparations in the Vitrase model. Vol 7, Page 018

VIT 04-17-97: Probe study: Comparison of biozyme hyaluronidase Lot 130B vs Lot 200C in vitreous model. Vol. 7, Page 063

VIT 06-12-97: Probe study: Comparison of current and proposed low dose preparations for Vitrase including a low phosphate arm. Vol. 7, Page 081

VIT 09-02-97: Probe study: Comparison of current and single use formulation for Vitrase manufactured lots. Vol. 7, Page 095

VIT 05-07-98II: Probe study: Comparison of intravitreal injections of biozyme hyaluronidase Lot 130B vs Lot 224B with negative controls in rabbits. Vol. 7, Page 179

PT-VIT-0606011: Investigation into histopathological abnormalities in Vitrase studies. Vol. 7, Page 202

VIT 03-11-98: Comparison of biozyme hyaluronidase Lot 130B vs. Lot 224B in vitreous model. Vol. 7, Page 110

Studies NOT reviewed:

The following studies were designed for another indication

[redacted]

Comparison of intravitreal injections of biozyme hyaluronidase Lot 130B vs Lot 224C in rabbits. Vol. 6, Page 1

Key study findings: Intravitreal injection of hyaluronidase (single dose, 75 iu/50 µl) produced a transient inflammatory response characterized by an increase in the mean scores for aqueous cells and flare in hyaluronidase-treated eyes compared to normal saline and vehicle treatment. There were no significant differences between the eyes treated with Lot 224C and Lot 130B.

Study No: P0798018
Vol/Page: Volume 6, Page 001
Conducting laboratory and location: [redacted]

Date of study initiation: July 31, 1998
GLP compliance: Yes
Animal: 17 male New Zealand Red Cross rabbits, 10-13 weeks old, 1.58-2.49 kg
Route: Intravitreal injection
Dosage: 75 iu/50 µl, single dose
Drug: Hyaluronidase (Lot#: PHI-07-10, Biozyme Lot#: 224C)
Control: Hyaluronidase (Lot#: PHH-03-05, Biozyme Lot#: 130B)
Vehicle control [7.3 mg potassium phosphate (monobasic), 11.4 mg potassium phosphate (dibasic) and 30.1 mg lactose in 24 ml 0.9% sodium chloride injection, USP]. The formulation was similar to the clinical formulation.
0.9% Sodium chloride injection, USP

Study design:

Group	N	Treatment (one per eye)	Injection volume (µl)	Treatment day	Necropsy day
1	7	A and B	50	Day 1	Day 31
2	7	C and D	50	Day 1	Day 31
3	3	E and E	0	Untreated	Day 31

A = ACS hyaluronidase (Biozyme Lot# 224C)
B = ACS hyaluronidase (Biozyme Lot# 130B)
C = Vehicle control
D = 0.9% NaCl injection, USP
E = No treatment

The purpose of this study was to compare the relative safety of ocular injections of hyaluronidase made from Biozyme raw material Lot 224C with that of a previous lot (Biozyme Lot # 130B). The day of dosing was designated as Day 1. Toxicity was assessed as shown below.

Toxicity assessment

Parameter	Procedure
Mortality	Twice daily
Clinical observations	Daily
Body weights	Days 1 and 31
Slit lamp biomicroscopy	Groups 1 and 2 animals: Pretreatment and on Days 2, 4, 8, 11, 15, 22 and 31 Group 3 animals: Pretest and on Days 15 and 31
Indirect ophthalmoscopy	Groups 1 and 2 animals: Pretreatment and on Days 2, 4, 8, 11, 15, 22 and 31 Group 3 animals: Pretest and on Days 15 and 31
Fluorescein angiography	Groups 1 and 2 animals: Pretreatment and on Days 15 and 31 Group 3 animals: Pretest and on Day 31
Gross pathology	Day 31, eyes
Histopathology	Eyes

Mortality: One Group 1 animal (Biozyme Lot#s 224C and 130B) died on Day 4. The cause of the death was considered as necrotizing pneumonia. On Day 3, the animal was observed as small and not eating. None of the other animals died prior to scheduled euthanasia.

Clinical observations: No drug-related, toxicologically significant findings were noted.

Body weights: No drug-related, toxicological significant effects were noted.

Slit lamp examinations: Positive findings are summarized in the table below. The results showed that there was a transient inflammatory response to the injection of hyaluronidase. The inflammatory response was characterized by an increase in the mean scores for aqueous cells and flare for hyaluronidase-treated eyes compared to normal saline and vehicle treatment. In addition, treatment with hyaluronidase resulted in brief, significant increase in mean scores for vitreous cells.

Positive findings for slit lamp examinations

Mean score		Treatment			
	Day	Lot 224C	Lot 130B	Vehicle control	NaCl
Aqueous cell and flare	2	0.83	2.83	0.71	0
	4	2.00	2.00	0	0
	8	0	0	1.43	0
	31	0	0	0	0
Vitreous cells	4	3.00	3.67	0.71	0.71
	8	4.33	3.83	0.71	0.71
	22	1	0.667	0.71	0.71
	31	1.67	1.17	0.71	0.71

Criteria for the grading of aqueous cell and flare and vitreous cells: 0=No cells observed, trace=1-5 cells per slit beam field, 1=5-10 cells per slit beam field, 2=10-20 cells per slit beam field, 3=20-50 cells per slit beam field, 4>50 cells per slit beam field.
The same score system was used in other toxicity studies submitted in this NDA.

Indirect ophthalmoscopy examinations: Positive findings are summarized in the table below. Hyaluronidase treatment was associated with a greater amount of fluffy white precipitates (FWP) and granular white precipitates (GWP) than saline and vehicle treatment. The mean scores in the untreated control animals were 0.

Positive findings for indirect ophthalmoscopic examinations

Mean score		Treatment			
	Day	Lot 224C	Lot 130B	Vehicle control	NaCl
Fluffy white precipitates	4	2.00	2.33	0	0
	22	0.67	0.33	0.29	0.14
	31	0	0	0	0
Granular white precipitates	31	0.33	0.33	0	0
Haze	2	1.67	2.00	1.43	1.14
	4	2.67	2.67	1.14	1.43
	15	1.33	1.33	0.43	0.57
	22	0.33	0.50	0.29	0.29
	31	0	0	0.14	0.14

Grading for FWP and GWP: 0=No precipitation, Trace=Rare precipitation occupying <5% of the vitreous field, 1=Defined precipitation occupying 5-15% of the vitreous field, 2= Defined precipitation occupying 15-40% of the vitreous field, 3= Defined precipitation occupying 40-75% of the vitreous field, 4= Defined precipitation occupying 75-100% of the vitreous field (usually view of the vitreous may be completely obscured with this level of precipitation).

Grading for haze: 0=Crystal clear, Trace=Minimal obscuration of retinal details, 1=Definite obscuration of retinal details (slight granularity), 2= Obscuration of vitreous (a fluorescein angiogram is not clear), 3= Obscuration of vitreous (only prominent landmarks such as optic disc are clearly discernible), 4= Haze sufficient to preclude visualization of the retina.

The same score system was used in other toxicity studies submitted in this NDA.

Fluorescein angiography examinations: No treatment-related abnormalities were noted.

Gross necropsy: The eyes of all surviving animals appeared normal at necropsy.

Histopathology: Positive findings are summarized in the table below. No toxicologically significant, treatment-related changes were observed. The lymphoplasmacytic change was characterized by an occasional cell or a few cells adjoining vessels on the surface of the optic nerve and was seen in all groups. The significance of this change was unknown.

Histopathological findings in rabbits treated with hyaluronidase

Group	Vehicle	Saline	No treatment	Lot 130B	Lot 224C
Number of eyes	7	7	6	6	6
Cornea					
Erosion, focal, minimal	1	0	1	0	0
Limbus					
Mononuclear cell infiltration	0	0	1	2	1
Optic nerve head					
Lymphoplasmacytic cells, minimal, 1st examination	1	1	1	2	5
Lymphoplasmacytic cells, minimal, 2nd examination	0	2	2	5	6

In summary, New Zealand Red Cross rabbits were treated intravitreally with a single dose (75 iu/50 µl) of hyaluronidase with different lot numbers and were observed for 30 days. One animal died on Day 4 due to acute necrotizing pneumonia. No drug-related changes in clinical observations and body weights were noted. Ophthalmologic examination showed a transient inflammatory response to the injection of hyaluronidase characterized by an increase in the mean scores for aqueous cells and flare in hyaluronidase-treated eyes compared to normal saline and vehicle treatment. There were no significant differences between the eyes treated with Lot 224C and Lot 130B. Post-mortem examination did not show drug-related, toxicologically significant changes.

Comparison of intravitreal injections of current versus single use formulations with negative controls in rabbits. Vol. 6, Page 109

Key study findings: An ocular inflammatory response was noted following intravitreal injection of hyaluronidase. No differences in the toxicological profiles of hyaluronidase between F1 formulation or F2 formulation.

Study No: P0299028
Conducting laboratory and location [redacted]

Date of study initiation: March 2, 1999
GLP compliance: Yes
Animal: Female New Zealand Red rabbits, 12-13 weeks old, 2.1-2.6 kg
Route: Intravitreal injection (both eyes), single dose
Dosage: 75 iu/50 µl
Drug: Hyaluronidase [Lot#: PHH-09-05/Biozyme Lot#: 200C (Formulation 2, F2, single use formulation)]
Control: Hyaluronidase [Lot#: PHH-07-05/Biozyme Lot#: 200C (Formulation 1, F1, current formulation, similar to the clinical formulation)]
Vehicle control (for both Formulations 1 and 2)
0.9% Sodium chloride injection, USP
Untreated control

Study design:

Group	N	Treatment (OS)	Treatment (OD)	Injection volume	Treatment day	Necropsy day
1	2	No treatment	No treatment	N/A	Day 1	Day 33
2	5	0.9% NaCl	Formulation 1	50 µl	Day 1	Day 33
3	5	Vehicle for F1	Formulation 1	50 µl	Day 1	Day 33
4	5	0.9% NaCl	Formulation 2	50 µl	Day 1	Day 33
5	5	Vehicle for F2	Formulation 2	50 µl	Day 1	Day 33

The purpose of this study was to compare the relative toxicological profiles of 2 different drug formulations, in which lactose concentrations are different. In F2, lactose concentration was 20-fold of that in F1. The day of dosing was designated as day 1. Toxicity was assessed as shown below.

Toxicity assessment

Parameter	Procedure
Mortality	Once daily
Clinical observations	Once daily
Slit lamp biomicroscopy	Groups 2-5 animals: Pretreatment and on Days 2, 5, 8, 15, 22 and 33 Group 1 animals: Pretest and on Day 33
Indirect ophthalmoscopy	Groups 2-5 animals: Pretreatment and on Days 2, 5, 8, 15, 22 and 33 Group 1 animals: Pretest and on Day 33
Fluorescein angiography	Groups 2-5 animals: Days 1, 15 and 33
Histopathology	Eyes

Mortality: One Group 4 animal (OS: 0.9% NaCl, OD: Formulation 2) died on Day 19. Watery diarrhea with blood was observed in its cage. No necropsy was performed on this animal. The cause of the death cannot be conclusively attributed to treatment with hyaluronidase in Formulation 2. None of the other animals died prior to scheduled euthanasia.

Clinical observations: Four animals were observed to have soft stool on one or two days during the observation period: one in Group 2 (0.9% NaCl, hyaluronidase in F1), one in Group 3 (vehicle F1, hyaluronidase in F1) and two in Group 4 (0.9% NaCl, hyaluronidase in F2). One Group 3 (vehicle F1, hyaluronidase in F1) animal showed a decreased appetite for 2 days.

Slit lamp and indirect ophthalmoscopy examinations: Positive findings are summarized in the table below. The results showed that there was an inflammatory response to the injection of hyaluronidase. The inflammatory response was characterized by a significant increase in the mean scores for haze and vitreous cells for hyaluronidase-treated eyes compared to normal saline and vehicle treatments. In addition, treatment with hyaluronidase resulted in an increase in mean scores for cells and flare in anterior chamber. Hyaluronidase treatment was also associated with a greater amount of fluffy white precipitates and granular white precipitates than saline and vehicle treatments. The results suggested that hyaluronidase in both formulations might be responsible for the inflammatory response. No toxicologically significant differences were noted between F1-treated eyes and F2-treated eyes.

Positive findings for slit lamp examinations (mean score)

		Treatment					
	Day	No treatment	F1	PSS	F1 vehicle	F2	F2 vehicle
Aqueous cell and flare	2	NA	0 5	0 4	0 2	0.7	0
	5	NA	0.2	0	0	0 2	0
	22	NA	0	0	0	0	0
Vitreous cells	5	NA	2 0*	0	0	1.8*	0
	8	NA	4.4*	0	0	4.3*	0
	33	0	2.4*	0	0	2.0*	0
Haze	2	NA	1.4	0.6	0.2	1.2	0.2
	5	NA	1.4*	0.1	0.2	1.7*	0.2
	22	NA	0.4	0	0	0.2	0
	33	0	0 1	0	0	0	0
Fluffy white precipitates	2	NA	0.2	0	0	0	0
	5	NA	0.4	0	0	0.5	0
	33	0	0	0	0	0	0
Granular white precipitates	15	NA	0	0	0	0.1	0
	22	NA	0.1	0	0	0.1	0
	33	0	0	0	0	0.1	0

* Scores significantly higher than those in control groups ($P < 0.05$)

Fluorescein angiography examinations: No treatment-related abnormalities were noted in PSS and F2 groups. Trace leakage of fluorescein was noted in F2 vehicle (1/5), F1 vehicle (1/5) and F1 (2/10) groups. The leakage was possibly due to the vehicle, not the active substance.

Post-mortem examinations: Positive histological findings are summarized in the table below. No indication of inflammation was noted. The photoreceptor nuclear displacement was characterized by the finding of photoreceptor nuclei external to external limiting membrane. The sponsor considered this change was related to less than optimal fixation, a processing artifact, not a treatment-related effect.

Histopathological findings in rabbits treated with hyaluronidase

Group	No treatment	F1	PSS	F1 vehicle	F2	F2 vehicle
Number of eyes	4	10	10	5	10	5
Optic disc cupping, focal, minimal to mild					1	
Photoreceptor nuclear displacement, multifocal, minimal	1	1			1	1

In summary, New Zealand Red rabbits were treated intravitreously with a single dose of hyaluronidase (75 iu/50 µl, in 2 different formulations: F1 and F2) or PSS and vehicles, and were observed for 33 days. One animal (OS: 0.9% NaCl, OD: Formulation 2) died on Day 19. The cause of the death was unknown. Clinical observations showed short-term soft stool and/or inappetence in a few animals in 1-2 days. Ophthalmologic examinations showed an inflammatory response to the injection of hyaluronidase in both formulations. The inflammation was characterized by an increase in the mean ophthalmic scores for haze and vitreous cells for hyaluronidase-treated eyes compared to normal saline and vehicle treatments. Other evidence of inflammation (cells and flare in anterior chamber) was also noted. Angiogram assay showed trace leakage in the eyes treated with F1 vehicle, F2 vehicle and hyaluronidase in F1 formulation on the average of 1 out of 5 eyes. The leakage was not considered due to the treatment with hyaluronidase. Histopathologic examinations showed no treatment-related effects. In conclusion, there were no differences in the toxicological profiles of hyaluronidase in either F1 formulation or F2 formulation. However, a few changes (e.g., higher mean scores for haze and vitreous cells) were seen between hyaluronidase (in both formulations) and vehicle-treated eyes.

Probe study: Dose escalation of Vitrase in rabbits. Vol. 6, Page 194

Key study findings: Significant abnormalities in ophthalmoscopy, angiography and histopathology were observed in the eyes treated at 750 iu of Vitrase. Less serious effects were noted in the eyes treated at 75 or 150 iu.

Study No: TOX-07-08961X
Conducting laboratory and location: [redacted]

Date of study initiation: October 15, 1998
Date of report: July 23, 1999
GLP compliance: No
Animal: Female New Zealand Red rabbits, 1.8-2.2 lb., 6/group
Route: Intravitreal injection, single dose
Dosage: 75, 150, or 750 iu/50 µl
Drug: Hyaluronidase (Lot#: 224B/PHI-05-06) in saline, right eye
Control: 0.9% Sodium chloride injection, USP, left eye

The purpose of this study was to determine the toxicological effects of three doses of Vitrase from Lot 224C in a rabbit model. Toxicity was assessed as shown below.

Toxicity assessment

Parameter	Procedure
Slit lamp biomicroscopy	Pretreatment and 1, 4, 7, 11, 14, 28, 36, 48, 63, 83, and 104 days after injection
Indirect ophthalmoscopy	Pretreatment and 1, 4, 7, 11, 14, 28, 36, 48, 63, 83, and 104 days after injection
Fluorescein angiography	Pretreatment and 1, 4, 7, 11, 14, 28, 36, 48, 63, 83, and 104 days after injection
Histopathology	2 animals/group were terminated on Days 28, 55 and 104. Ocular tissues were examined histopathologically.

Results:

Slit lamp and indirect ophthalmoscopy examinations: Early (1st day) aqueous cell and flare were the most common signs in the treated groups. Vitreous haze, fluffy white precipitates and persistent vitreous cells were also observed in the treated groups in a dose-dependent manner. In HD group, iritis, papillitis/optic nerve edema were observed.

Positive findings for ophthalmoscopic examinations (mean score)

		Treatment					
	Day	75 iu	Saline	150 iu	Saline	750 iu	saline
Aqueous cell and flare	1	2 67	0	4.17	0	4	0
	4	1.17	0	1.5	0	2	0
	7	0	0	0	0	0.17	0
Vitreous cells	4	4 67	0	4.33	0.33	5	0 17
	28	2.33	0	2.33	0	4.5	0
	48	0 25	0	1 5	0	2 75	0 5
	63	0	0	0	0	2	0
Haze	1	1 33	1	1.83	0.5	3	0 5
	4	1.83	0 17	2.83	0	3.83	0 17
	14	0.67	0	1.17	0	3	0
	63	0	0	0.5	0	1	0
Fluffy white precipitates	4	0 83	0	3	0	3 83	0
	7	0 5	0	2.17	0	3.33	0
	48	0	0	0	0	1.5	0

Fluorescein angiography examinations: On Day 14, trace leakage of fluorescein was noted in 1 LD (1/6) and 1 HD (1/6) eyes. Mild leakage was noted in 4 HD (4/6) eyes. One HD eye also showed possible microaneurysm formation. All eyes were normalized by termination (Days 28, 55 and 104).

Histopathologic examinations: Positive histological findings are summarized in the table below. Papillitis and possibly secondary hyalitis were noted in the treated eyes. Retinal degeneration and retinitis were also noted in the treated eyes. These changes were not observed in the untreated left eyes. Other findings, including minimal to mild, focal optic disc cupping and minimal to moderate, focal subjunctival lymphoid proliferation, were found in both control and treated eyes, and were considered physiologic.

Histopathological findings in rabbits treated with hyaluronidase

Group	75 iu	150 iu	750 iu
Number of eyes (treated eyes)	6	6	6
Retinal degeneration, focal	1 mild		1 mild, 1 mild to moderate
Retinitis		1 mild, 1 minimal	1 minimal
Papillitis, diffuse			3 minimal, 1 mild
Hyalitis, multifocal			1 minimal
Iridocyclitis, multifocal			1 mild

In summary, New Zealand Red rabbits were treated intravitreously with a single dose of hyaluronidase (75, 150 and 750 iu/50 µl, right eyes) or PSS (left eyes), and were observed for up to 104 days. Ophthalmologic examinations showed inflammatory responses to the injection of hyaluronidase in a dose-dependent manner. The inflammation was characterized by an increase in the mean ophthalmic scores for haze and vitreous cells, cells and flare in anterior chamber. Iritis and papillitis/optic nerve edema were also noted in HD eyes. Angiogram assay showed mild, reversible leakage in 4/6 eyes treated at 750 iu. Histopathological examination showed retinal degeneration, retinitis, papillitis, hyalitis, and iridocyclitis, mainly in the HD group animals.

An pilot intravitreal injection toxicity study of hyaluronidase for intravitreal ophthalmic injection in the cynomolgus monkey. Vol. 9, Page 001

Key study findings: The administration of a single 75 iu intravitreal injection, or two 75 iu intravitreal injections of hyaluronidase resulted in an acute ocular inflammatory reaction. The inflammation was reversible.

Project No: 56380
Conducting laboratory and location: [redacted]

Date of study initiation: September 22, 1999
GLP compliance: No
Animal: Two male cynomolgus monkeys (*Macaca fascicularis*) 2-3 years old, 3.6-4.7 kg
Route: Intravitreal injection
Dosage: 75 iu/50 µl, right eye only
Drug: Hyaluronidase for Intravitreal Ophthalmic Injection, USP (Lot#: PHI-05-006)
Study design:

Group	Test article	N/sex/group	Dosage (iu/µl)	Dosing regimen
1	Hyaluronidase	1 male	75/50	Single dose (day 1)
2	Hyaluronidase	1 male	75/50	2 doses at a 28-day interval (days 1 and 29)

The purpose of this study was to determine the toxicity potential of hyaluronidase following up to two intravitreal injections in the monkey. The day of the first dosing was designated as day 1. Toxicity was assessed as shown below. The Group 1 animal received a single injection followed by a 28-day recovery period. The Group 2 animal received 2 injections followed by a 56-day recovery period.

Toxicity assessment

Parameter	Procedure
Mortality	Twice daily
Clinical observations	Twice daily
Body weights	Weekly
Ophthalmology/ Tonometry/ERG	Pre-test, days 3, 8, 14, 21 and 28 (both groups) and on days 31, 35, 42, 49, 56, 70 and 84 (Group 2 only)
Gross pathology	Complete necropsy was performed on all animals at the end of the study.
Histopathology	Both eyes

Results:

Mortality: No mortality occurred during the study period.

Clinical observations: Constriction of the pupil in the treated eye was observed in both animals. For Group 1 male, constriction of the pupil remained evident until scheduled sacrifice on day 29. For Group 2 male, the constriction of the pupil lasted 8 and 4 days after the 1st and 2nd injections, respectively. The pupil constriction was correlated with the moderate uveitis, and was possibly indicative of residual inflammation following the injection.

Body weights: No drug-related effects were noted.

Slit lamp biomicroscopy and funduscopy examinations: The untreated eye was normal. A moderate (Group 2) to severe (Group 1) anterior uveitis was noted in the treated eyes (right eyes) of both animals. The inflammation was evidenced by aqueous flare and cells, presence of fibrin, mild swelling of the iris. Blurring of the fundus precluded retinal evaluation at these times. Subsequent examinations on days 8 and 35 showed vascular congestion and swelling of the optic nerve head. The degree of inflammation after the 2nd injection was comparable to that after the 1st injection. However, transient vitreous opacity and slight tortuosity of the retinal venules were observed. The inflammation was resolved 21-28 days after the 1st injection and 41 days after the 2nd injection with only a few vitreous cells left.

ERG: There were no apparent differences between a-wave and b-wave amplitude or latency in the treated and untreated eyes for the Group 2 male. For the Group 1 male, the b-wave response from the treated eye was diminished in the scotopic filter sequence and 29 Hz flicker relative to the left eye on days 3 and 8 by 80% and 75%, respectively. No oscillatory potentials were visible following the 0 dB (no filter) scotopic single flash ERG at these 2 timepoints. By day 14, the oscillatory potentials were visible. The b-wave amplitude was comparable to the left eye for the 0 dB scotopic single flash ERG and appeared recovering toward untreated eye levels at lower light intensities and the flicker. By day 21, the filter sequence ERG was similar to the untreated eye, but the 29 Hz flicker results still remained lower.

Tonometry: Reductions in IOP occurred in all treated eyes (see table below). On days 3 and 31 (after the 1st and 2nd injections, respectively), the IOP was immeasurably low. For the Group 1 male, the reduced IOP in the treated eye persisted until day 21. For Group 2 male, no differences in IOP was observed between the treated and untreated eyes on days 8 and 42. The reduced IOP was considered to be associated with the moderate uveitis after each injection.

IOP changes in monkeys treated with hyaluronidase (mm Hg)

Group	Pre-test		Day 3		Day 8		Day 14		Day 21		Day 28	
1st treatment	OD	OS	OD	OS	OD	OS	OD	OS	OD	OS	OD	OS
1	11	9	-	6	6	8	5	7	8	8	8	9
2	16	16	-	8	10	9	10	10	6	7	9	10
2ND treatment	Day 31		Day 35		Day 42		Day 49		Day 56		Day 70	
	OD	OS	OD	OS	OD	OS	OD	OS	OD	OS	OD	OS
2	-	10	7	12	10	12	14	17	9	10	12	14

Gross necropsy: There were no treatment-related gross observations.

Histopathology: Positive findings are listed in the table below. The inflammation was slight to mild, subacute, and was characterized by a perivascular infiltration of a lower number of neutrophils, eosinophils, macrophages, lymphocytes and/or plasma cells. The monkey with 56-day recovery period showed no inflammation in retina, ciliary body, iris, vitreous body and optic nerve. The number of pigment-laden macrophages was reduced. These changes suggested that the inflammatory responses might be reversible.

Histopathological findings in monkeys treated with hyaluronidase

Males	Right eye (treated)		Left eye	
Group	1	2	1	2
Number of eyes	1	1	1	1
Inflammation: conjunctiva	1 slight	1 mild	1 slight	
Inflammation. retina	1 slight			
Inflammation: vitreous body	1 slight			
Pigment laden macrophage: retina	1 mild	1 slight		
Pigment laden macrophage: vitreous	1, slight			
Inflammation: iris	1 mild			
Inflammation: ciliary body	1 slight			
Inflammation: optic nerve	1 slight			

In summary, Cynomolgus monkeys were treated intravitreously with a single dose (75 iu/50 µl) or double doses (75 iu, separated by 28 days) of hyaluronidase and were observed for 28 and 56 days, respectively. Clinical observations and ophthalmologic examination showed an acute, transient inflammatory response to the injection of hyaluronidase characterized by constricted pupils and moderate anterior uveitis. The inflammation precluded detailed evaluation of posterior ocular structures on days 3 and 31. Subsequent examinations on days 8 and 35 showed vascular congestion and swelling of the optic nerve head. ERG changes included reduced b-wave amplitude in Group 1 male. A decrease in IOP was also noted in both animals and was considered as drug-related. The inflammatory reaction, ERG and IOP changes were improved over the course of the study. Histopathological examination showed inflammation in the animal with 28-day recovery period. No inflammation was noted in the retina, iris, ciliary body, vitreous body and optic nerve in the animal with a 56-days recovery period, indicating that the inflammatory responses were reversible.

An intravitreal injection toxicity study of hyaluronidase for intravitreal ophthalmic injection (with a 90-day observation period) in the cynomolgus monkey. Vol. 9, Page 080

Key study findings: The administration of a single 75 iu or 150 iu intravitreal injection, or two 75 iu intravitreal injections of hyaluronidase resulted in an ocular inflammatory reaction. The inflammation seemed to be reversible.

Project No: 56359
Conducting laboratory and location: [redacted]

Date of study initiation: June 6, 2000
GLP compliance: Yes
Animal: Cynomolgus monkeys (*Macaca fascicularis*) 3-5 years old, 2.8-4.0 kg for males and 2.6-3.4 kg for females
Route: Intravitreal injection
Dosage: 75 or 150 iu/50 µl, right eye only

Drug: Hyaluronidase for Intravitreal Ophthalmic Injection, USP (Lot#: PHJ-06-03/224C) The formulation was similar to the clinical formulation.

Study design:

Group	Test article	N/sex/group	Dosage (iu/eye/µl)	Dosing regimen
1	Control	3	0/50	Single dose
2	Hyaluronidase	4	75/50	Single dose
3*	Hyaluronidase	3	75/50	2 doses 43 or 44 days apart
4	Hyaluronidase	3	150/50	Single dose

* Two Group 3 animals (1 male and 1 female) were considered unsuitable to receive a second dose due to persistent ocular inflammation in the treated eyes. These two animals were not dosed any more and were evaluated with Group 2 animals. Two Group 2 animals were transferred to Group 3 for the second dose.

The purpose of this study was to determine the toxicity potential of hyaluronidase following up to two intravitreal injections in the monkey. The day of the first dosing was designated as day 1. Toxicity was assessed as shown below.

Toxicity assessment

Parameter	Procedure
Mortality	Twice daily
Clinical observations	Twice daily
Body weights	Weekly
Ophthalmology/ Tonometry/ERG	Slit lamp biomicroscopy, IOP, and funduscopy pretreatment, on the days of dosing, and Weeks 1, 5, 9 and 13 on all animals, and on Day 14/16 (Group ¾), Days 22, 35, 42 and 49/50 (Group 3) ERG pretreatment and Weeks 1, 5, 9 and 13 on all animals, and on Day 15/17 for Group ¾ animals
Clinical pathology	Pretest, Week 4 and at termination
Immunoglobulin assay	Pretest, Weeks 2 and 4, and at termination. Group 3 animals were also sampled 4 weeks after the 2nd dose.
Gross pathology	Complete necropsy was performed on all animals at the end of the study (Day 90 or 91).
Histopathology	Both eyes
TK	Not performed.

Results:

Mortality: One Group 1 female and one Group 2 male died due to anesthetic complications during ERG recording on Days 59 and 86, respectively. There was no drug-related mortality.

Clinical observations: Constriction of the pupil was observed 1 to 2 weeks following the initial and the second intravitreal injection in all treated eyes. This was followed by dilation of the pupil for 1 to 7 weeks. One Group 3 animal showed general redness of the eye 2 to 3 weeks post dose. In the observations in Group 3 animals after the second injection, an opaque appearance of the treated eye was noted in 2/4 males and 4/4 females and lasted for up to 2 weeks. In Group 4 animals (150 iu), an opaque appearance of the treated eye was first observed in 5/6 animals 1 to 2 weeks post dose. In two of these animals, the effect lasted for about 5 and 11 weeks, respectively. These two animals also showed partially closed eyes one week post dose. Most of these positive observations, not seen in the control animals, disappeared four weeks after the injection.

Body weights: No drug-related, toxicological significant effects were noted.

Slit lamp biomicroscopy and funduscopy examinations: In Groups 2 and 3 animals, the initial injection produced acute, moderate to severe anterior uveitis characterized by dense aqueous flare and cells in the treated eye of most animals. The presence of a hypopyon or a clot in the anterior chamber was detected in most animals. Focal retinal infiltrates and sheathing of retinal vessels were also observed. The inflammatory response appeared to resolve over 2 to 3 weeks. By Week

4, 9/14 eyes treated at 75 iu showed either no change at all or minimal effects such as slight vitreous cells and/or pigment on the lens capsule and/or irregular foveal pigmentation. The other three Group 2 and two Group 3 animals demonstrated moderate to severe numbers of vitreous cells, blurry fundus, dense focal or diffuse vitreous opacity, posterior synechiae, and anterior capsular opacity. The two Group 3 animals were more severely affected with persistent anterior uveitis and severe infiltration of the vitreous with inflammatory cells. The fundus could not be evaluated. The sponsor switched these two animals with two Group 2 animals for the second injection. By Week 13, 2/8 eyes treated with a single dose of 75 iu were normal. Two eyes showed a small number of vitreous cells. A focal retinal infiltrate was noted in one eye. The other 3 eyes showed more severe lesions including pigmentation of the lens capsule, slight to severe numbers of vitreous cells, and a dense focal vitreous opacity.

For the animals experienced the second intravitreal injection (Group 3), moderate to severe anterior uveitis was noted in all of the six animals. Two weeks after the dosing, the inflammation was reduced or resolved. Slight to severe numbers of vitreous cells and fundic lesion (focal retinal infiltrates and/or sheathing of the retinal vessels) were present in 6/6 animals, pigment on the lens capsule in 4/6 animals, anterior capsular opacity in 2/6 animals, and dense focal vitreous opacity in 1/6 animals. By the beginning of the Week 13, lesions were minimal and similar in nature to those of the singly dosed animals at the same time period.

For the animals dosed at 150 iu, the reaction was similar to the 75 iu dosed animals. Vitreous cells were present in all animals and sheathing of the retinal vessels was noted in one animal. Four weeks post dose, three animals showed moderate numbers of vitreous cells, blurry fundus, and a dense focal vitreous opacity. By the beginning of the Week 13, two animals had no observable lesions, two animals had only slight numbers of vitreous cells, and the other two animals had slight to moderate vitreous cells, a dense focal vitreous opacity and pigment on the lens capsule. One animal also had a slightly blurry fundus.

ERG: Each ERG occasion consisted of a series of single flash stimuli with different light intensity. The following ERG changes were considered as drug-related.

Scotopic a-wave: There was a dose-related effect on a-wave amplitude and latency 2 days following the initial injection of hyaluronidase. The reduction in magnitude of mean a-wave amplitude between treated and contralateral untreated eyes was greatest at –20 dB, ranging from 40-60% in males and 60-80% in females. At maximal flash intensity (0 dB) and –10 dB, animals at 75 iu had reductions in a-wave approximately 15-30% in males and 7-52% in females. At 150 iu, animals had reductions of approximately 47-55% in males and 47-52% in females. In many animals, the a-wave latency was also prolonged. At –10 dB, mean a-wave latency was prolonged by approximately 15-40% across treated groups, while at 0 dB, latency was prolonged by about 5-18%. The ERG changes following the second injection was similar to that after the initial injection. The improvement of the reduction in the magnitude and the prolongation in latency was noted during Week 5. In Week 13, the a-wave amplitude and latency values between treated and contralateral control eyes were comparable with the exception of Group 2 males where the mean a-wave amplitude was 28% smaller in the treated eye at 0 dB.

Scotopic b-wave: There was a drug-related effect on b-wave amplitude 2 days following the initial injection. The mean b-wave amplitude was reduced by about 55-85% at –20 dB and 40-

70% at –10 dB and 0 dB compared to the contralateral control eyes. The b-wave latency was slightly prolonged at –20 dB but at 0 to –10 dB, the latency became shorter by about 5-30%. During Week 5, the reduction in mean b-wave amplitude in the treated eyes tended to be less severe. In the animals experienced the second injection, an 11 to 60% decrease in b-wave amplitude and an up to 35% prolongation of the latency were noted in the treated eyes. During Week 13, the mean b-wave amplitude in the treated eye was comparable to the contralateral eye and/or the pretreatment baseline for the most flash intensities. The exception included Group 2 and Group 3 males that had 25-30% reductions in mean b-wave amplitude. In addition, Group 3 females showed a 10% prolongation in the mean b-wave latency at 0 dB.

Photopic 29 Hz flicker: Two days following the initial dose, the treated eyes showed 50-65% and 60-75% reductions in the mean flicker amplitude at 75 iu and 150 iu, respectively, relative to the contralateral eyes. The mean latency was slightly prolonged in the treated eye by 4-14% across the treated groups. Four days following the second injection, the flicker amplitude was severely reduced in 6/6 treated eyes by a range of 20-85%. The latency was also prolonged in 3/6 eyes by a range of approximately 15 to 30%. By Week 5, reductions in mean flicker amplitude compared to the contralateral control eyes improved to 35-45% in Groups 2 and 3, and 45-50% in Group 4. During Week 13, the reduced mean flicker amplitude in the treated eye was further improved. For Group 3 males and Group 4 females, the reductions in the amplitude was within 30% and 20% of the contralateral control eye, respectively. All other groups were comparable to the Group 1 control and/or the pretreatment baseline.

Tonometry: Reductions in IOP occurred in all treated animals the day following the initial injection (-25-60%) and the second injection (-40-70%). The recovery of the IOP took about 4-8 weeks for the initial injection and 2 weeks for the second injection, respectively. The sponsor indicated that the decrease in the IOP could be due to liquification of the vitreous and inflammatory reaction in the eye.

Clinical pathology: There were no drug-related effects on hematology, clinical chemistry and urinalysis.

Immunoglobulin assay: When measuring the hyaluronidase-specific IgG antibodies, the mean OD value was 0.072-0.304 for pre-treatment monkey serum samples. Measurement performed on Days 14 and 22 showed no abnormal findings. Only samples from two Group 3 animals showed high responses for hyaluronidase-specific IgG antibodies following the second injection [1 male (mean OD = 0.830) and 1 female (mean OD = 0.315)] on Day 71 (29 days after the second dosing). By Day 92, the female was normal (mean OD = 0.229), but the male still had a high response (mean OD = 0.442). The positive results were confirmed with Western Blot analysis. For the other animals, the hyaluronidase-specific IgG remained within background levels. No adverse effects were noted in association with the antibody response.

Gross necropsy: There were no treatment-related gross observations.

Histopathology: Positive findings, including histiocytic cell inflammation within the vitreous body, mononuclear cell infiltration, and peripheral degenerative retinal lesions, are summarized in the table below. The severity was minimal in all of these findings. Retinal degeneration, also

noted in one untreated eye, was described as non-progressive, and with no impact on the vision of the animals.

Histopathological findings in rabbits treated with hyaluronidase

Males	Right eye (treated)				Left eye (untreated)			
Group	1	2	3	4	1	2	3	4
Number of eyes	3	4	3	3	3	4	3	3
Degeneration: peripheral retina			2	2				
Inflammation: vitreous body		1	2	1				
Infiltration: mononuclear cell		1	1					
Females								
Number of eyes	3	4	3	3	3	4	3	3
Degeneration peripheral retina		2		1				1
Inflammation· vitreous body			1					
Infiltration mononuclear cell			2					

In summary, Cynomolgus monkeys were treated intravitreously with a single dose (75 or 150 iu/50 µl) or double doses (75 iu) of hyaluronidase and were observed for 90 days. Clinical observations and ophthalmologic examination showed an acute, transient inflammatory response to the injection of hyaluronidase characterized by constricted pupils, opaque appearance of the eye, and moderate to severe anterior uveitis (aqueous cells and flare). ERG changes including reduced amplitude and prolonged latency were considered as drug-related. A decrease in IOP was also noted and was considered as drug-related. The inflammatory reaction, ERG and IOP changes were improved over the course of the study. At the end of the study, only slight vitreous cells, pigment on the lens capsule and irregular foveal pigmentation were observed in all treated groups. The significant presence of drug-specific antibodies in two Group 3 animals did not change the severity and type of the drug-related effects. Histopathological examination showed minimal histiocytic cell inflammation within the vitreous body, mononuclear cell infiltration, and peripheral degenerative retinal lesions in the treated eye. The sponsor indicated that the higher incidence of the microscopic findings in Group 3 animals (two treatments) was likely related to the difference in recovery time (about 7 weeks) between dosing and termination. In conclusion, intravitreal treatment with hyaluronidase at either a single dose up to 150 iu or two doses of 75 iu caused similar inflammatory effects. The inflammatory responses were reversible.

Safety of multiple injections of Vitrase in rabbits. Vol. 9, Page 407

Key study findings: The administration of three consecutive 75 iu intravitreal injections of hyaluronidase resulted in similar transient inflammatory reactions with higher magnitude and longer duration.

Project No: TOX-08-08961X
Conducting laboratory and location: [redacted]

Date of study initiation: February 2, 1999
GLP compliance: Yes
QAU: No
Animal: Ten female New Zealand Red rabbits, 1.8-2.2 bl.
Route: Intravitreal injection
Dosage: 75 iu/50 µl, right eye only on Days 0, 28 and 63 (the left eye was treated with 0.9% sodium chloride solution.)

Drug: Hyaluronidase for Intravitreal Ophthalmic Injection, USP (Lot#: PHI-05-06/224B)

The purpose of this study was to determine the toxicity potential of hyaluronidase following three sequential intravitreal injections vs three saline injections in rabbits. The day of the first dosing was designated as day 0. Toxicity was assessed as shown below.

Toxicity assessment

Parameter	Procedure
Slit lamp biomicroscopy	Pretreatment and 1, 3, 7, 11, 14 and 28 days after each injection
Indirect ophthalmoscopy	Pretreatment and 1, 3, 7, 11, 14 and 28 days after each injection
Fluorescein angiography	Pretreatment, days of 2nd and 3rd injections, 14 days after each injection, and at termination (day 161)
Histopathology	Eyes
ERG	At the end of the observation period in 8 animals

Results:

Slit lamp and indirect ophthalmoscopy examinations: Positive findings are summarized in the table below. Following the 1st injection, ocular examinations showed inflammatory responses including aqueous cell and flare, precipitates, vitreous haze, and vitreous cells in the treated eye. These findings, with the exception of vitreous cells, disappeared within 28 days.

After the 2nd and 3rd injections, similar inflammatory reactions with greater magnitude and longer duration were noted. The resolution of these abnormal findings took 35 days after the 2nd injection, and 43 days after the 3rd injection. Vitreous membrane was noted in two animals after the 2nd injection. One of these animals also had retinal detachment. These two animals were terminated before the 3rd injection. For the remaining eight animals, vitreous membrane was noted in five animals with retinal detachment found in one of these animals.

Positive findings by slit lamp and indirect ophthalmoscopic examinations

	Aqueous cell/flare		Iris synechiae		Haze		FWP		Vitreous cells	
Day	75 iu	Saline	75 iu	Saline	75 iu	Saline	75 iu	Saline	75 iu	Saline
1	**2.4**	**0**	**0.4**	**0.2**	**1.2**	**0.4**	**0.4**	**0**	**0**	**0**
3	0.8	0	2 3	0 7	2.7	0	2 0	0	3 6	0
7	0	0	0.6	0.3	1.9	0	0.5	0	4.8	0.3
28	0	0	0	0	0	0	0.3	0	2.7	0
29	**3.0**	**0**	**0.1**	**0**	**2.5**	**0**	**0.2**	**0**	**2.9**	**0.1**
31	2.9	0	0.8	0.1	3.6	0	0.9	0	3 6	0
35	0.1	0	1.4	0.2	2.9	0	2.7	0	4.7	0
63	0	0	0 5	0.38	0	0	0	0	2.38	0
64	**3.25**	**0**	**2.25**	**0.13**	**2.83**	**0.13**	**0.5**	**0**	**3**	**0**
66	3	0	0.63	0.63	3.63	0	2.5	0	5	0
70	0.38	0	1.63	0.63	3.25	0	3.75	0	5	0
73	0.13	0	2 63	1.13	2.5	0	3.13	0	5	0
77	0	0	2 13	0.5	1.75	0	2.38	0	5	0.13
106	0	0	0.88	0.13	0.5	0	0 38	0	3.5	0.13
120	0	0	0.13	0.25	0.38	0	0.88	0	2.5	0.5
161	0	0	2.63	1.75	0.13	0	1.13	0	2	0.38

Grading for iris synechiae: 0=no synechiae seen, 1-12=the number of clock hours of synechiae noted with each clock hr corresponding to 30° of iris involvement.
Grading for lens: 0=no opacification or mechanical defect present, 1=cataract or lens defect present.
The same score system was used in other toxicity studies submitted in this NDA.

Fluorescein angiography examinations: All angiograms from the saline treated eyes were normal. For the treated eyes, microaneurysms or retinal windows were seen. In angiograms taken near the

time of injection, excessive vitreous haze or very rarely, mild leakage of fluorescein was noted. All leakage was resolved later.

Positive angiogram findings in the treated eyes

Day of examination	Findings
3	Vitreous haze in 1 animal
14	Vitreous haze in 1 animal
29	Vitreous haze in 1 animal
30	Vitreous haze in 2 animals, vitreous membrane in 1 animal
43	Microaneurysms in 2 animals, vitreous haze and membrane in 2 animals, mild leak in 1 animal
63	Microaneurysms in 3 animals, retinal detachment in 1 animal
64	mild leak in 1 animal
80	Microaneurysms in 1 animal, vitreous haze in 1 animal, vitreous membrane in 2 animals, mild leak in 1 animal
120	Microaneurysms in 1 animal, vitreous membrane in 2 animals
161	Vitreous membrane in 1 animal

Histopathologic examinations: No abnormal findings were noted in saline-treated eyes. Positive histological findings including hyalitis, retinal detachment and retinal disruption, are summarized in the table below.

Histopathological findings in rabbits treated with hyaluronidase

Group	Control	After 2 injection	After 3 injections
Number of eyes (treated eyes)	**10**	**2**	**8**
Papillitis, diffuse		1 minimal, 1 mild	3 minimal, 1 mild
Hyalitis, multifocal			5 minimal, 1 mild
Reactive RPE with swelling and separation of RPE cells, multifocal		2 minimal	
Retinal disruption/detachment, multifocal			2 minimal
Pigmented epithelial proliferation, focal			1 minimal

ERG: There were two consistent findings in the drug-treated eyes: lower implicit a-wave times (latency) and higher b-wave amplitude. There was no evidence of damage to the photoreceptors or midretina of the Vitrase treated eyes. [**Reviewer's Comments**: The ERG results were different from those in Study CTBR 56359 conducted in monkeys. No data were provided. Although ERG data did not indicate retinal damage, histopathologic and angiographic data showed the damage.]

In summary, New Zealand Red rabbits were treated intravitreously with hyaluronidase (75 iu/50 µl, right eyes) or PSS (left eyes) for three times and were observed for up to 161 days. No abnormal findings were noted in the saline treated eyes. Ophthalmologic examinations showed acute, self-limited inflammatory reactions to the injection of hyaluronidase. The reactions included aqueous cell and flare, vitreous haze, precipitates, and vitreous cellular infiltration. The second and third injections tended to cause similar responses with higher magnitude and longer duration. In addition, 2/10 animals and 6/8 animals developed vitreous membranes after the 2nd injection and 3rd injection, respectively. Retinal detachment was also noted in 2 of these animals. Angiogram assay showed mild leakage, vitreous haze and/or membrane, and microaneurysms in the treated eyes. Histopathological examination showed retinal disruption/detachment, papillitis, and hyalitis.

Probe study: determining the toxicology of "old" vs "new" ACS hyaluronidase. Vol. 9, Page 443

Key study findings: There was no excess toxicity related to the increased concentrations of phosphate and lactose in the new formulation.

Project No: VIT 07-22-96
Conducting laboratory and location: [redacted]

Date of study initiation: July 22, 1996
GLP compliance: No
QAU: Not signed
Animal: Dutch belted rabbits, 1.5-2.5 kg
Route: Intravitreal injection
Dosage: 50, 75 or 100 U/30 µl, Days 0, 30 and 60
Drug:

	Old formulation			New formulation		
Lot #	PHD-06-02			PHG-03-01		
Dose (U)	50	75	100	50	75	100
Lactose (%)	0 15	0.22	0.29	0.42	0 62	0.83
Potassium phosphate (mM)	5 8	8.8	11 7	20.8	31 2	41 5

Study design:

Group	N	Treatment (OD)	Treatment (OS)	Injection volume	Treatment day	Necropsy day
1	10	No treatment	vehicle	30 µl	0, 33,61	3/group on Days 30 and 60, and 4/group on Day 90
2	10	Old formulation, 50 U	New formulation, 50 U	30 µl	0, 33,61	
3	10	Old formulation, 75 U	New formulation, 75 U	30 µl	0, 33,61	
4	10	Old formulation, 100 U	New formulation, 100 U	30 µl	0, 33,61	

The purpose of this study was to determine the toxicity potential of hyaluronidase with different formulations and multiple intravitreal injections in rabbits. The day of the first dosing was designated as day 0. Toxicity was assessed as shown below.

Toxicity assessment

Parameter	Procedure
Slit lamp biomicroscopy	Pretreatment and days 1, 2, 4, 7, 15, 33, 36, 45, 60, 64, 67, 75 and 90
Indirect ophthalmoscopy	Pretreatment and days 7, 15, 33, 36, 45, 60, 64, 67, 75 and 90
Fluorescein angiography	Pretreatment and days 7, 15, and 45

Results:

Slit lamp and indirect ophthalmoscopy examinations: Inflammatory reactions were noted in all hyaluronidase treated groups. The reactions included aqueous cells and flare, vitreous haze, vitreous fluffy white precipitates (FWP), and vitreous cells. There was no difference in ocular reactions between these two formulations. The magnitude of the peak aqueous cell/flare reaction following the 2nd and 3rd injections was similar to that after the 1st injection. By the final scheduled examination for the last 2 phases, the reactions were presented as a trace level (score: 0.5-0.8) for the 2nd injection or absent (score: 0) for the 3rd injection. Vitreous haze also showed a pattern of declining values with no haze noted 29 days after the 3rd injection. For vitreous FWP and cells, the scores were persistent over the observation periods across injections/doses. The magnitude of the vitreous FWP was higher (2.0-3.8) after the 2nd and 3rd injections compared to that after the 1st injection (0.4-2.9).

Fluorescein angiography: In the examination carried on day 7, questionable leakage and vitreous haze were noted in one animal each in Groups 2 (OS, new formulation, 50 U), 3 (OD, old formulation, 75 U) and 4 (OS, new formulation, 100 U). No drug-related toxicity to the RPE or retinal vasculture was noted.

In summary, Dutch belted rabbits were treated intravitreously with two formulations of hyaluronidase (50, 75 or 100 iu/30 µl, 1/month x 3) or vehicle for the new formulation and were observed for up to 90 days. Ophthalmologic examinations showed acute inflammatory reactions to the injection of hyaluronidase. The reactions included aqueous cell and flare, vitreous haze, precipitates, and vitreous cells. The second and third injections tended to cause similar responses with slightly higher magnitude in vitreous FWP. There was no differences in toxicity between the tested two formulations, suggesting higher concentrations of lactose and potassium phosphate would not elicit greater toxicity.

Comparative ocular irritation and toxicity study of Vitrase and heat-stressed Vitrase given by intravitreal injection to Dutch belted rabbits. Supplement 1, Page 027

Key study findings: There was no toxicologically significant, treatment-related changes noted for Vitrase or heat-stressed Vitrase in this study. No differences were noted between these two treatments.

Project No: 152-002
Conducting laboratory and location: [redacted]

Date of study initiation: February 21, 2002
GLP compliance: Yes
Animal: Female Dutch belted rabbits, 12 weeks old, 1282-1559 g
Route: Intravitreal injection
Dosage: 75 or 150 iu/50 µl, left eye only. The right eye was treated with vehicle control.
Drug: Vitrase (Lot#: 242D/IVS002) The formulation was similar to the clinical formulation.
Heat-stressed Vitrase [Lot#: IVS002. Heat stressed Vitrase showed a loss of activity (10-15%) compared to the native product.]

Study design:

Group	Test article (OS)	Dose (iu)	N	Terminated on day 30	Terminated on day 64
1	Vitrase	75	8	4	4
2	Heat-stressed Vitrase	75	8	4	4
3	Vitrase	150	8	4	4
4	Heat-stressed Vitrase	150	8	4	4

The purpose of this study was to determine the toxicity potential of Vitrase and heat-stressed Vitrase following a single intravitreal administration to female Dutch belted rabbits. The day of the first dosing was designated as day 1. Toxicity was assessed as shown below.

Toxicity assessment

Parameter	Procedure
Mortality	Twice daily
Clinical observations	Once daily
Body weights	Weekly
Food consumption	Daily
Ophthalmology/ Tonometry	Slit lamp biomicroscopy/indirect ophthalmoscopy: pretreatment, on days 2, 5, 7, 10, 14, 28, 42 and 63 IOP: pretreatment, on days 1, 2, 5, 7, 10, 15, 28, 42, and 63
Angiography	Left eyes: days 5, 12, 29, and 62; right eyes: days 6, 13, 30 and 63
Gross pathology	All animals
Histopathology	Both eyes

Results:

Mortality: There was no mortality during the study period.

Clinical observations: No drug-related clinical signs were noted. In the first 7 days, conjunctival congestion, discolored conjunctiva, and conjunctivitis were noted in both treated and control eyes with the similar incidence in all groups. These changes were not considered as drug-related.

Body weights: No toxicologically significant effects were noted.

Food consumption: There were no toxicologically significant effects on food consumption.

Slit lamp biomicroscopy and funduscopy examinations: The findings are summarized in the table below. No significant ocular abnormalities were developed. The changes in Groups 1 and 3 animals were due to contact of the dosing needle. The peripapillary depigmentation was a variation of normal pigmentation and was noted before the study started. The reason for the fibrin clot was unknown. This change was resolved by day 28. Over the 63-day study period, there were no significant drug-related ocular effects at the doses tested. **[Reviewer's Comments**: The ophthalmoscopic examination results in this study were too "clean", and were different from those obtained from other toxicity studies. The reviewer noted that the conducting lab was not the same labs for other studies.]

Ophthalmic examination findings in rabbits treated with Vitrase and heat-stressed Vitrase

Males	Right eye (control)				Left eye (treated)			
Group	1	2	3	4	1	2	3	4
Retinal hemorrhage near the optic nerve					1, day 2			
Slight retinal elevation with focal hemorrhage and choroidal depigmentation					1, day 7			
Focal area of depigmented choroid at the site of previous lesion (fundic scar)					1, days 14-63			
Peripapillary depigmentation		1, days 7-28						1, days 7-28
Hole in retina inferior temporal to optic nerve							1, days 7-28	
Linear fibrin clot inferior to the optic nerve								1, day 14

IOP: The normal IOP in rabbits should be 19.5 ± 1.84 mmHg. In this study, all groups showed increased IOP values before (29.3-32.3 mmHg) and after treatment. Over the 63-day study period, there were no consistent drug-related ocular effects on IOP at the doses tested. On day 2, the mean IOP values of all groups were slightly lower (24.69-28.06 mmHg) compared to the pre-test values. These changes were noted in both eyes, and were not considered as drug-related. On day 5, the IOP values in the treated eyes (left eyes) were 22 ± 5.2 mmHg in Group 3 and 14.1 ±

2.79 mmHg in Group 4 animals. The IOP values went back to the pre-test levels on day 7. There was no trend toward increased IOP.

Fluorescein angiography: There was no significant, consistent vascular abnormality developed during the course of the study. One animal in Group 1 showed hypofluorescence inferior to the disc of the left eye on study days 29 and 62. Two animals in Group 2 showed hyperfluorescence superior to the disc of the left eye on study days 5 and 12, and days 5 and 62, respectively.

Gross necropsy: There were no treatment-related gross observations in gross necropsy examinations performed on day 30 and day 64.

Histopathology: In the left eye of one Group 1 animal terminated on day 30, a single bulla of the cornea was noted. A small focus of inflammatory cells, mainly lymphocytes, was presented in the bulbar conjunctiva in a few left and right eyes from all groups. These changes were considered incidental and spontaneous. No toxicologically significant findings were noted.

In summary, this study was designed to evaluate the effects of drug product degradants. Dutch belted rabbits were treated intravitreously with a single dose (75 or 150 iu/50 µl) of naïve or heat-stressed Vitrase (left eye) and were observed for 2 months. The right eye was treated with vehicle. No treatment-related effects were noted in clinical observations, body weights, food consumption, ophthalmic examinations (including slit-lamp biomicroscopy, indirect ophthalmoscopy, and fluorescein angiography), necropsy, and histopathological examinations. Both Vitrase and heat-stressed Vitrase might have an effect on lowering the IOP temporarily. In conclusion, no toxicologically significant, treatment-related effects were noted for Vitrase or heat-stressed Vitrase in this study. No differences were noted between these two treatments.

Comparative ocular irritation and toxicity study of Vitrase given by intravitreal injection to Dutch belted rabbits. Supplement 2, Page 001

Key study findings: Transient, mild inflammation in the treated eye was noted. There were no treatment-related changes for the four different lots of Vitrase in this study.

Project No: 152-003
Conducting laboratory and location: [redacted]

Date of study initiation: June 4, 2002
GLP compliance: Yes
Animal: Male Dutch belted rabbits, 6 months old, 2-2.36 kg
Route: Intravitreal injection
Dosage: 75 or 300 iu/50 µl, left eye only. The right eye was treated with vehicle control.
Drug: Vitrase with different lot numbers (Lots 130B/PHH-03-05, 224C/PHI-09-07 and 242D/IVS-002 were reconstituted with 0.9% NaCl for injection, USP; Lot 242B was reconstituted with vehicle.) The formulations for all of the lots were similar to the clinical formulation.

Study design:

Group	Lot #	Dose (iu/50 µl)	N	Terminated on day 30
1	PHH-03-05	75	3	3
2	PHI-09-07	75	3	3
3	242B	75	3	3
4	IVS-002	75	3	3
5	IVS-002	300	3	3

The purpose of this study was to determine the toxicity potential of four different lots of Vitrase following a single intravitreal administration to male Dutch belted rabbits. The day of the first dosing was designated as day 1. Toxicity was assessed as shown below.

Toxicity assessment

Parameter	Procedure
Mortality	Twice daily
Clinical observations	Once daily
Body weights	Weekly
Food consumption	Daily
Ophthalmology/ Tonometry	Slit lamp biomicroscopy/indirect ophthalmoscopy: pretreatment, on the days 2, 5, 8, 10, 15, and 29 IOP: pretreatment, on days 2, 5, 7, 10, 15, and 27
Angiography	Left eyes: days 5, 12, and 28, right eyes: days 6, 13, and 29
Gross pathology	All animals, day 30
Histopathology	Both eyes

Results:

Mortality: There was no mortality during the study period.

Clinical observations: No drug-related clinical signs were noted. Conjunctival congestion, discolored conjunctiva, and conjunctivitis were noted from days 3 to 7 in both treated and control eyes with the similar incidence in all groups.

Body weights: There were no toxicologically significant differences among different animal groups.

Food consumption: There were no toxicologically significant differences among different animal groups.

Slit lamp biomicroscopy and funduscopy examinations: On day 8, 10 and 15, diffuse, fine vitreal granularity was seen in all drug-treated eyes but the group 4 animals. The change, indicating liquified vitreous, was an expected result of the drug, and was not considered as an abnormal finding. The degree of liquification was decreased by day 29. The left eye of one Group 3 animal and one Group 5 animal had a depigmented foci noted in the choroid. The significance was unknown. One Group 3 animal showed trace value of fluffy white precipitates in the left eye.

IOP: The normal IOP in rabbits should be 19.5 ± 1.84 mmHg. In this study, all animals showed increased IOP values before the treatment (23.8-31.2 mmHg, average: 28.4 mmHg). After treatment, decreased IOP values were noted in the left eyes (drug-treated). The lowest values were observed on day 5 (8.8-12.3 mmHg) relative to the right eyes (17.0-26.2 mmHg). None of the rabbits developed glaucoma. The sponsor's explanation for the increased IOP was due to the degree of restraint needed to obtain the pressure in rabbits that were not preconditioned for the

procedure. The increased IOP was not the result of treatment. On day 15, the IOP levels were similar between control and treated eyes.
[**Reviewer's comments**: The sponsor indicated that the lowered IOP in the left eyes was consistent with mild inflammation in the eye. However, the sponsor claimed that the ocular inflammation scores for all the rabbits for all the categories were zero.]

Fluorescein angiography: There was no significant, consistent vascular abnormality developed during the course of the study. The positive findings are summarized in the table below. Hyperfluorescence might indicate the leakage of fluid from the blood vessels. Hypofluorescence might indicate a decrease in vascular perfusion or masking of the normal blood supply. No retinal lesions were associated with these problems. The significance of these changes were not known.

Fluorescein angiography examination findings in rabbits treated with Vitrase

Males	Right eye (control)		Left eye (treated)	
Group	1	3	3	5
Circular hyperfluorescent spot	1, day 29			
Superior circular hyperfluorescent lesion		1, pre-test and day 29		
Superior circular hypofluorescent lesion			1, day 28	
Hyperfluorescent foci inferior to the disc				1, day 5
Hypofluorescent lesion lateral optic nerve and hyperfluorescent lesion inferior to the disc				1, day 12

Gross necropsy: There were no treatment-related gross observations in gross necropsy examinations.

Histopathology: Minimal focal retinal detachment and atrophy were noted in the left eye of one Group 2 animal. Focal retinal atrophy was noted in one Group 3 animal (mild, left eye) and one Group 4 animal (minimal, right eye). The sponsor indicated that the single retinal lesion in three rabbits was not considered as drug-related, but could have resulted from the injection procedure. No positive findings were noted in the other animals.

Summary: This study was designed to determine if there were any differences in the toxicological response to various finished product lots of Vitrase, representing the clinical and commercial products. Dutch belted rabbits were treated intravitreously with a single dose (75 or 300 iu/50 µl, left eyes) of four different lots of Vitrase and were observed for 1 month. The right eye was treated with vehicle. No toxicologically significant, treatment-related effects were noted in clinical observations, body weights, food consumption, ophthalmic examinations (including slit-lamp biomicroscopy, indirect ophthalmoscopy, and fluorescein angiography), necropsy, and histopathological examinations. The IOP in the left eye was decreased on day 5 and returned to normal by the end of the study. In conclusion, no non-reversible, treatment-related effects were noted when comparing the four different lots of Vitrase in this study.

Reviewer's comments: In the two studies conducted by . ———— , the ophthalmic and histological examination did not show intraocular inflammatory responses seen in other toxicity studies performed by other laboratories. The reviewer does not know the reasons. From these two studies, however, it could be concluded that there were no differences in the drug effects between naïve and heat-stressed Vitrase products, and among four different lots of Vitrase.

The following studies reviewed are non-GLP probe studies. The studies had some deficiencies, and the reports were not well organized and written. The reviewer summarized these studies in this review for the purpose of reference.

Probe study: Safety of intravitreal hyaluronidase injections. Vol. 7, Page 003

Key study findings: The eyes treated with Wydase (containing thimerosal) and thimerosal showed retinal damage and necrosis in a dose-dependent manner, suggesting that thimerosal, a known mercury compound, is an etiologic agent for acute retinal necrosis.

Study No: 95C-01869-00
Conducting laboratory and location:

Date of study initiation: January 16, 1995
Date of report: March 20, 1999
GLP compliance: No
Animal: Dutch Belted Cross rabbits, 2.0-2.5 lb., 2/sex/group
Route: Intravitreal injection, single dose
Dosage: 1, 15, 30, 50 and 150 U/100 µl, both eyes
Drug: Hyaluronidase (Lot#: 130B) diluted in BSS (Lot 130B was depleted later.)
Wydase (containing thimerosal) diluted in BSS
Thimerosal diluted in BSS
Study design:

Group	Treatment
1	Balanced salt solution (BSS)
2	BSS + 0.0075 mg thimerosal (equivalent to dosage in 15 U Wydase)
3	BSS + 0.025 mg thimerosal (equivalent to dosage in 50 U Wydase)
4	Wydase 1 U
5	Wydase 15 U
6	Wydase 30 U
7	Wydase 50 U
8	Wydase 150 U
9	Hyaluronidase ACS 1 U
10	Hyaluronidase ACS 15 U
11	Hyaluronidase ACS 30 U
12	Hyaluronidase ACS 50 U
13	Hyaluronidase ACS 150 U

The purpose of this study was to determine the safety of intravitreal injections of varying concentrations of ACS hyaluronidase, Wydase and thimerosal (a preservative). Toxicity was assessed as shown below.

Toxicity assessment

Parameter	Procedure
Fundus photography	Pretreatment, and 2 and 7 days after the injection
Fluorescein angiography	Pretreatment, and 2 and 7 days after the injection
Histopathology	Eyes, 1/sex/group, 2 and 7 days after the injection

Results:

No abnormalities were noted in Group 1 (BSS) eyes.

Two days after the treatment, no visible effects were noted in most groups. In Group 3 (BSS+ thimerosal) multiple retinal and intravitreal hemorrhages were present. In animals treated with Wydase 50 and 150 U, the major retinal vessels appeared engorged and obstructed. Fluorescein leakage was noted.

The results obtained at 7 days after injection are summarized in the table below. It seemed that similar retinal damages (leakage of fluorescein, retinal necrosis) were seen in Wydase (at 30-150 U) and thimerosal treated eyes. The eyes treated with hyaluronidase-ACS at 1-50 U were free of toxic effects.

Reviewer's comments: There are a few deficiencies in this study. The sponsor indicated that the Lot 130B was depleted after this study. So this study was not very meaningful. In the sponsor's Summary of Findings, the Groups 4-8 were labeled hyaluronidase-ACS, which was different from the protocol. No detailed data were provided. The reviewer believes that the study results can only be taken as a reference.

Positive findings following intravitreal injection of Wydase, thimerosal and hyaluronidase-ACS

Group	Treatment	Ophthalmoscopy and angiography (day 7)	Histology (day 7)
1	Balanced salt solution (BSS)	Normal	Capillary ectasia, inner retinal surface-1
2	BSS + 0 0075 mg thimerosal	Leakage of fluorescein, obscuring visualization of retina	Regional retinal necrosis-4
3	BSS + 0 025 mg thimerosal	Detached retina, filling defects of vessels, leakage of fluorescein, obscuring most retinal detail	Regional retinal necrosis and degeneration all layers-4
4	Wydase 1 U	Slightly swelling of optic disk, leakage of fluorescein	Normal
5	Wydase 15 U	Details of optic disc indistinct, leakage of fluorescein, obscuring visualization of retina	Perivascular histiocytic inflammation-2
6	Wydase 30 U	Significant disruption of the optic disk, leakage of fluorescein, obscuring visualization of retina	Regional retinal necrosis and degeneration all layers-4
7	Wydase 50 U	Detached retina, filling defects of vessels, leakage of fluorescein, obscuring most retinal detail	Perivascular histiocytic inflammation-1, regional retinal necrosis and degeneration with focal retinal detachment-3
8	Wydase 150 U	Detached retina, filling defects of vessels, leakage of fluorescein, obscuring most retinal detail	Regional retinal necrosis and degeneration all layers-4
9	Hyaluronidase ACS 1 U	Normal	Capillary ectasia, inner retinal surface-1, perivascular histiocytic inflammation-1
10	Hyaluronidase ACS 15 U	Normal	Normal
11	Hyaluronidase ACS 30 U	Normal	Capillary ectasia, inner retinal surface-1
12	Hyaluronidase ACS 50 U	Normal	Capillary ectasia, inner retinal surface-1
13	Hyaluronidase ACS 150 U	Preretinal material present (inflammation or drug itself?), leakage of fluorescein, obscuring visualization of retina	Perivascular histiocytic inflammation-1

1: minimal; 2: mild; 3: moderate; 4: severe

In summary, Dutch Belted Cross rabbits were treated intravitreously with a single dose of hyaluronidase (1, 15, 30, 50 or 150 U/100 µl), Wydase (1, 15, 30, 50 or 150 U/100 µl) or BSS plus thimerosal, and were examined with ophthalmoscopy, fluorescein angiography and histopathology at 2 days and 7 days after the injection. The eyes treated with Wydase (containing thimerosal) at $\geq$ 30 U and thimerosal showed significant leakage, retinal damage and necrosis in a dose-dependent manner. These changes were not seen in the hyaluronidase-ACS treated eyes, suggesting that thimerosal, a known mercury compound, might be an etiologic agent for acute retinal necrosis.

Specific activities of various types of hyaluronidase

Type	Source	Activity (U/mg)	Total protein (mg protein/mg)	Specific activity (U/mg protein)
[redacted]	Ovine	3971	0.258	15393
Type I	Bovine	192	0.956	203
Type IV	Bovine	775	0.690	1123
Type V	Ovine	2676	0.591	4528
Type VI	Bovine	2721	0.260	10465

Probe study: Comparison of biozyme hyaluronidase Lot 130B vs Lot 200C in vitreous model. Vol. 7, Page 063

Key study findings: Lot 130B produced more serious ocular inflammatory reactions than Lot 200C.

Study No: VIT 04-17-97
Conducting laboratory and location: [redacted]

Date of study initiation: February 26, 1997
Date of report: August 15, 1997
GLP compliance: No

The purpose of this study was to compare the toxicity potential of two lots of hyaluronidase following a single intravitreal injection (100 iu/30 µl, Lots 130B-right eyes and Lot 200C-left eyes) to six Dutch belted rabbits. The animals were observed for 30 days. Toxicity was assessed with slit lamp biomicroscopy (pre-test and days 1, 4, 7, 14, 21 and 30), indirect ophthalmoscopy (pre-test and days 1, 4, 7, 14, 21 and 30), fluorescein angiography (pre-test and days 14 and 30), and histopathologic examination.

Ophthalmoscopic examinations: Intravitreal injection of hyaluronidase caused an anterior chamber reaction (aqueous cells and flare, fibrin) within one day of injection. Vitreous haze and precipitates peaked on day 4 post the injection and followed a waning course to resolution by the study conclusion. Vitreous cells peaked on day 7 post the injection and declined to trace levels by study termination. In general, Lot 130B had higher scores (see table below).

Peak scores of ocular changes following the injection of 2 lots of hyaluronidase

Treatment	Lot 130B	Lot 200C
Aqueous cells/flare	2.0± 1.4	0.3± 0.8
Fibrin	0.8± 0.8	0.5± 0.5
Synechiae	2.0± 2.8	1.0± 2.0
Haze	1.5± 0.5	1.0± 0
FWP	0.7± 0 5	0.2± 0.4
GWP	0	0
Vitreous cells	3.7± 0.5	2.4± 0.5

Grading for fibrin: 0=no fibrin seen, trace: thin, delicate strand identified, 1 thick or 2-3 thin delicate strands present, 2: 2 thick or more than 3 thin delicate strands present, 3: multiple strands of various size present, 4: thick, opaque, 3-dimensional strands of fibrin present.
The same score system was used in other toxicity studies submitted in this NDA.

The fluorescein angiography examination: Small amount of leakage was noted on day 14 in one Lot 200C treated eye. The leakage was resolved by day 30. No other abnormality was reported.

Histopathological examination: The histopathological examination was performed on only rabbits. The sponsor indicated that 2 rabbits were dead and the eyes from one rabbit were lost. No abnormality was noted.

In conclusion, both lots of hyaluronidase produced ocular inflammatory reactions (aqueous cells/flare, fibrin, synechiae, haze, FWP, GWP, and vitreous cells) in ophthalmoscopic examinations. The inflammatory reactions in Lot 130B treated eyes were more severe. No remarkable toxicity was noted in fluorescein angiographic and histopathologic examinations.

Reviewer's comments: The sponsor did not indicate the causes of death in 2 rabbits. The eye samples from the 3rd animal were lost. This study was not well conducted.

Probe study: Comparison of current and proposed low dose preparations for Vitrase including a low phosphate arm. Vol. 7, Page 081

Key study findings: The proposed formulation with 20-fold increase in lactose did not increase the ocular toxicity.

Study No: ViIT06-12-97
Conducting laboratory and location: [redacted]

Date of study initiation: July 24, 1997
Date of report: October 2, 1997
GLP compliance: No

The purpose of this study was to compare the relative safety of two formulations of hyaluronidase (Lot #: 200C) following a single intravitreal injection (100 iu/50 µl, current formulation-right eyes and single use formula-left eyes) to five Dutch belted rabbits. The rabbits were examined with slit lamp biomicroscopy and indirect ophthalmoscopy (days 1, 4, 7, 15, 21 and 32 post the injection), fluorescein angiography (pre-test and days 15 and 30 post the injection), and histopathology (eyes only).

Formulations used in Study VIT 06-12-97

Ingredients (mg)	Current formulation	Proposed single use formulation
Hyaluronidase (ovine)	0.9	0.9
Lactose monohydrate NF/EP	5	[redacted]
Potassium phosphate, monobasic, NF/EP	1.22	1.22
Potassium phosphate, dibasic, USP/EP	1.92	1.92
0.9% NaCl for injection USP	3 ml	3 ml

Ophthalmoscopic examinations: Waning course of aqueous cell and flare, fibrin, vitreous haze, formation of precipitates was noted in both groups. Peak scores for vitreous cells and haze were higher in high lactose group (see table below). However, in general, no toxicologically significant differences were noted in the eyes treated with two different formulations.

Peak scores of ocular changes following the injection of 2 formulations of hyaluronidase

Treatment	Current formulation	Days of peak score noted	Proposed formulation	Days of peak score noted
Aqueous cells/flare	2.8± 1.3	4	2.6± 0.5	4
Haze	2.0± 1.2	7	3.0± 0	4
FWP	1.0± 1.0	4	2 0± 1.4	4
GWP	0 6± 0.9	21	0.2± 0.4	21
Vitreous cells	2.4± 1.3	7	3 2± 0 8	4

Fluorescein angiography examination: The films were flawed by artifact caused by white light leaking. The sponsor indicated that the angiograms appeared "largely normal" with the exception that one eye in the single use group had minimal leakage on day 30.

Histopathological examination results were not available because of the significant processing and fixation artifact.

In conclusion, the proposed formulation with 20-fold increase in lactose did not increase the ocular toxicity profile. This study was not complete.

Reviewer's comments: The sponsor indicated the leakage observed in the angiographic examination was in "current" group in study report. However, the angiography report by [redacted] showed the leakage was in the single use group. In both angiography and histopathology examinations, artifact was noted that precluded normal examination. The sponsor also mentioned in the summary that there was an arm of 10-fold decrease in phosphate. However, no data were provided. This study was not well performed.

Probe study: Comparison of current and single use formulation for Vitrase manufactured lots. Vol. 7, Page 095

Key study findings: For the eyes treated with different formulations of hyaluronidase, similar findings were noted in ophthalmoscopic and angiographic examinations.

Study No: VIT 09-02-97
Conducting laboratory and location: [redacted]

Date of study initiation: September 2, 1997
GLP compliance: No

The purpose of this study was to compare the ocular toxicity of two formulations of hyaluronidase following a single intravitreal injection (75 U/50 µl, current formulation vs single use formula) to 10 Dutch belted rabbits (one formulation/eye, two formulations/animal). The difference of the two formulations was the lactose concentration. The rabbits were examined with slit lamp biomicroscopy and indirect ophthalmoscopy (pre-test and days 1, 4, 7, 14, 21 and 29 post the injection), fluorescein angiography (pre-test and days 15 and 30 post the injection), and histopathology (eyes only).

Formulations used in Study VIT 09-02-97

Ingredients (mg)	Single use formulation	Current formulation
Lot #	PHH-09-01C	PHH-07-05
Hyaluronidase (ovine)	450 U	480 U
Lactose monohydrate NF/EP	10	0.4
Potassium phosphate, monobasic, NF/EP	0.098	0.098
Potassium phosphate, dibasic, USP/EP	0.154	0.154
0 9% NaCl for injection USP	0 3 ml	0.3 ml

Ophthalmoscopic examinations: The magnitude of the ocular findings including aqueous cells/flare, haze, fluffy white precipitates, and vitreous cells was identical across time. The two formulations shared the same ocular toxicity profile.

Peak scores of ocular changes following the injection of 2 formulations of hyaluronidase

Treatment	Current formulation	Days of peak score noted	Single use formulation	Days of peak score noted
Aqueous cells/flare	0 9± 1 0	7	0.5± 0 5	7
Synechiae	2.1± 3 3	4	1.8± 3 2	4
Haze	2.1± 1.2	7	1 8± 0 6	7
FWP	1 1± 1 6	4	0.6± 0.8	4
Vitreous cells	2.4± 0 9	7	2.4± 0.7	7

Fluorescein angiography examination: The films were flawed by artifact caused by white light leaking. The sponsor indicated that the angiograms appeared "largely normal" with the exception that 2/10 eyes in each group had trace leakage on day 30.

Histopathological examination: Severe retinal detachment and degeneration was noted in the left eye in one rabbit. In another rabbit, questionable mild multifocal detachment of the retina with no significant changes in overlying retina was noted in the right eye. In the 3rd animal, mild focal perivascular mononuclear cell infiltration in choroid was noted in left eye. The sponsor did not mention what treatment was received for each eye. The sponsor indicated that some globe trauma (rupture/questionable detachment) occurred during enucleation.

In conclusion, for the eyes treated with different formulations of hyaluronidase, similar findings were noted in ophthalmoscopic and angiographic examinations.

Reviewer's comments: This study was not well performed:

The sponsor indicated that the animals were mistakenly dosed for only 25 U on day 1. On day 4, the animals were dosed for another 25 U.

No treatment was indicated in histopathological examination. In both angiography and histopathology examinations, artifact was noted that precluded normal examination.

VIT 03-11-98: Comparison of biozyme hyaluronidase Lot 130B vs. Lot 224B in vitreous model. Vol. 7, Page 110

Key study findings: Lot 224B and Lot 130B caused similar ocular inflammatory responses.

Study No: VIT 03-11-98

Conducting laboratory and location: [redacted]

Date of study initiation: March 24, 1998
Date of final report: November 13, 2001
GLP compliance: No
Animal: 7 male New Zealand Red rabbits
Route: Intravitreal injection
Dosage: 100 iu/50 µl, single dose (1 lot/eye, 2 lots/animal)
Drug: Hyaluronidase (Biozyme Lot#: 224B/PHG-03-05 and Lot#: 130B/PHG-03-05)

Lot 130B was raw materials from a harvest of ovine testes in 1985. The old clinical and nonclinical studies were performed with this lot. The sponsor has prepared several new lots of raw material including Lot 224B from ovine testes in [redacted] Lot 224B was chosen to be used in phase III clinical trials. The purpose of this study was to compare the ocular toxicity of two lots of hyaluronidase following a single intravitreal injection (100 iu/50 µl) to seven New Zealand Red rabbits. The rabbits were examined with slit lamp biomicroscopy and indirect ophthalmoscopy (pre-test and days 1, 3, 7, 10, 14, 21 and 30 post the injection), fluorescein angiography (pre-test and days 14 and 30 post the injection), and histopathology (eyes only).

Result:

Ophthalmoscopic examinations: Inflammatory responses including aqueous cells/flare, haze, vitreous cells, FWP, GWP, and iris synechiae were observed in the eyes treated with both lots of hyaluronidase. The severity for both lots was similar with the exception of aqueous cells and flare on day 1 (see table below). Lens opacity was not noted. The fibrin deposition was only noted at a very low level (Mean score = 0.1429) in Lot 130B group on the 1st day post-injection.

Positive findings for ophthalmoscopic examinations (mean score)

	Cells and flare		Vitreous haze		Vitreous cells		FWP	
Day	224B	130B	224B	130B	224B	130B	224B	130B
1	0.1429	1.5714	1.7143	2.0	0.1429	0.7143	0.5714	0.4286
3	0.7143	0 8571	1.4286	1.7143	1.2857	0.8571	0.7143	0.7143
7	0.1429	0	1.7143	1 5714	2.1429	2.0	0.4286	0.1429
10	0	0	1.7143	1 2857	2.1429	2.4286	0	0
14	0	0	0.8571	1.1429	1.5714	1.7143	0	0
30	0.1429	0	0.5714	0.2857	1.4286	1.4286	0	0
	GWP		Iris synechiae					
Day	224B	130B	224B	130B				
1	0	0	0	0.4286				
3	0	0	0.1429	0				
7	0.4286	0.4286	0.1429	0.1429				
10	0.2857	0.1429	1 1429	0.5714				
14	0.1429	0.1429	0.5714	0				
30	0	0 1429	0.1429	0.7143				

Fluorescein angiography examination: All fluorescein angiography examinations were within normal limits.

Histopathological examination: The results are summarized in the table below. Retinal separation or retinal changes characterized by minimal to moderate, multifocal vacuolar changes in both groups (5/group) were reported by 1 pathologist. Due to these findings, the tissues were

subjected to multiple examinations from multiple histopathologists. The overall opinion was that the retinal findings were the results of processing artifacts and fixation in 3% glutaraldehyde.

Incidence of histopathologic findings

	130B	224B
Optic nerve, eyes checked	6	7
--vacuolar change	1 trace, 4 mild, 1 moderate	7 mild
Retina, eyes checked	7	7
--vacuolar change	6 mild, 1 moderate	6 mild, 1 moderate

In summary, seven male New Zealand Red rabbits were intravitreally treated with two lots of hyaluronidase (each lot per eye, 100 iu/50 µl) and were observed for 30 days. Ophthalmoscopic examinations showed similar manner of inflammatory responses in the eyes treated with both lots of hyaluronidase. All fluorescein angiographic examinations were within normal limits. Histopathologic examinations showed retinal changes (vacuolar changes) in both groups. The sponsor indicated that the changes were due to processing artifacts and fixation. In conclusion, Lot 130B and 224B had similar responses after intravitreal injection to rabbits.

Probe study: Comparison of intravitreal injections of biozyme hyaluronidase Lot 130B vs Lot 224B with negative controls in rabbits. Vol. 7, Page 179

Key study findings: Lot 224B was safer than or at least equal to Lot 130B regarding ocular toxicity in this study.

Study No: VIT 05-07-98II
Conducting laboratory and location: [redacted]

Date of study initiation: June 15, 1998
Date of report: July 20, 1998
GLP compliance: No

The purpose of this study was to compare the ocular toxicity of two lots of hyaluronidase following a single intravitreal injection (75 iu/50 µl, Lot #s: 130B/PHH-03-05 and 224B/PHI-05-06, 6/group, both eyes) to male Dutch Cross rabbits. The study was conducted with 3 negative controls: vehicle, 0.9% NaCl injection, USP, and no treatment (7, 7 or 3 animals/group, respectively). The rabbits were examined with slit lamp biomicroscopy and indirect ophthalmoscopy (pre-test and days 1, 4, 7, 11, 14, and 30 post the injection), fluorescein angiography (pre-test and days 14 and 30 post the injection), and histopathology (eyes only).

Study Design

Group	Treatment	Lot	Dose	Volume	N of animals
1	Hyaluronidase	130 B	75 iu	50 µl	6
2	Hyaluronidase	224B	75 iu	50 µl	6
3	Vehicle			50 µl	7
4	PSS			50 µl	7
5	No treatment				3

Ophthalmoscopic examinations: Similar to the results found in other studies, inflammatory responses were noted. These responses were reversible. At the end of the study

(day 30), all of the positive observations were improved or disappeared. Lot 130B caused more serious responses in almost all the categories (see table below).

Peak scores of ocular changes following the injection of 2 lots of hyaluronidase

Treatment	Lot 130B	Lot 224B	Vehicle	saline
Aqueous cells/flare	2.83	0.50	0	0.71
Fibrin	0 33	0	0.57	0
Synechiae	0.17	0	0	0.29
Haze	2.00	1.67	0 86	0.86
FWP	1.50	0.33	0	0.14
Vitreous cells	3.83	4	1 29	1.43

Fluorescein angiography examination: The films were flawed by artifact caused by white light leaking. The sponsor indicated that at least 50% of the vascular field was visible and no abnormality was noted.

Histopathological examination: Minimal to mild mononuclear cell infiltration at the limbus was noted in many animals in all groups. The sponsor indicated that this was a common finding in rabbit eyes. Minimal perivascular mononuclear cell infiltration was noted in both eyes of one animal.

In conclusion, Lot 224B was safer than or at least equal to Lot 130B regarding ocular toxicity in this study.

Reviewer's comments: No treatment was indicated in histopathological examination. In angiography examination, artifact was noted. This study was not well performed.

Investigation into histopathological abnormalities in Vitrase studies. Vol. 7, Page 202

Key study findings: The histopathological findings (retinal change or retinal degeneration) in some toxicity studies were a consequence of fixation and/or processing artifact.

In the previous toxicity studies with Vitrase, histopathological examinations had occasionally documented findings as "retinal change", "retinal degeneration", "retinal nuclear displacement" or "photoreceptor displacement". The sponsor reviewed and summarized histopathological data from mid 1998 to mid 1999 (see table below). Based on the table, the sponsor indicated that the overall incidence was 16.3% in hyaluronidase injected eyes, 37.5% in saline/vehicle injected eyes, and 40.0% in naïve eyes. The sponsor concluded that the histopathological findings represented a consequence of fixation and/or processing artifact. The findings were not drug- or procedure-related.

Retinal Pathology Summary

Study	# eyes examined: HAse	# eyes examined: Injected Ctrl	# eyes examined: No Rx	# eyes + retinal lesions: HAse	# eyes + retinal lesions: Injected Ctrl	# eyes + retinal lesions: No Rx	Action Taken	New Findings	Comments
Vit02-17-97	12	0	0	3	0	0	3 positive and 1 control section sent to Schmidt (I couldn't locate all + sections)	lesions felt to probably represent artifact, different from findings in later studies	
Vit04-17-97	6	0	0	0	0	0	none		
Vit06-12-97	10	0	0	0	0	0	none		significant fixation artifact
Vit09-02-97	20	0	0	0	0	0	none		
Vit12-12-97	15	5	0	0	1	0	5 control blocks sent for deep cuts	1/5 control blocks positive for photoreceptor displacement	
Vit02-26-98	10	0	5	1	0	5	5 naive blocks sent for deep cuts	5/5 naive eyes positive for photoreceptor displacement	
VC03-11-98	12	0	0	7	0	0	Sent to 2 other pathologists with untreated controls from Vit02-26-98	Opinions differed as to the cause of the widespread vacuolar change	Safety study repeated
Vit05-07-98II	12	14	6	0	0	0	Control blocks deep cut	no retinal lesions found in deep cuts of controls	
Vit05-07-98C	14	14	6	9	8	0	Blocks from untreated controls and fellow eyes of rabbits in which one eye was + sent for deep cuts	No change from original report	
P0798018	12	14	6	0	0	0	All blocks sent for deep cuts to investigate few mononuclear cells, no retinal lesions found		
TOX-07-98961X	18	18	0	3	5	0	7 control blocks deep cut. Remaining 11 blocks sent for deep cuts	No other lesions found in 7 blocks. 5/11 of the remaining control blocks positive for photoreceptor dislocation	No real dose response (1/6 75IU, 0/0 150IU, 2/6 750IU)
RDS04-14-99	0	7	7	0	13	7			
TOTALS	141	72	30	23	27	12			

Overall incidence in hyaluronidase injected eyes is 16.3%.
Overall incidence in saline/vehicle injected eyes is 37.5%.
Overall incidence in naive eyes is 40.0%.

Summary and conclusion:

A number of toxicity studies were included in this NDA submission and relatively consistent toxicity results were observed in all studies. The studies were conducted with slit lamp biomicroscopic, indirect ophthalmoscopic, angiographic and histopathologic examinations. Inflammatory responses in ocular tissues, evidenced by aqueous cells and flare, FWP, GWP, synechiae, vitreous cells and haze, were noted in most studies. ERG changes (lowered amplitude and increased latency) for a- and b-waves and decreased IOP levels after the intravitreal injection were also observed. These changes were considered to be related to the inflammatory reactions in the intraocular tissues. Retinal changes (retinal degeneration) were noted in several studies. The

ocular inflammation was reversible. In several studies, retinal disruption/detachment, microaneurysms, vitreous membrane, papillitis and hyalitis were noted in angiographic and histopathologic examinations. The sponsor concluded that the histopathological retinal findings represented a consequence of fixation and/or processing artifact, and were not drug- or procedure-related. The reviewer believes that some changes might possibly be procedure-related. Positive results were seen in 2 of 6 monkeys following 2 intravitreal injections in an analysis for the presence of hyaluronidase-specific antibodies. No adverse effects were noted in association with the presence of the antibodies. The clinical relevance of these results is unknown.

IX. DETAILED CONCLUSIONS AND RECOMMENDATIONS:

Conclusions: Hyaluronidase is an endogenous enzyme that hydrolyzes intercellular matrix substance. Hyaluronidase has been approved by the agency for the aid in the absorption and dispersion of injected drugs. The recommended dose is 150 U of hyaluronidase added to the vehicle containing the drug. Doses of 50 to 1500 U have been used as an aid in the resolution of hematomas, transudates, and edema. It is reported that purified hyaluronidase has little toxicity. No cardiovascular, capillary, or renal effects have been reported following single large doses. Skin hypersensitivity is rare and it has low antigenicity. Based on this information, systemic toxicity is not a concern in the development of Vitrase.

In this NDA submission, the sponsor is trying to develop the enzyme (from ovine testes) as a drug for the treatment of vitreous hemorrhage. In pharmacologic studies with the rabbit vitreous clot model, hyaluronidase was effective on clot clearance, optic nerve and retinal clarity, and red reflex in a dose-dependent manner. In toxicity studies, temporary inflammatory reactions were observed following intravitreal injection of hyaluronidase. The inflammatory response to Vitrase may facilitate clot clearance.

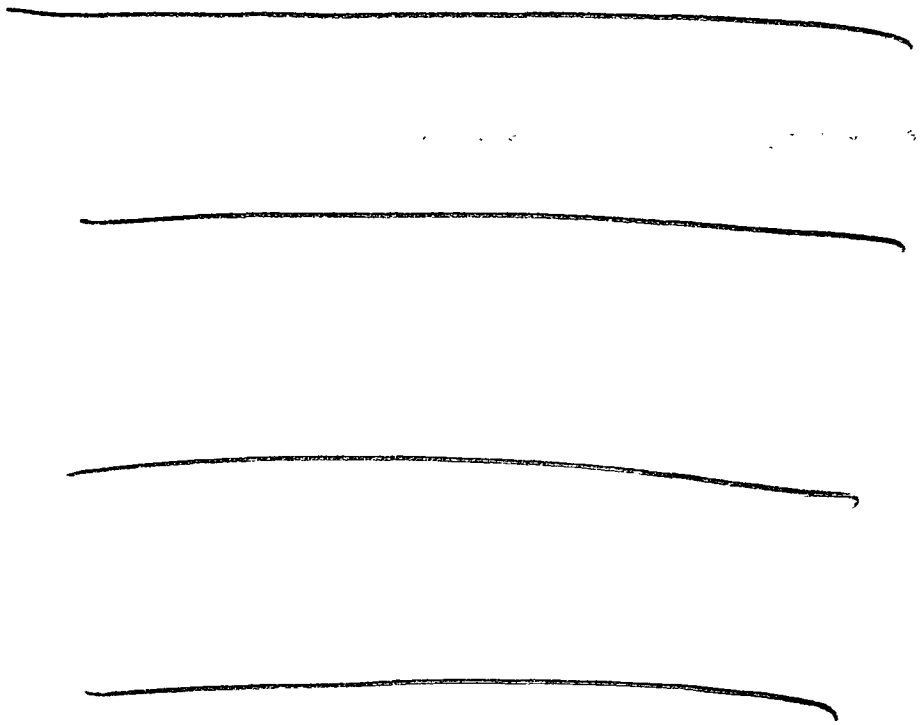
Based on the nonclinical study results, it is concluded that Vitrase was effective on the treatment of vitreous hemorrhage in the animal model, and reversible ocular inflammatory responses occurred following intravitreal injection of Vitrase.

The reviewer talked with Dr. Jennifer Harris, Medical Officer at HFD-550, about the ocular inflammation following the intravitreal injection. Both Dr. Harris and the reviewing pharmacologist considered that the inflammation resulted from the intravitreal injection, and the inflammation might facilitate the treatment of vitreous hemorrhage.

General Toxicology Issues: Acute inflammatory reactions were observed in the ocular tissues following the intravitreal injection of Vitrase. The reactions were expected, and reversible. Retinal changes were noted in several studies. It is not determined whether the retinal changes were drug-related. Positive results were seen in 2 of 6 monkeys following 2 intravitreal injections in an analysis for the presence of hyaluronidase-specific antibodies. The clinical relevance of these results is unknown.

Recommendations:

Labeling with basis for findings:



X. APPENDIX/ATTACHMENTS:

Addendum to review: No

Other relevant materials (Studies not reviewed, appended consults, etc.): No

Any compliance issues: No

This is a representation of an electronic record that was signed electronically and this page is the manifestation of the electronic signature.

/s/

Zhou Chen
2/14/03 03:12:40 PM
PHARMACOLOGIST

Josie, Please sign this review. Thanks. Zhou

Josie Yang
2/20/03 09:35:55 AM
PHARMACOLOGIST