CENTER FOR DRUG EVALUATION AND RESEARCH

APPLICATION NUMBER:

21-859

PHARMACOLOGY REVIEW

DEPARTMENT OF HEALTH AND HUMAN SERVICES
PUBLIC HEALTH SERVICE
FOOD AND DRUG ADMINISTRATION
CENTER FOR DRUG EVALUATION AND RESEARCH

PHARMACOLOGY/TOXICOLOGY REVIEW AND EVALUATION

NDA NUMBER:	**21-859**
SERIAL NUMBER:	000
DATE RECEIVED BY CENTER:	3/24/05
DRUG NAME:	**Enhanze SC™** [Recombinant human hyaluronidase, recombinant human PH20 (**rHuPH20**)]
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
SPONSOR:	Halozyme Therapeutics, Inc., 11588 Sorrento Valley Road, #17, San Diego, CA 92121 Tel: 858-794-8889x208; Fax: 858-259-2539
DOCUMENTS REVIEWED:	"Pharmtox" part of the submission
REVIEW DIVISION:	Division of Anti-Infective and Ophthalmology Drugs (HFD-520)
PHARM/TOX REVIEWER:	Zhou Chen, MD, PhD
PHARM/TOX SUPERVISOR:	Robert Osterberg, PhD
DIVISION DIRECTOR:	Janice Soreth, MD
PROJECT MANAGER:	Lori Gorski

Date of review submission to Division File System (DFS): June 14, 2005

TABLE OF CONTENTS

EXECUTIVE SUMMARY

I. Recommendations

A. Recommendation on approvability

This application is approvable from a nonclinical perspective with some minor modifications of labeling as revised in the "Clinical Pharmacology" section, "Carcinogenesis, Mutagenesis, Impairment of Fertility" section and "Pregnancy" section.

B. Recommendation for nonclinical studies

No recommendation is necessary.

C. Recommendations on labeling

Minor modifications of labeling in the "Clinical Pharmacology" section, "Carcinogenesis, Mutagenesis, Impairment of Fertility" section and "Pregnancy" section (see Labeling Review) are recommended.

II. Summary of nonclinical findings

A. Brief overview of nonclinical findings

Three pharmacology studies were conducted by the sponsor. rHuPH20 demonstrated equivalent diffusion efficacy and dermal barrier reconstitution to the USP hyaluronidase reference standard in nude mice. The ability to achieve and sustain akinesia was also demonstrated when rHuPH20His6, a modified form of rHuPH20, was co-injected (by peribulbar injection) with local anesthetics in monkey eyes.

In a systemic toxicity study conducted in SD rats, no toxicologically significant abnormal findings were noted following iv administration of the drug at 10500 Units/kg. Histopathological examinations showed slight renal tubule dilatation in all 5 drug treated males with amorphous material in the lumina consistent with hyaline casts. Similar changes were not seen in female and control animals. In two toxicity studies conducted in monkeys via both peribulbar and sc injections, the drug was well tolerated. In ophthalmologic examinations, minimal to moderate (score = 1 or 2) conjunctival congestion, swelling, and/or discharge were seen in both control and treated animals with similar incidence and severity. These changes were reversible and not considered as toxicologically significant.

B. Pharmacologic activity

Hyaluronidase is a naturally occurring family of enzymes that hydrolyze hyaluronic acid. In this NDA submission, the sponsor is developing Enhanze SC for the same indications as Wydase, a DESI drug product prepared from bovine testes [Federal Register Vol. 35, No 185, p14800-14801 for hyaluronidase (Wydase, NDA 6-343)]. Wydase was marketed in the United States for many years and the drug was withdrawn for reasons unrelated to safety and efficacy. Enhanze SC contains a neutral pH active human hyaluronidase, rHuPH20, a 447 amino acid single chain polypeptide with N-linked glycosylation structures

that is generated by recombinant DNA technology in Chinese Hamster Ovary (CHO) cells. The proposed route of delivery and clinical dose (150 Units) for the Enhanze SC™ formulation are the same as those for Wydase (150 Units, subcutaneous injection).

C. Nonclinical safety issues relevant to clinical use

There are no drug-related safety issues relevant to clinical use.

2.6 PHARMACOLOGY/TOXICOLOGY REVIEW

2.6.1 INTRODUCTION AND DRUG HISTORY

NDA number: NDA 21-859
Review number: 000
Sequence number/date/type of submission: 000/March 18, 2005/Commercial
Information to sponsor: Yes (X) No ( )
Sponsor and/or agent: Halozyme Therapeutics, Inc., 11588 Sorrento Valley Road, Suite 17, San Diego, CA 92121
Manufacturer for drug substance: [redacted]

Reviewer name: Zhou Chen
Division name: Division of Anti-Infective and Ophthalmology Drugs
HFD #: 520
Review completion date: June 14, 2005

Drug:

Trade name: **Hylenex (recombinant human hyaluronidase injection)**
Generic name: Recombinant human hyaluronidase
Code name: rHuPH20
Chemical name: 36-482-Hyaluronoglucosaminidase PH20 (human)
CAS registry number: 757971-58-7
Molecular formula/molecular weight: $C_{2327}H_{3565}N_{589}O_{667}S_{20}$, MW: [redacted]
Structure:

The drug substance, rHuPH20, is a 447 amino acid single chain polypeptide with N-linked glycosylation structures.

Relevant INDs/NDAs/DMFs: IND 66,888

Drug class: Human recombinant protein

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 the resorption of radiopaque agents

Targeted clinical formulation:

Ingredient	Quality Standard	Function	Unit Formula 1-mL vial (mg)
rHuPH20 (recombinant human hyaluronidase)	In house		(~150 U)
Sodium Phosphate Dibasic Dihydrate	USP, Ph. Eur.		1.78
Sodium Hydroxide	NF, Ph. Eur., JP		
Albumin (Human)	USP, Ph. Eur.		1.0
Calcium Chloride Dihydrate	USP, Ph. Eur.		0.40
Sodium Chloride	USP, Ph. Eur., JP		8.5
Edetate Disodium Dihydrate	USP, Ph. Eur.		1.0
Water for Injection	USP, Ph. Eur.		qs to 1.0 ml

Route of administration: Subcutaneous injection

Proposed use: 150 USP units, single injection

Studies reviewed within this submission:

Pharmacology:

R03001: An efficacy study of optiphase in cynomolgus monkeys
R03002: Trypan blue diffusion assay test
R03003: Dermal reconstitution following administration of rHuPh20

PK:

No PK studies were submitted.

Toxicology:

Single dose studies

R03005: A preliminary IV acute toxicology study in rats with OptiphaseTM

Repeated dose studies

RDH00007: Pilot ascending dose peribulbar and subcutaneous tolerability study in rhesus monkeys
RDH00006: A single/repeat dose toxicity study of hyaluronidase administered by peribulbar and subcutaneous injection to rhesus monkeys, with a 3-week recovery period

Studies <u>not</u> reviewed within this submission:

MC04373: Method validation report: Validation of a method for the qualitative detection of nAbs in primate serum using [redacted] detection
MC04552: Method validation report: Validation of a method for the qualitative detection [redacted] activity in primate serum using [redacted] detection

2.6.2 PHARMACOLOGY

2.6.2.1 Brief summary

Three pharmacology studies were conducted by the sponsor. The studies were designed to demonstrate the efficacy of rHuPH20 in various presentations. Study R03001 demonstrated the ability to achieve and sustain akinesia (nerve block) when a modified form of rHuPH20, rHuPH20His6, was co-administered (by peribulbar injection) with lidocaine and bupivacaine in monkey eyes. rHuPH20 demonstrated equivalent diffusion efficacy and dermal barrier reconstitution to the USP hyaluronidase reference standard in nude mice. These studies provide evidence of rHuPH20's biological equivalence.

2.6.2.2 Primary pharmacodynamics

R03001: An efficacy study of Optiphase in cynomolgus monkeys

The purpose of this study was to test the efficacy of rHuPH20His6 (Optiphase) by determining the time to and duration of akinesia (complete inhibition of movement of the eyeball and associated musculature) following administration of peribulbar anesthesia in male cynomolgus monkey eyes.

rHuPH20His6 was an early preparation of rHuPH20 with histidine tagged to facilitate purification. rHuPH20His6 was a research and development batch.

Local anesthetic of lidocaine (2%) and bupivacaine (0.5%) were mixed with the test or control compound at 4.5:4.5:1 (final volume =2 ml). Complete akinesia was observed at Optiphase OP500 (500 U/ml hyaluronidase) and OP300 (300 U/ml hyaluronidase) which were the highest and second highest dose concentrations of the Optiphase. Akinesia was sustained up to periods greater than 60 min. In one eye injected with OP300 and one eye with OP500, akinesia was observed immediately following peribulbar administration. Only partial block to eye movement was observed with the injection of OP150 (150 U/ml hyaluronidase), with carrier control or with Bovine Compounding Pharmacy Hyaluronidase CP150 (150 U/ml hyaluronidase produced by Compounding Pharmacy).

Summary of time to and duration of akinesia in monkeys treated with hyaluronidase

Treatment	Animal #	Eye	Time to akinesia	Duration of akinesia
OP300	**20997**	Left	Immediately	> 60 min
	20945	Right	4 min	11 min
OP500	**20997**	Right	Immediately	> 20 min
	20991	Left	4 min	26 min

In conclusion, OP300 and OP500 showed the greatest potential for enhancing spread of the local anesthetic to accomplish an extended period of akinesia, even though there was no clear indication as to the time to akinesia.

R03002: Trypan blue diffusion assay test

The purpose of this study was to compare rHuPH20, a recombinant CHO derived human hyaluronidase, to a bovine testes-derived USP hyaluronidase for the spreading ability (spreading factor activity) in nude mice. Study design is summarized in the table below. Each test article was diluted 1:10 with trypan blue and injected subcutaneously into female nude balb/c mice. Measurement of the dye areas was taken at 2.5, 5, 10, 15 and 20 min post injection.

Study design

Test article	Number of sites	Final dose (Units/ml)	Dose volume (µl)	Total dose (USP Units)
rHuPH20	3	100	50	5
rHuPH20	3	10	50	0.5
rHuPH20	3	1	50	0.05
USP hyaluronidase	3	100	50	5
USP hyaluronidase	3	10	50	0.5
USP hyaluronidase	3	1	50	0.05
Vehicle	4	0	50	0

Results are summarized in the table below. rHuPH20 showed a dose-dependent spreading activity similar to that of the USP enzyme. Both enzymes at doses ≥ 10 Units/ml induced a significantly increased diffusion over control injections. In conclusion, USP and rHuPH20 hyaluronidase enzymes showed equivalent activity in the mouse trypan blue diffusion assay.

Summary of dye area

Dose	Time (min)	OPTIPHASE™ Mean Area (mm2)	SD	USP Mean Area (mm2)	SD	Carrier Mean Area (mm2)	SD
100U/ml	2.5	80.5	13.0	54.9	5.2	37.0	7.4
	5.0	88.5	9.5	83.3	7.2	44.6	7.3
	10.0	80.2	11.7	84.6	23.1	47.7	6.3
	15.0	99.3	14.4	92.4	32.4	52.2	4.7
	20.0	108.8	18.6	115.3	39.1	56.7	7.4
10U/ml	2.5	44.2	9.3	42.7	7.5		
	5.0	51.4	5.0	53.0	8.6		
	10.0	62.8	2.0	63.5	10.0		
	15.0	68.4	5.7	72.2	7.6		
	20.0	76.0	6.9	76.4	8.6		
1U/ml	2.5	38.7	5.4	33.9	5.3		
	5.0	46.9	3.7	44.9	5.4		
	10.0	53.3	1.7	51.9	3.6		
	15.0	59.2	1.4	57.8	1.2		
	20.0	68.5	1.2	68.3	2.3		

R03003: Dermal reconstitution following administration of rHuPh20

The purpose of this study was to demonstrate the transient nature of the spreading factor activity of recombinant CHO derived human hyaluronidase, rHuPH20, compared to a bovine testes-derived USP hyaluronidase and control in nude female balb/c mice. Study design is summarized in the table below. Six sites were utilized for each test article and four for the carrier control article. Test article and carrier control article was injected into 2 and 3 animals, respectively. The same article was injected intradermally on opposing lateral sides of each animal. Measurement of the dye area was calculated 5 and 15 min from dye injection. Dye (trypan blue) was injected at 0.5, l, 24 and 48 hours post enzyme or carrier control injection.

Study design

Test article	Number of sites	Final dose (Units/ml)	Dose volume (µl)	Total dose (USP Units)
rHuPH20	6	100	50	5
USP hyaluronidase	6	100	50	5
Control	4	0	50	0

Results are summarized in the table below. Both rHuPH20 and USP hyaluronidase induced significantly increased diffusion at 30 and 60 min post injection. There was no significant difference between these two groups. The spreading activity completely diminished to control levels after 24 hr and 48 hr. In conclusion, USP and rHuPH20 hyaluronidase enzymes showed equivalent activity in the mouse dermal reconstitution assay. Dermal diffusion subsided in both enzyme treated groups within 24 hr of administration. Dermal reconstitution was seen in all enzyme injected sites after 24 hr.

Summary of dye area in mice treated with hyaluronidase and trypan blue (mm^2, mean ± SD)

Time (hr after test article or control)	rHuPH20	USP hyaluronidase	Vehicle control
5 min after trypan blue			
0.5	86.21±10.5	86.02±10.8	39.73±7.49
1	79.52±13.3	83.92±6.06	37.86±4.89
24	39.65±8.06	48.75±8.83	42.58±12.7
48	47.83±14.3	43.49±7.56	49.18±6.84
15 min after trypan blue			
0.5	114.2±14.9	116.9±22.2	51.52±11.3
1	101.9±11	108.7±8.53	46.67±10.7
24	53.56±5.16	62.46±6.28	56.5±16.6
48	59.33±18.8	58.02±8.93	66.51±14.2

2.6.3 PHARMACOKINETICS/TOXICOKINETICS

No PK studies were provided by the sponsor.

2.6.4 PHARMACOKINETICS TABULATED SUMMARY

No tabulated summary was provided by the sponsor.

2.6.5 TOXICOLOGY

2.6.5.1 Overall toxicology summary

In a systemic toxicity study conducted in SD rats, animals were treated with a single iv dose of rHuPH20 at 10500 Units/ml/kg and were observed for 14 days. No drug-related abnormal findings in clinical observations, body weights, and necropsy examinations were noted. Histopathological examinations showed slight renal tubule dilatation in all 5 drug treated males with amorphous material in the lumina consistent with hyaline casts. Similar changes were not seen in female and control animals.

Two toxicity studies in monkeys were performed via both peribulbar and sc injections. In both studies, the drug was well tolerated. In ophthalmologic examinations, minimal to moderate (score = 1 or 2) conjunctival congestion, swelling, and/or discharge were seen in both control and treated animals with similar incidence and severity. These changes were reversible and not considered as toxicologically significant. No systemic toxicity findings were noted.

2.6.5.2 Single-dose toxicity

R03005: A preliminary IV acute toxicology study in rats with OptiphaseTM

Key study findings: Histopathological examinations showed slight renal tubule dilatation in all 5 drug treated males with amorphous material in the lumina consistent with hyaline casts. Similar changes were not seen in female and control animals.

Report N^{o}: R03005
Compound: CHO derived rHuPH20
Dose: 10500 Units/ml/kg
Dosing regimen: Single dose
Route: Intravenous
Animal: Sprague Dawley rats (150-175 g, 1/sex for vehicle control and 5/sex for test article groups)
Study site:
Study initiation: 11/11/2003
GLP: No

The purpose of this study was to determine the potential toxicity of rHuPH20 following a single iv injection in rats. The day of dosing was designated as Day 0. Animals were observed for 14 days after dosing. Toxicity was assessed as shown below.

Observation and Times:

Clinical signs: Daily
Body weights: Days 0, 7 and 14
Necropsy: Day 14
Histopathology: The following organs from all animals were examined histopathologically: liver, lung, spleen, kidney, brain and skin.

Results:

Clinical observations: No toxicologically significant abnormal findings were noted.

Body weights: No toxicologically significant changes were noted.

Necropsy: There were no drug-related abnormal findings.

Histopathological examinations: Slight renal tubule dilatation was noted in all 5 drug treated males with amorphous material in the lumina consistent with hyaline casts. Similar changes were not seen in female and control animals. In addition, the dose used in this study was high (10500 Units/ml/kg). The toxicological significance was not determined. No treatment-related changes were noted in the other organs.

Summary and conclusion: SD rats were treated with a single iv dose of rHuPH20 at 10500 Units/ml/kg and were observed for 14 days. No drug-related abnormal findings in clinical observations, body weights, and necropsy examinations were noted. Histopathological examinations showed slight renal tubule dilatation in all 5 drug treated males with amorphous material in the lumina consistent with hyaline casts. Similar changes were not seen in female and control animals. In addition, the dose used in this study was high (10500 Units/ml/kg). The toxicological significance was not determined.

2.6.5.3 Repeated-dose toxicity

RDH00007: Pilot ascending dose peribulbar and subcutaneous tolerability study in rhesus monkeys

Key study findings: Peribulbar and subcutaneous injections of rHuPH20 at doses up to 4500 U in monkeys were well tolerated with only minimal to mild conjunctival swelling, erythema and discharge in HD (45000 U) animals at 24 hr after dosing. A NOAEL was considered as 4500 U for peribulbar injection and 45000 U for subcutaneous injection, respectively.

Study no.: RDH0007
Conducting laboratory and location: C

3

Date of study initiation: Not indicated
GLP compliance: Yes
QA report: yes (X) no ( )
Test article, lot #, and % purity: rHuPH20 recombinant human hyaluronidase, Lot: HUA0406CA
Control article: Vehicle (Enhanze placebo), Lot: 67-19-A1
Route: Peribulbar and subcutaneous (on the scapula) injections

Methods

Species/strain: Rhesus monkeys
Number/sex/group or time point (main study): 1/sex
Satellite groups used for toxicokinetics or recovery: No
Age: 3-5 years old
Weight: 3.1-4.7 kg
Unique study design or methodology (if any): Only one eye was treated at each time.

The purpose of this study was to determine the local tolerability of a recombinant form of hyaluronidase via peribulbar injections or subcutaneous injections in rhesus monkeys. This was a pilot dose ranging study. Two monkeys (1/sex) were treated with ascending doses of the drug as shown in the table below.

Dose (U/injection/animal)	Route	# of injection	Concentration (U/ml)	Volume/injection (ml)	Extrapolated dose* (U/kg)
150	Peribulbar	1: Day 1	150	1	38
150	sc	1: Day 1	150	1	38
4500	Peribulbar	1: Day 4	4500	1	1200
4500	sc	1: Day 4	4500	1	1200
45000	Peribulbar	1: Day 8	45000	1	12000
45000	sc	1: Day 8	45000	1	12000

*Based on an average of approximate 3.9 kg body weight

Observation and Times:
Clinical signs: Twice daily through Day 11
Body weights: Days 1, 4, 8, and 11
Serum anti-drug antibodies: Pretest and on Day 32
Ophthalmoscopy: Slit lamp biomicroscopic and indirect ophthalmologic examinations were conducted at pretreatment, 24 hr following each dose, and on Day 32.

Results:

Clinical observations: No toxicologically significant abnormal findings were noted.

Body weights: There were no effects on body weights throughout the study.

Serum anti-drug antibodies: No data were provided.

Ophthalmoscopy: No drug-related fundus or vitreous changes were noted. Slit lamp examinations showed mild congestion and swelling of the conjunctiva (score = 1) in both eyes in the male animal 24 hr after the second injection (4500 U). Since these changes were seen in both eyes (treated and untreated) in only one animal, they were not considered as drug-related, but likely secondary to handling or anesthetic recovery. Twenty-four hr after the high dose (45000 U) peribulbar injection, mild congestion, minimal swelling and mild discharge of the conjunctiva (all scores = 1) were noted in the treated eye in both animals, and were considered treatment-related. The response could be due to extremely high concentrations of the test article. These changes were fully reversible by about 48 hr after the dose.

In summary, rhesus monkeys (1/sex) were treated via peribulbar and sc injections with rHuPH20 at ascending doses of 150 U, 4500 U, and 45000 U. No abnormal findings related to sc injections were noted. For peribulbar injections, no drug-related abnormal findings in clinical observations and body weights were noted. In ophthalmologic examinations, mild congestion and swelling of the conjunctiva were seen in one

animal 24 hr after the second dose. Since these changes were seen in both eyes (treated and untreated) in only one animal, they were not considered as drug-related. Twenty-four hr after the high dose peribulbar injection, mild congestion, minimal swelling and mild discharge of the conjunctiva were noted in the treated eye in both animals, and were considered treatment-related. These changes were fully reversible by about 48 hr after the dose. A NOAEL was considered as 4500 U for peribulbar injection and 45000 U for subcutaneous injection, respectively.

RDH00006: A single/repeat dose toxicity study of hyaluronidase administered by peribulbar and subcutaneous injection to rhesus monkeys, with a 3-week recovery period

Key study findings: Single peribulbar injections in one or both eyes and once weekly subcutaneous injections (up to 2 weeks) of rHuPH20 at doses up to 38000 U in monkeys were well tolerated.

Study no.: RDH00006
Conducting laboratory and location: C

3

Date of study initiation: June 14, 2004
GLP compliance: Yes
QA report: yes (X) no ( )
Test article, lot #, and % purity: rHuPH20 recombinant human hyaluronidase (107,968 U/mg), Lot: HUA0406CA
Control article: Vehicle (placebo)

Methods

Species/strain: Rhesus monkeys
Number/sex/group or time point (main study): 4/sex
Satellite groups used for toxicokinetics or recovery: No
Age: 2.6-8.1 years old for males and 3.2-7.1 years old for females
Weight: 3.6-8.6 kg for males and 3.3-6.3 kg for females
Unique study design or methodology (if any): No

The purpose of this study was to determine the potential toxicity of a recombinant form of hyaluronidase when administered as a single or repeat dose (sc only) by peribulbar injections or subcutaneous injections to rhesus monkeys followed by a 3-week recovery period. The study design is listed in the table below. All animals were treated by peribulbar (right eye) and sc injections on Day 1. Groups 2 and 6 animals received additional doses via peribulbar (left eye) and sc injections on Day 8.

Group	n/sex	Dose (U)	Dosing regimen	Concentrations (U/ml)	Number of sacrificed animals (n/sex)		
					Day 3	Day 10	Day 29
1	4	0	Day 1	0	2	2	2
2	4	0	Days 1 and 8	0		2	2
3	4	130	Day 1	130	2	2	
4	4	3880	Day 1	3880	2	2	
5	4	38800	Day 1	38800	2	2	
6	4	38800	Days 1 and 8	38800		2	2

Observation and Times:
Clinical signs: Twice daily
Food consumption: Once daily
Body weights: Pretest, prior to dosing and necropsy

ECG: Pretest, on Day 1 (4 hr post-dose, all animals), Day 9 (Groups 1, 3, 4, and 5 animals), and Day 13/14 (Groups 2 and 6 animals)
Heart rate and blood pressure: Pretest and 4 hr post-dose on Day 1
Ophthalmoscopy: Slit lamp biomicroscopic and indirect ophthalmologic examinations were conducted at pretreatment, 24 hr following each dose, and prior to necropsy.
Clinical pathology: Days 3, 10 and 29
TK: Pretest and 30 min after dose on Day 1 (all animals), and 24 hr after dose on Days 1 and 8 (Groups 2 and 6 animals)
Serum anti-drug antibodies: Days 21 and 28
Necropsy: At termination, all animals
Organ weights: At termination, all animals (see table below)
Histopathology: The tissues and organs of all animals listed in the table below were examined histopathologically.

Adrenals*	Optic nerves	Heart*	Spinal cord
Aorta	Ovaries*	Ileum	Spleen*
Bone marrow	Pancreas	Injection site	
Bone (femur)	Parathyroid*	Jejunum	Stomach
Brain*	Pituitary*	Kidneys*	Testes*
Cecum	Prostate	Liver*	Thymus*
Cervix	Rectum	Lung*	Thyroid*
Colon	Salivary gland	Lymph nodes, axillary	Tongue
Duodenum	Sciatic nerve	Lymph nodes mandibular	Trachea
Epididymis*	Seminal vesicles	Lymph nodes, mesenteric	Urinary bladder
Esophagus	Skeletal muscle	Gall bladder	Uterus
Eye	Skin	Gross lesions	Vagina

*, organ weight obtained

Results:

Clinical observations: No toxicologically significant abnormal findings were noted.

Local effects after sc injections: No toxicologically significant effects were noted.

Local effects after peribulbar injections: Clinical signs including transient redness and/or swelling were noted under or around the dosed eye one hr after dosing in both control and drug-treated groups (see table below). The signs resolved within 24 hr after the initial dosing (Day 1, right eyes) and within 48 hr after the second dose (Day 8, left eyes). These were considered as expected results due to the volume injected at the peribulbar site. Clinical signs were less severe in hyaluronidase-treated groups.

Post-dosing observations

Group	1(control)	2(control)	3 (LD)	4 (MD)	5 (HD)	6 (HD)
N/sex	4	4	4	4	4	4
Slight swollen under right eye (1 hr post-dosing, Day 1)	2f	1m, 1f	2m, 4f	4m, 4f	3m, 2f	1m, 1f
Moderate swollen under right eye (1 hr post-dosing, Day 1)	3m, 2f	1f	1m		1m, 2f	
Severe swollen under right eye (1 hr post-dosing, Day 1)	1m	1m				
Slight swollen under left eye (1 hr post-dosing, Day 8)						3m, 3f
Moderate swollen under left eye (1 hr post-dosing, Day 8)		2m, 3f				1m, 1f
Severe swollen under left eye (1 hr post-dosing, Day 8)		2m, 1f				

Food consumption: There were no effects on food consumption throughout the study.

Body weights: There were no abnormal findings in body weights throughout the study.

ECG, HR, and BP: There were no drug-related effects on ECG, heart rate and blood pressure examinations.

Clinical pathology: No drug-related effects on hematology, clinical chemistry, and urinalysis examinations were noted.

Ophthalmoscopy: No drug-related fundus, optic disc and vitreous changes were noted. Twenty-four hr after dosing on either Day 2 or Day 9, mild to moderate conjunctival changes including congestion, swelling (minimal to mild), and/or discharge in both control (1/16 on Day 2 and 3/8 on Day 9) and HD (5/16 on Day 2 and 4/8 on Day 9) animals were observed. There were no differences regarding severity. All of the observed effects were reduced or fully resolved at the next evaluation time point. Since these effects were noted in both control and rHuPH20-treated eyes with similar severity, it was possible that these effects were caused by peribulbar injection procedure and were not toxicologically significant.

Positive findings in slit lamp examinations

		Group 2	Group 6
Day 2	Conjunctival congestion (right eye) mild, score = 1	1	2
	Conjunctival discharge, mild (right eye) score = 1	1	3
	Conjunctival discharge, moderate (right eye) score = 2		1
	Conjunctival discharge, mild (left eye) score = 1		2
	Total animals affected	1	5
Day 9	Conjunctival congestion (left eye) mild, score = 1	1	3
	Conjunctival congestion (left eye) moderate, score = 2	2	1
	Conjunctival swelling, minimal (left eye) score = 1	1	3
	Conjunctival swelling, mild (left eye) score = 2	2	1
	Conjunctival discharge, mild (left eye) score = 1	2	2
	Total animals affected	3	4
Pre-necropsy	Conjunctival congestion (left eye) mild, score = 1	1	1
	Conjunctival swelling, minimal (left eye) score = 1	1	1
	Total animals affected	1	1

Serum TK and antibody: No serum antibody and hyaluronidase activity were detected.

Necropsy: No toxicologically significant effects were noted. Local injection site findings (discolored, red, subcutaneous injection site observations) were seen in all groups with similar incidence and severity, and were not considered as drug-related.

Necropsy findings

Group	1(control)	2(control)	3 (LD)	4 (MD)	5 (HD)	6 (HD)
Day 3						
Lung, adhesion	1m, 1f			1m	1f	
Liver, accentuated lobular pattern, diffuse, minimal to mild	1m		1m, 1f			
Spleen, accentuated follicular pattern, diffuse, minimal to moderate	1f		2f	2f	2m	
Injection site, discoloration, red, subcutaneous	1f			1m, 1f	1m, 1f	
Day 10						
Lung, adhesion	1m					1m
Liver, accentuated lobular pattern, diffuse, minimal to mild			1f			
Spleen, accentuated follicular pattern, diffuse, minimal to moderate	1m, 2f	1m, 1f	1m, 1f	1f	1m, 2f	1m, 1f
Injection site, discoloration, red, subcutaneous		1m		1f		
Day 29						
Injection site, discoloration, red, subcutaneous		1m				

Organ weights: No toxicologically significant effects on organ weights were noted.

Histopathology: No toxicologically significant effects were noted.

In summary, rhesus monkeys (4/sex/group) were treated via peribulbar (single dose) and sc (single or double doses) injections with rHuPH20 at doses of 130 U, 3880 U, and 38800 U. No toxicologically significant abnormal findings were noted in clinical observations, food consumption, body weights, ECG, HR, Bp, clinical pathology, necropsy and histopathological examinations. For peribulbar injections, transient redness and/or swelling were noted under or around the dosed eye one hr after dosing in both control and drug-treated groups. The signs resolved within 24 hr or 48 hr after dosing. In ophthalmologic examinations, no drug-related fundus, optic disc and vitreous changes were noted. Twenty-four hr after dosing on either Day 2 or Day 9, mild to moderate conjunctival changes including congestion, swelling (minimal to mild), and/or discharge in both control and HD animals were observed with similar severity. All of the observed effects were reversible. Since these effects were noted in both control and rHuPH20-treated eyes with similar severity, it was possible that these effects were caused by peribulbar injection procedure and were not toxicologically significant. In conclusion, single peribulbar injections in one or both eyes and once weekly subcutaneous injections (up to 2 weeks) of rHuPH20 at doses up to 38000 U in monkeys were well tolerated.

2.6.5.4 Discussion and Conclusions

In a single dose iv toxicity study conducted in SD rats, no toxicologically significant abnormal findings were noted. Histopathological examinations showed slight renal tubule dilatation in all 5 drug treated males with amorphous material in the lumina consistent with hyaline casts. Similar changes were not seen in female and control animals. In addition, the dose used in this study was high (10500 Units/ml/kg). The toxicological significance was not determined. In two toxicity studies conducted in monkeys via both peribulbar and sc injections, the drug was well tolerated. In ophthalmologic examinations, minimal to moderate (score = 1 or 2) conjunctival congestion, swelling, and/or discharge were seen in both control and treated animals with similar incidence and severity. These changes were reversible and not considered as toxicologically significant. In conclusion, data from all three toxicity studies support the safety of the clinical application of the drug. No safety concerns were raised.

OVERALL CONCLUSIONS AND RECOMMENDATIONS

Conclusions: Pharmacology studies showed evidence of rHuPH20's biological equivalence to the USP hyaluronidase reference standard regarding diffusion efficacy and dermal barrier reconstitution. The ability to achieve and sustain nerve block (akinesia) was also demonstrated when rHuPH20 was co-injected (by peribulbar injection) with local anesthetics in monkey eyes.

In a single dose iv toxicity study conducted in SD rats, histopathological examinations showed slight renal tubule dilatation in all 5 drug treated males with amorphous material in the lumina consistent with hyaline casts. Similar changes were not seen in female and control animals. In two toxicity studies conducted in monkeys via both peribulbar and sc injections, the drug was well tolerated. Data from all three toxicity studies support the safety of the clinical application of the drug.

Nonclinical studies showed no toxicologically significant events. From pharmacology/toxicology standpoint, an "approvable" was recommended with some minor labeling modifications.

Unresolved toxicology issues (if any): No

Recommendations:

This application is approvable from a nonclinical perspective with some minor modifications of labeling as revised in the "Clinical Pharmacology" section, "Carcinogenesis, Mutagenesis, Impairment of Fertility" section and "Pregnancy" section.

Suggested labeling:

Minor modifications of labeling are recommended in the "Clinical Pharmacology" section, "Carcinogenesis, Mutagenesis, Impairment of Fertility" section and "Pregnancy" section.

Clinical Pharmacology

Hyaluronidase is a spreading substance which modifies the permeability of connective tissue through the hydrolysis of hyaluronan, a polysaccharide found in the intercellular ground substance of connective tissue, and of certain specialized tissues, such as the umbilical cord and vitreous humor. Hyaluronic acid is also present in the capsules of type A and C hemolytic streptococci. Hyaluronidase hydrolyzes hyaluronic acid by splitting the glucosaminidic bond between C1 of the glucosamine moiety and C4 of glucuronic acid. This temporarily decreases the viscosity of the cellular cement promotes diffusion of injected fluids or of localized transudates or exudates, thus facilitating their absorption.

Knowledge of the mechanisms involved in the disappearance of injected hyaluronidase is limited. It is known, however, that the blood of a number of mammalian species brings about the inactivation of hyaluronidase. *Studies have demonstrated that hyaluronidase is antigenic; repeated injections of relatively large amounts of this enzyme from animal sources may result in the formation of neutralizing antibodies.*

CARCINOGENESIS, MUTAGENESIS, IMPAIRMENT OF FERTILITY

Long-term animal studies have not been performed to assess the carcinogenic ~~or mutagenic~~ potential of hyaluronidase.

~~Long-term a~~Animal studies have not been performed to assess whether hyaluronidase impaired fertility; however, it has been reported that testicular degeneration may occur with the production of organ specific antibodies against this enzyme following repeated injections. Human studies on the effect of intravaginal hyaluronidase in sterility due to oligospermia indicated that hyaluronidase may have aided conception. ~~Thus, it appears that hyaluronidase may not adversely affect fertility in females.~~

PREGNANCY

Teratogenic Effects—Pregnancy Category C

No adequate and well controlled animal studies have been conducted with Hylenex to determine reproductive effects. No adequate and well controlled studies have been conducted with Hylenex in pregnant women. Hylenex should be used during pregnancy only if clearly needed.

Signatures (optional):

Reviewer Signature _____

Supervisor Signature_____ Concurrence Yes ___ No ___

APPENDIX/ATTACHMENTS

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

/s/

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6/14/05 10:09:13 AM
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