CENTER FOR DRUG EVALUATION AND RESEARCH

APPLICATION NUMBER:

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PHARMACOLOGY REVIEW(S)

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

PHARMACOLOGY/TOXICOLOGY BLA REVIEW AND EVALUATION

Application number:	125422
Supporting document/s:	0
Applicant's letter date:	April 16, 2012
CDER stamp date:	April 17, 2012
Product:	JETREA (ocriplasmin) Intravitreal Injection,
Indication:	Treatment of symptomatic vitreomacular adhesion including macular holes
Applicant:	Thrombogenics, Inc
Review Division:	Transplant and Ophthalmology Products
Reviewer:	María I. Rivera, PhD
Supervisor/Team Leader:	**Lori Kotch, PhD, DABT**
Division Director:	Renata Albrecht, MD
Project Manager:	Jacquelyn Smith

Disclaimer

Except as specifically identified, all data and information discussed below and necessary for approval of BLA 125422 are owned by Thrombogenics or are data for which Thrombogenics has obtained a written right of reference. Any information or data necessary for approval of BLA 125422 that Thrombogenics does not own or have a written right to reference constitutes one of the following: (1) published literature, or (2) a prior FDA finding of safety or effectiveness for a listed drug, as reflected in the drug's approved labeling. Any data or information described or referenced below from reviews or publicly available summaries of a previously approved application is for descriptive purposes only and is not relied upon for approval of BLA 125422.

MEMORANDUM

To: The file
Cc: Renata Albrecht, M.D., Division Director, Division of Transplant and Ophthalmology Products, Center for Drug Evaluation and Research
FROM: Lori E. Kotch, PhD, DABT, Toxicologist/Team Leader
Office of Antimicrobial Products, Division of Transplant and Ophthalmology Products

NDA #: 125422
Submission Type: original BLA application
Product: JETREA (ocriplasmin)
Date: October 2, 2012

SYNOPSIS:
Thrombgenics, Inc has submitted an original BLA application for ocriplasmin, a recombinant human protein with similar enzymatic activity as intact plasmin. The proposed indication is treatment of symptomatic vitreomacular adhesions including macular holes. In the Highlights section of the label, Ocriplasmin is defined as a proteolytic enzyme as its pharmacologic class. Ocriplasmin has proteolytic activity against protein components of the vitreous body and the vitreoretinal interface (e.g. laminin, fibronectin and collagen), thereby dissolving the protein matrix responsible for the abnormal vitreomacular adhesion (VMA). The proposed dosing regimen is a single intravitreal dose of 125µg**.**

The nonclinical data in support of *JETREA* for the proposed indication were reviewed by the primary reviewer, Maria Rivera, Ph.D., and are briefly summarized in the "Executive Summary" and "Integrated Summary and Safety Evaluation" sections of her review. Pharmacology, Safety pharmacology, Ocular toxicity and Systemic toxicity studies were conducted in support of the current BLA. The proteolytic activity of Ocriplasmin was demonstrated with proteolytic effects reported on fibrinogen, fibronectin, gelatin, laminin and collagen; all relevant components in the vitreous and vitreoretinal interface. Intravitreal administration of ocriplasmin was demonstrated to induce vitreous liquefaction and posterior vitreous detachment (PVD) in various animal models and human donor eyes.

Safety Pharmacology studies in dogs showed a significant decrease in blood pressure, a slight increase in QT/QTc intervals and P-wave amplitude, and a slight decrease in tidal volume. The exposure margin at the no-observed-effect level (NOEL) of 1.5 mg/kg is >130-fold the estimated systemic concentration of 46 ng/mL in humans after a single intravitreal dose.

The systemic toxicity of ocriplasmin was evaluated in rats and dogs after IV

administration. The adverse findings observed were related to the thrombolytic action of the drug. The NOELs were 220- and 675-fold the estimated systemic concentration of 46 ng/mL in humans (after a single intravitreal clinical dose of 125 µg). As such, systemic toxicity of ocriplasmin appears unlikely following a single 125µg intravitreal injection in humans, based on nonclinical data.

The intravitreal toxicity of ocriplasmin was evaluated in rabbits, monkeys and minipigs. Findings after a single intravitreal injection included narrowing of the retinal vessels with associated retinal atrophy in rabbits only, lens subluxation in all 3 species, and changes in intraocular pressure (IOP), inflammation, and electroretinography (ERG) changes in rabbits and monkeys. Pathological changes related to intraocular hemorrhage were also observed in rabbits and monkeys; however it is uncertain whether this effect is a result of the injection procedure itself or a pharmacologic effect of ocriplasmin. The exposure margins for the findings of inflammation, ERG changes and lens subluxation observed in rabbits and monkeys after a single intravitreal dose were modest (0.1-fold to 1.5-fold). A more favorable exposure margin (3.7-fold) was observed for the microscopic retinal changes observed in the monkey. With the exception of lens subluxation, the nonclinical findings tended to resolve over time after administration of a single intravitreal dose.

A second intravitreal administration of ocriplasmin (28-days apart) in monkeys at doses of 75 µg/eye (41 µg/mL vitreous) or 125 µg/eye (68 µg/mL vitreous) was associated with lens subluxation in **all** ocriplasmin treated eyes, sustained increases in IOP and associated glaucoma in two animals with severe lens subluxation, and multiple adverse microscopic findings in the eye including vitreous liquefaction, degeneration/disruption of the hyaloideocapsular ligament (with loss of ciliary zonular fibers), lens degeneration, mononuclear cell infiltration of the vitreous, and vacuolation of the retinal inner nuclear cell layer. These doses were 1.4-fold and 2.3-fold the intended clinical concentration of 29 µg/mL vitreous, respectively.

Recommendations: I concur with Dr. Rivera's conclusions regarding the nonclinical findings for JETREA, her current recommendation that the licensing application be approved for marketing, and her recommendations regarding the language for the prescribing information. Additionally, I concur with her recommendation to include in the *Warnings and Precautions* section, the animal data that demonstrated that a second intravitreal dose (28 days apart) in monkeys substantially increases the incidence of lens subluxation and associated ocular findings. A copy of Dr. Rivera's review, with supervisory sign-off, has been conveyed to the regulatory project manager for inclusion in the final action package, and has been uploaded into the DARRTS database.

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

/s/

LORI E KOTCH
10/02/2012

DEPARTMENT OF HEALTH AND HUMAN SERVICES
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1 EXECUTIVE SUMMARY

1.1 INTRODUCTION

Thrombogenics, Inc. developed a truncated form of plasmin, ocriplasmin, a recombinant human protein with similar enzymatic activity as intact plasmin (although less potent with regard to fibrinolytic activity). The proposed indication is treatment of symptomatic vitreomacular adhesions including macular holes. Ocriplasmin has proteolytic activity against protein components of the vitreous body and the vitreoretinal interface (e.g. laminin, fibronectin and collagen), thereby dissolving the protein matrix responsible for the abnormal vitreomacular adhesion. Ocriplasmin offers the first pharmacological treatment option for this progressive and potentially sight-threatening condition. The drug has not been approved outside the USA. The intended dosing regimen is a single intravitreal dose of 125 µg.

1.2 BRIEF DISCUSSION OF NONCLINICAL FINDINGS

The intravitreal toxicity of ocriplasmin has been evaluated in rabbits, monkeys and minipigs. Findings after a single intravitreal injection included narrowing of the retinal vessels with associated retinal atrophy in rabbits only, lens subluxation in all 3 species, and changes in intraocular pressure (IOP), inflammation, and electroretinography (ERG) changes in rabbits and monkeys. One monkey developed a hyphema and retinal atrophy, however, a relationship to ocriplasmin treatment is uncertain.

A second intravitreal administration of ocriplasmin (28 days apart) in monkeys was associated with an increase incidence of lens subluxation, sustained increases in IOP, and a series of adverse microscopic findings in the eye. The lens subluxation was associated with degeneration/disruption of the hyaloideocapsular ligament observed microscopically, accompanied by loss of the ciliary zonular fibers. Iridodenesis (quivering of the iris) was noted in most animals with lens subluxation, as expected due to lack of support from the lens. Therefore, the lens subluxation is considered a consequence of the proteolytic activity of ocriplasmin. In monkeys and minipigs, vitreous gel breakdown was reported. This finding was an expected pharmacological action of the drug.

The exposure margins (0.1-1.5-fold) for the findings of inflammation, ERG changes and lens subluxation observed in rabbits and monkeys after a single intravitreal dose are low. A more favorable exposure margin (3.7-fold) was observed for the microscopic retinal changes observed in the monkey. However, except for lens subluxation, the nonclinical findings were reversible after administration of a single intravitreal dose.

Safety Pharmacology studies in dogs showed a significant decrease in blood pressure, a slight increase in QT/QTc intervals and P-wave amplitude, and a slight

decrease in tidal volume. Except for P-wave amplitude, all findings showed a trend toward recovery. The exposure margin at the no-observed-effect level (NOEL) of 1.5 mg/kg is >130-fold the estimated systemic concentration of 46 ng/mL in humans after a single intravitreal dose, indicating low concern for similar effects to be observed in humans. In addition, no effects were observed in electrocardiographic (ECG) parameters in a 14-day repeated-dose toxicology study in dogs at intravenous (IV) doses up to 10 mg/kg every other day.

The systemic toxicity of ocriplasmin was evaluated in rats and dogs after IV administration. The adverse findings observed were related to the thrombolytic action of the drug. The NOEL levels were 10 mg/kg every other day in rats and 2 mg/kg every other day in dogs. These doses are 220- and 675-fold the estimated systemic concentration of 46 ng/mL in humans after an intravitreal clinical dose of 125 µg. Therefore, the nonclinical data provides support to conclude that systemic toxicity of ocriplasmin is unlikely following a single 125 µg intravitreal injection in humans.

Additional support for the systemic safety of ocriplasmin at the proposed human dose is provided by the following observations:

- If ocriplasmin was completely systemically absorbed after intravitreal injection, the amount (46 ng/mL) would be miniscule compared to the amount of plasminogen in the human blood (200 µg/mL).
- If systemic bioavailability of the intraocular dose is 100% (i.e., 4.6 nmol), there would be sufficient α2-antiplasmin present in the blood to neutralize all ocriplasmin, based on the normal plasma concentration of the serine protease inhibitor α2-antiplasmin (1 nmol/mL of plasma),

1.3 RECOMMENDATIONS

1.3.1 Approvability

Ocriplasmin can be approved from the nonclinical perspective at a dosing regimen of a single intravitreal dose of 125 µg.

1.3.2 Additional Non Clinical Recommendations

None

1.3.3 Labeling

Note: *Information recommended by the reviewer is presented in italic style.*

INDICATIONS AND USAGE

Jetrea *is a proteolytic enzyme* indicated for the treatment of symptomatic vitreomacular adhesion (b) (4) (1).

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(b) (4)

8.1. Pregnancy

Teratogenic Effects

Pregnancy Category C. Animal reproduction studies have not been conducted with ocriplasmin. There are no *adequate and well-controlled studies o* (b) (4) in pregnant women. It is not known whether ocriplasmin can cause fetal harm when administered to a pregnant woman or can affect reproduction capacity. The systemic exposure to ocriplasmin is expected to be (b) (4) low after intravitreal injection of a single 125 (b) (4) dose. *Assuming 100% systemic absorption (and a plasma volume of 2700 mL), the estimated plasma concentration is 46 ng/mL*. JETREA should be (b) (4) *given to a pregnant woman only if clearly needed.*

13. NONCLINICAL TOXICOLOGY

13.1. Carcinogenesis, Mutagenesis, Impairment of Fertility

No carcinogenicity, mutagenicity or reproductive and developmental toxicity (b) (4) *studies were conducted with ocriplasmin*.

13.2. Animal Toxicology and/or Pharmacology

The (b) (4) *ocular* toxicity of ocriplasmin *after a single intravitreal dose* has been evaluated in rabbits, monkeys and minipigs. Ocriplasmin induced an inflammatory response and transient ERG changes in rabbits and monkeys, *which* (b) (4)

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(b) (4)

Lens subluxation was observed in the 3 species at (b) (4) ocriplasmin concentrations at or above 41 (b) (4) mL vitreous, a concentration *1.4-fold* above the intended clinical concentration of 29 (b) (4) /mL (b) (4) . (b) (4)

—

(b) (4)

A second intravitreal administration of ocriplasmin (28-days apart) in monkeys at doses of 75 (b) (4) /eye (41 (b) (4) /mL vitreous) or 125 (b) (4) /eye (68 (b) (4) /mL vitreous) was associated with lens subluxation in all ocriplasmin treated eyes, sustained increases in IOP and associated glaucoma in two animals with (b) (4) lens subluxation, (b) (4) microscopic findings in the eye including vitreous liquefaction, degeneration/disruption of the hyaloideocapsular ligament (with loss of ciliary zonular fibers), lens degeneration, mononuclear cell infiltration of the vitreous, and vacuolation of the retinal inner nuclear cell layer. These doses are 1.4-fold and 2.3-fold the intended clinical concentration of 29 (b) (4) /mL vitreous, respectively.

2 DRUG INFORMATION

2.1 DRUG

CAS Registry Number: 1048016-09-6

Generic Name: Ocriplasmin

Chemical Name: Microplasmin; recombinant truncated human plasmin

Molecular Formula/Molecular Weight: Ocriplasmin is a protein of 249 amino acid residues; 27,237 Da

Structure or Biochemical Description: The protein consists of two peptide chain (19 and 230 amino acids long) and 6 disulfide bonds, (b) (4)

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Schematic amino acid sequence of ocriplasmin written from N-termi the C-terminus. All six sulfide bridges are shown (full lines). Th peptides are separated by (/)

```
APSFDCGKPQVEPKKCPGR/VVGGCVAHPHSWPWQVSLRTR   40

FGMHFCGGTLISPEWVLTAAHCLEKSPRPSSYKVILGAHQ    80

EVNLEPHVQEIEVSRLFLEPTRKDIALLKLSSPAVITDKV   120

IPACLPSPNYVVADRTECFITGWGETQGTFGAGLLKEAQL   160

PVIENKVCNRYEFLNGRVQSTELCAGHLAGGTDSCQGDSG   200

GPLVCFEKDKYILQGVTSWGLGCARPNKPGVYVRVSRFVT   240

WIEGVMRNN   249
```

Pharmacologic Class: Proteolytic enzyme

- Serine protease that selectively cleaves peptide bonds on the carboxy-terminal side of lysine and arginine residues of protein and peptide substrates

2.2 RELEVANT INDS, NDAS, BLAS AND DMFS

IND 100,370 (Microplasmin)

2.3 DRUG FORMULATION

The drug product is a sterile, clear and colorless solution with no preservatives in a single use glass vial containing 0.5 mg of ocriplasmin at a concentration of 2.5 mg/mL solution. For intravitreal administration, ocriplasmin drug product solution is to be diluted with an equal volume of 0.9% (w/v) sodium chloride prior to use. The composition of the formulation is shown in the table below.

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Table 1: Ocriplasmin Drug Product Formulation

Ingredient	Unit Formula (mg/0.200mL)	Concentration (mg/mL)	Function
Drug Substance			
Ocriplasmin	0.500	2.50	Active ingredient
Excipients			
(b) (4) Mannitol	0.750	3.75	(b) (4)
Citric acid (b) (4)	0.210	1.05	
Sodium hydroxide[a]	(b) (4)		
Water for injection			

(b) (4)

(b) (4)

2.4 COMMENTS ON NOVEL EXCIPIENTS

There are no novel excipients in the drug formulation.

2.5 COMMENTS ON IMPURITIES/DEGRADANTS OF CONCERN

Defer to the review of the product quality reviewer.

2.6 PROPOSED CLINICAL POPULATION AND DOSING REGIMEN

The proposed clinical population is subjects with symptomatic vitreomacular adhesion (VMA) including macular hole. The recommended dose is a single intravitreal injection of 125 µg corresponding to 0.1 mL of the diluted solution.

2.7 REGULATORY BACKGROUND

The non-clinical program was discussed at the end-of Phase 2 meeting with FDA on September 24, 2008. The Division indicated that no additional non-clinical studies were anticipated other than those completed and planned for intravitreal use of ocriplasmin. The Division agreed that genetic toxicity studies are not generally required for biologic products and that they will not be necessary for ocriplasmin. In addition, carcinogenicity studies with ocriplasmin were not considered necessary as the applicant was not seeking a clinical indication entailing chronic use. The Division agreed that reproductive and developmental toxicity studies would not be necessary if significant systemic exposure was not observed in humans following an intravitreal injection of ocriplasmin.

3 STUDIES SUBMITTED

3.1 STUDIES REVIEWED

Reference ID: 3198420

Pharmacology

- Evaluation of Enzymatic Properties and Substrate Specificity of Recombinant Human Microplasmin Preparations in Comparison to Natural Human Plasmin (Study # R04-TX-002)
- *In Vitro* Comparison of the Efficiency and Specificity of Substrate Hydrolysis for Recombinant Human Microplasmin and Natural Plasmin (Study # R04-TX-003)
- Dynamic Light Scattering Evaluation of the Dynamic Properties of Vitreous following Pharmacologic Vitreolysis with Microplasmin (Study R04-TG-001)

Safety Pharmacology

- Evaluation of the Effect of Microplasmin on the Modified Irwin Screen Test in the Rat (Study # 257003)
- Effect on the Cardiovascular and Respiratory System in the Anaesthetized Dog (Study # 843343)

Pharmacokinetics/ADME

- Kinetics of Inactivation of Microplasmin in Porcine Eyes (Study # SR 10/mPI18/ItP; Non-GLP)
- Dose- and Time-Dependent Inactivation of Microplasmin in Post-Mortem Porcine Eyes (Study # B000137; GLP)
- Kinetics of Inactivation of Microplasmin in Homogenized Porcine Eye Vitreous Fluid (Study # SR 10/mPI17/ItP; Non-GLP)
- Kinetics of Inactivation of Microplasmin in Homogenized Human Eye Vitreous Fluid (Study # SR 10/mPI16/ItP; Non-GLP)
- Auto-Proteolytic Degradation of Microplasmin (Study # SR 09/mPI09/ItP)

General Toxicology

- A Multiple Dose Intravitreal Toxicity Study of Microplasmin in the Cynomolgus Monkey with a 12-Week Observation Period (Study # 570221)
- A Single Dose Intravitreal Toxicity Study of Microplasmin in the Cynomolgus Monkey with a 4-Week Observation Period (Study # 570256)
- Recombinant Human Microplasmin 7-Day Intravenous Dose Range Finding Study in Rats (Study # 662288)

Special Toxicology

- Toxicology Study of Microplasmin and *Pichia Pastoris* Yeast Expression System Products in the Rat (Study # R02-TX-002)
- Validation of ELISA Methodology to Detect Dog and Rat IgG Antibodies to Microplasmin (Study # 767995)

3.2 STUDIES NOT REVIEWED

- Microplasmin: Tolerance Test (Paravenous Route) in Rabbits (Study # 511268)

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3.3 PREVIOUS REVIEWS REFERENCED

- IND 100,370 by Dr. Amy Ellis (signed December 12, 2006)
- IND 100,370 by Dr. Maryam Rafie-Kolpin (signed March 15, 2007)

4 PHARMACOLOGY

4.1 PRIMARY PHARMACOLOGY

In vitro studies demonstrated the proteolytic activity of ocriplasmin. Intravitreal administration of ocriplasmin was demonstrated to induce vitreous liquefaction and posterior vitreous detachment (PVD) in various animal models and human donor eyes. Ocriplasmin showed proteolytic effects on fibrinogen, fibronectin, gelatin and, to a lesser extent, laminin and collagen, all relevant components in the vitreous and vitreoretinal interface. Ocriplasmin showed similar enzymatic activity as intact plasmin, although ocriplasmin was less potent with regard to fibrinolytic activity. There were also differences in potency depending on the substrate. Based on the concentration to induce 50% hydrolysis, ocriplasmin was more effective on collagen type IV compared to plasmin, whereas plasmin was more effective on fibrinogen, gelatin, laminin and fibronectin.

These studies are summarized below.

Study R04-TX-002: Evaluation of Enzymatic Properties and Substrate Specificity of Recombinant Human Microplasmin Preparations in Comparison to Natural Human Plasmin

Description: Enzymatic activity of ocriplasmin towards synthetic substrates (S-2304, S-2444), fibrin, fibrinogen, and ocriplasmin inhibition by α2-antiplasmin were compared with the properties of natural human plasmin *in vitro*.

Main findings:

- Both enzymes had similar affinity and enzymatic activity towards the 2 synthetic substrates.
- The fibrinolytic potency of ocriplasmin appeared 4- to 5-fold lower compared with plasmin on a molar basis: the concentration required for 50% clot lysis within 3 hrs was estimated to be ~100 nM (2.7 µg/mL) for ocriplasmin and 20 nM (1.6 µg/mL) for plasmin.
- Both enzymes showed an increase in degradation of fibrinogen over time, with more degradation apparent for ocriplasmin than for plasmin.
- Both ocriplasmin and plasmin formed a complex with the serine protease inhibitor, α2-antiplasmin. The complex formation occurred at a slower rate for ocriplasmin (formed after a 3 min incubation for plasmin compared to >30 min for ocriplasmin).
- The second order rate constants of the binding of α2-antiplasmin to ocriplasmin or plasmin were calculated to be 2.5, 2.7, and 3.0 x 10^5 $M^{-1}s^{-1}$ for the 3 ocriplasmin batches and 1.0 x 10^7 $M^{-1}s^{-1}$ for plasmin.

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Study R04-TX-003: *In Vitro* Comparison of the Efficiency and Specificity of Substrate Hydrolysis for Recombinant Human Microplasmin and Natural Plasmin
Description: Enzymatic activity of ocriplasmin towards S-2304, fibrinogen, collagen type IV, gelatin, laminin and fibronectin *in vitro* were compared with the properties of natural human plasmin.
Main findings:

- Ocriplasmin and plasmin demonstrated the same efficacy towards the hydrolysis of the synthetic chromogenic substrate S-2304 (K_m of 0.195 and 0.179 mM, respectively).
- The *in vitro* effects on different substrates was measured as the concentration to induce 50% hydrolysis (defined as IC_{50}) of the natural substrates; ocriplasmin was more effective on collagen type IV (1.3-fold) compared to plasmin, whereas plasmin was more effective on fibrinogen (2.0-fold), gelatin (3.7-fold), laminin and fibronectin (4.8-fold). Overall, the IC_{50} values for both enzymes ranged between 0.3-447 nM
- The rate of hydrolysis of fibrinogen and fibronectin was higher (~1.7-fold) for plasmin than ocriplasmin, similar between both enzymes for gelatin, and higher for ocriplasmin than plasmin for collagen type IV (1.3-fold) and laminin (1.7-fold). Overall, the % substrate lysis per hour for both enzymes ranged between 8.6-33%, under the conditions of the study.
- The relative order of activity (as measured by rate of substrate hydrolysis) was identical for ocriplasmin and plasmin (from highest rate of activity to lowest rate of activity, taking into account drug concentration used: fibrinogen > fibronectin, gelatin > collagen type IV > laminin.

Study R04-TG-001: Dynamic Light Scattering Evaluation of the Dynamic Properties of Vitreous following Pharmacologic Vitreolysis with Microplasmin
Description: The biophysical effects of ocriplasmin on porcine vitreous were characterized *in vitro* and *in-situ* using the non-invasive technique of dynamic light scattering (DLS). DLS allows the measurement of reductions in the diameter of particles suspended in a solution, in this case, porcine vitreous macromolecules.

Main Findings:
<u>Dissected porcine eyes</u>:

- At 125 µg, one-third reduction in the overall average particle size after 210 min
- At 600 µg, 80% reduction in particle size after 60 min
- There was a substantial diminution in normalized average particle size, thus, ocriplasmin application to the vitreous of the porcine eye led to a breakdown of vitreous macromolecules and ultimately to liquefaction.

<u>Intact porcine eyes</u>:

- Dose-dependent diminution in the average particle size after 30-min incubations: no effects at 12.5 µg, one-third diminution at 125 µg, 7-fold diminution at 800 µg
- After 2-hr incubations, ~87% diminution at 600 µg (highest dose)

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- Generally, more intensive degree of particle size breakdown after longer incubation period (2 hrs *vs.* 30 min)

Whole porcine vitreous subfractions:

- Significant shift in the particle size distribution with considerably higher amounts of lower particle size species after incubation with ocriplasmin (500 µg) compared to the control

A series of publications were submitted to support the activity of ocriplasmin in inducing posterior vitreous detachment (PVD) and vitreous liquefaction. In addition to vitreous liquefaction, ocriplasmin caused a dose-dependent PVD without notable effect on the internal limiting membrane (ILM) and/or retina as demonstrated in rat, rabbit and cat eyes *in vivo,* as well as in porcine eyes *ex vivo* and in human donor eyes *ex vivo*. There was no morphological damage to the ILM and/or retina at doses up to 1.87 µg/eye (34 µg/mL) in the rat *in vivo*, 250 µg/eye (119 µg/mL) in the rabbit *in vivo*, 250 µg/eye (76 µg/mL) in the pig *ex vivo*, and 25 µg/eye (9 µg/mL) in the cat *in vivo*. The highest dose tested in the *ex vivo* porcine eye (400 µg or 121 µg/mL) was associated with the appearance of dendritic-like cells on the retinal surface and circumscribed elevations of the retina and RPE in serous-like detachments in ~25% of the eyes examined.

In human donor eyes, vitreous collagen fibrils still covered the ILM after 30 min of incubation with 62.5 µg ocriplasmin (14 µg/mL, using a vitreous volume in humans of 4.36 mL). Treatment with 125 or 188 µg ocriplasmin (29 and 43 µg/mL, respectively) led to complete PVD with ILM surface completely devoid of residual vitreous collagen and no damage to the retina. The dose of 125 µg/eye, equivalent to 29 µg/mL, was selected as the clinical dose.

4.2 SECONDARY PHARMACOLOGY

Literature publications to support cardiovascular indications were included. These were not considered relevant for the ophthalmic indication. If microplasmin enters the systemic circulation (unlikely to occur at a significant level following intravitreal injection), it can perturb the clotting cascade given its fibrinolytic effects. In clinical trials at IV doses of ≥1 mg/kg, an allergic-type reaction was observed in several healthy volunteers. The applicant believes these findings were due to high ocriplasmin doses that resulted in the depletion of α2-antiplasmin and subsequent activation of complement C5 to C5a. The dose patients are likely to receive intravitreally even in overdose situations is unlikely to deplete systemic α2-antiplasmin and therefore, the risk of pseudo-allergic-type reactions is considered low.

SAFETY PHARMACOLOGY

The following studies were previously reviewed by Dr. Maryam Rafie-Kolpin with the initial IND submission. The review is attached in the Appendix. Several edits (denoted in italics) were made to the previous review by the current reviewer.

Reference ID: 3198420

Study title: Evaluation of the Effect of Microplasmin on the Modified Irwin Screen Test in the Rat (Study # 257003): Male Sprague-Dawley rats (4/group) were administered recombinant human ocriplasmin at single IV doses of 0, 2, 6, and 10 mg/kg. In ocriplasmin-treated groups, increased startle response, vocalization during handling, tachypnea, diarrhea, increased alertness, and increased exploratory activity were observed in some animals at isolated time points. These behavioral changes did not follow a dose-response relationship. *Therefore, it was difficult to clearly establish an association to ocriplasmin treatment.*

Study title: **Effect on the Cardiovascular and Respiratory System in the Anaesthetized Dog (Study # 843343):** The study evaluated the potential effects of the test article on the cardiovascular, respiratory system, and hematological parameters in anesthetized beagle dogs (4 males). Dogs were administered doses of 0, 0.15, 1.5 or 15 mg/kg ocriplasmin as sequential 15-min IV infusions with at least 30-min time intervals between doses.

The following findings were observed at 15 mg/kg. The systolic, diastolic, and mean blood pressures were significantly decreased within the first 5 min of infusion and throughout the duration of the study. Compared to the pre-dose values, peak decreases of 52.4% and 51% were observed in the systolic and diastolic blood pressures, respectively, 5 min after the end of the infusion. The mean blood pressures fell by 51% at the end of the 15 min infusion. *The blood pressures started to recover during the 30 min after the end of the infusion, but were still lower than the predose values. Heart rate was decreased with peak occurring 5 min after the end of the infusion (15%). The heart rate returned to predose values 30 min after the end of the infusion. A slight but significant increase in the QT interval and QTc interval was observed when compared to predose values. The increase corresponded to 12% and 8% at peak (i.e., 5 min after the end of the infusion), respectively. Values started to recover during the 30 min after the end of the infusion, but were still higher than the predose values. The P-wave amplitude was also increased at 15 mg/kg (12% above the predose value by the end of the infusion) and was still elevated (23.8%) 30 min after the end of the infusion.*

No significant effects were observed in the respiratory rate or minute volume at any of dose levels*. The tidal volume significantly decreased, reaching its lowest point of -28% compared to predose values at 10 min after the end of the infusion. At 30 min after the end of the infusion, tidal volume was 24% below the predose value.* The blood pH was decreased from 7.196 control value to 7.04 at 15 mg/kg. *This was accompanied by non-significant increases in $pC0_2$ (25%) and decreases in pO_2 (18%).*

Significant increases in the RBC (37%), hemoglobin (42%), hematocrit (38%), *glucose (52%), creatinine (14%), and triglyceride (190%) were observed at 15 mg/kg*, compared to control values. *Treatment with 1.5 mg/kg ocriplasmin significantly increased prothrombin time (10%) and significantly decreased fibrinogen (40%). PT, APTT and fibrinogen could not be determined at the higher dose since the blood did not coagulate.*

Reference ID: 3198420

Ocriplasmin plasma concentrations are summarized in the table below. The minimum detection limit was 0.625 ng/mL. The NOAEL was 1.5 mg/kg (6.16-7.22 µg/mL). In humans, the determination of the systemic availability after intravitreal injection was not conducted as the applicant indicated the expected amount in the systemic circulation was below the lower limit of quantitation (2.5 µg/mL). If the systemic bioavailability of the intravitreal dose was 100%, the applicant estimated a plasma concentration of 35 ng/mL would be expected (assuming a plasma volume of 3600 mL in an 80 kg person). Using a more conservative value of 2700 mL plasma volume for a 60 kg person, the plasma concentration is 46 ng/mL. Therefore, there is safety margin of >130-fold, which suggests minimal concern for similar systemic effects to be observed in humans.

Table 2: Ocriplasmin Plasma Concentrations (µg/mL) in the Dog Cardiovascular and Respiratory Safety Pharmacology Study

Dose (mg/kg)	0	0.15	1.5	15
Animal				
1	<LOQ	1.31	6.87	54.25
2	<LOQ	1.61	6.88	68.96
3	<LOQ	1.50	7.22	112.36
4	<LOQ	1.76	6.16	250.72

LOQ= Limit of quantitation of the assay

5 PHARMACOKINETICS/ADME/TOXICOKINETICS

5.1 PK/ADME

Absorption: Studies to investigate the systemic absorption of ocriplasmin after intravitreal administration were not conducted. The following studies were conducted to investigate the decline of microplasmin over time in the vitreous. Ocriplasmin, like many proteases, is subject to autocatalytic proteolytic degradation in the vitreous which follows second-order kinetics subject to product inhibition.

Kinetics of Inactivation of Microplasmin in Porcine Eyes (Study #SR 10/mPl18/ltP; Non-GLP) – Ocriplasmin was administered by intravitreal injection at concentrations of 50-250 µg (15-76 µg/mL) to porcine eyes *ex vivo* and incubated at 37°C for up to 180 min. Ocriplasmin was rapidly inactivated with 40-80% and 14-29% of the initial active ocriplasmin concentrations remaining at 60 min and at the end of the incubation period. At higher doses of ocriplasmin, there was more inactivation. .

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Dose- and Time-Dependent Inactivation of Microplasmin in Post-Mortem Porcine Eyes (Study # B000137; GLP) – Ocriplasmin activity was measured after injection at doses of 50, 125, 175, and 250 µg/eye and incubation up to 180 min at 37°C. A rapid decrease in ocriplasmin activity was observed after ocular administration with 33, 17, 13, and 9% of the initial active ocriplasmin concentrations remaining at the end of the study, respectively.

Kinetics of Inactivation of Microplasmin in Homogenized Porcine Eye Vitreous Fluid (Study # SR 10/mPI17/ltP; Non-GLP) – The concentration of active ocriplasmin was determined in porcine vitreous fluid and in PBS following the incubation of 50, 125, 175, and 250 µg ocriplasmin at 37°C for up to 24 hrs. The inactivation of ocriplasmin in porcine eyes followed a second-order process. The rate constants for PBS and porcine vitreous were calculated to be 207 ± 60 and 81 ± 15 M^{-1} s^{-1}, respectively. At the end of the 24-hr incubation period, 27, 13, 10, and 8% of the initial active ocriplasmin concentrations remained in the vitreous, compared to 10, 5, 3, and 2% in PBS, at 50, 125, 175, and 250 µg ocriplasmin, respectively.

Kinetics of Inactivation of Microplasmin in Homogenized Human Eye Vitreous Fluid (Study # SR 10/mPI16/ltP; Non-GLP) - The concentration of active ocriplasmin was determined in human vitreous fluid following the incubation of 18.75, 47, and 75 µg ocriplasmin at 37°C for 3, 5, and 3 hrs, respectively. At the end of the incubation period, 23%, 36% and 7% of the initial active ocriplasmin concentrations were left at 18.75 µg, 47 µg, and 75 µg, respectively. The lack of a dose response is probably due to the fact that the actual concentrations at 18.75 and 47 µg at Time 0 were very similar (24.98 and 26.64 µg/mL, respectively).

The study also investigated the decline of ocriplasmin activity in human vitreous fluid following addition of 125 µg ocriplasmin (the intended clinical dose) and incubated for up to 72 hrs at 37°C. After 5 hrs, ~16% of the initial actual active ocriplasmin concentrations remained. At the end of the incubation period (72 hrs), <0.6% of the initial actual concentrations remained. The data is presented in the table below.

Table 3: Concentrations of Active Ocriplasmin in Pooled Human Vitreous Incubated with 125 µg Ocriplasmin

		Time (min/h as indicated)											
		0 min	15 min	30 min	45 min	1 h	2 h	3 h	4 h	5 h	24 h	48 h	72 h
Pool 1	AVG	37.84	30.40	25.02	21.51	18.33	12.31	9.31	7.10	6.16	0.77	0.31	0.22
	SD	0.09	0.24	0.22	0.20	0.22	0.21	0.08	0.25	0.01	0.04	0.03	0.05
Pool 2	AVG	42.19	34.40	28.85	24.55	19.75	14.88	10.07	8.89	6.58	0.91	0.31	0.20
	SD	0.14	0.32	0.11	0.22	0.14	0.37	0.37	0.01	0.01	0.10	0.08	0.05

Values represent the mean ± SD (n=2); Units = µg/mL.

The inactivation of microplasmin in pooled human eye vitreous (n=2) followed a second-order process characterized by a rate constant of 210 M^{-1} s^{-1} for Pool 1 and 180 M^{-1} s^{-1} for Pool 2.

Reference ID: 3198420

Auto-Proteolytic Degradation of Microplasmin (Study # SR 09/mPl09/ltP) – The study investigated the degradation profile of ocriplasmin (42 µg/mL) in buffer and in porcine vitreous fluid after incubation for 0, 0.5, 0.75, 1, 2, 3, and 24 hrs. No difference was observed in degradation profile between PBS and vitreous fluid. The break-down fragments observed were consistent with autoproteolytic processing. The report states that a difference in time course was observed, whereby degradation in PBS occurred at a faster rate compared to degradation in vitreous fluid. This assessment was done by visual inspection of a Coomassie blue stained gel. However, the reviewer considered it was difficult to reach this conclusion because there was a band in the blank vitreous co-eluting with the ocriplasmin band. This band may have lead to an underestimation of the degree of ocriplasmin that was degraded in the vitreous fluid. On the other hand, a Western blot of the 3-hr incubation did show more degradation in PBS compared to vitreous fluid.

A clinical study following intravitreal injection of 125 µg ocriplasmin in 40 patients undergoing vitrectomy (Study # TG-MV-010) was performed demonstrating a rapid loss of activity of ocriplasmin at a similar rate as was observed in human and porcine vitreous fluid as well as in post mortem pig eyes (see Table 3 excerpted from the BLA Pharmacokinetics Written Summary).

Table 4: Ocriplasmin Levels in Vitreous Samples from Clinical Study TG-MV-010 Compared to those Observed in Homogenized Pig Vitreous

Time post injection	5-30 min	31-60 min	2-4h	24h	7 days
ocriplasmin levels in human vitreous (TG-MV-010)	12µg/mL	8.1µg/mL	2.6µg/mL	0.49µg/mL	<272ng/mL[a]
ocriplasmin levels in homogenized porcine vitreous[b]	9.3µg/mL	4.2µg/mL	2.8µg/mL	ND	ND

[a] below lower limit of quantification
[b] concentrations as obtained from injection of 125µg in the pig eye, most resembling to the human situation, data extracted from study B000137

ND = not determined

Distribution: The applicant indicated that no systemic distribution studies were conducted, as intravitreal administration of ocriplasmin is unlikely to result in significantly relevant systemic exposure.

The data from the 14-day IV repeated-dose studies in rats (Study # 662911) and dogs (Study # 662314) support the view that ocriplasmin in the circulation binds to

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serine protease inhibitors such as α2-antiplasmin. This is consistent with the *in vitro* observations in Study # R04-TX-002 above. Therefore, depending on the dose, ocriplasmin may be found primarily circulating as inactive ocriplasmin/α2-antiplasmin complex in the blood.

The applicant provided the following justification for not expecting significant levels of active ocriplasmin in the systemic circulation after a single intravitreal dose of 125 µg in humans:

"The normal plasma concentration of the serine protease inhibitor α2-antiplasmin is 1000 nM or 1 nmol/mL of plasma[1]. The intended dose of 125 µg for intravitreal administration of ocriplasmin is equivalent to 4.6 nmol of active substance. An average individual, 80 kg body mass with a normal blood volume of 72 mL/kg, has approximately 3600 mL plasma. Taken together, there is thus sufficient α2-antiplasmin present in as small a volume as 4.6 mL plasma to neutralize all ocriplasmin even if the systemic bioavailability of the intraocular dosage is 100%."

The reviewer agrees that the weight of evidence supports that significant levels of active ocriplasmin are unlikely to be observed after and intravitreal dose of 125 µg. The evidence includes the relatively rapid proteolytic degradation in the vitreous and the high levels of systemic protease inhibitors available to neutralize the intended clinical dose. In addition, even if ocriplasmin was completely systemically absorbed after intravitreal injection, the amount (46 ng/mL) would be miniscule compared to the amount of plasminogen in the human blood (200 mg/L).

Metabolism: No metabolism studies were conducted as it is expected that ocriplasmin enters the endogenous protein catabolism pathway through which it is rapidly inactivated via its interactions with protease inhibitors such as α2-antiplasmin (Study # R04-TX-002) or α-macroglobulin (as has been shown for plasmin)[2].

Excretion: Since ocriplasmin is a protein which is degraded by the endogenous catabolism, no excretion studies were conducted.

5.2 TOXICOKINETICS

The toxicokinetics of ocriplasmin, fibrinogen, and α2-antiplasmin were evaluated in the systemic route toxicity studies. Refer to the respective toxicology studies under *Section 6 - General Toxicology* for further details regarding study design.

7-Day Dose Range Finding in Sprague Dawley Rats (Study # 662288) – Rats (6/sex/group) were administered ocriplasmin by intravenous infusion at doses of 0, 10, 20, and 40 mg/kg/day for 7 days. Plasma concentrations of ocriplasmin were determined using a validated ELISA methodology. Blood samples for the determination

[1] Cederholm-Williams SA, *J Clin Pathol*, 34:979-81 (1981).
[2] Gyzander E and Teger-Nilsson AC, *Thromb Res*, 19:165-75 (1980).

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of ocriplasmin, fibrinogen and α2-antiplasmin were taken 0.5, 2, and 4 hrs post-infusion on Days 1 and 7. Toxicokinetic data collected in this study did not allow for an accurate interpretation of the results due to missing samples, lost samples, or insufficient sample volume available for analysis.

14-Day Toxicity Study in Sprague Dawley Rats (Study # 662911 or 21858) – Rats (10/sex/group) were administered ocriplasmin by intravenous infusion at doses of 0, 2, 7, and 10 mg/kg/day every other day for 14 days. Blood samples for the determination of plasma concentrations of ocriplasmin, fibrinogen (Day 1 only) and α2-antiplasmin were obtained at various time points (0.25, 0.5, 1, 2, and 4 hrs post-infusion) on Days 1 and 13. Ocriplasmin concentrations were analyzed *via* a validated ELISA assay, which recognizes ocriplasmin as well as ocriplasmin-α2-antiplasmin complexes. The results are presented on pages 11 and 12 of Dr. Rafie-Kolpin's review (see Appendix). Briefly, the increase in systemic exposure to ocriplasmin was higher than dose-proportional and there was accumulation (≤2-fold) with repeated dosing at the 2 higher doses (7 and 10 mg/kg every other day). The time for maximum plasma concentration (T_{max}) ranged from 1.5 to 5.25 hrs. The elimination half-life ($t_{1/2}$) ranged from 0.72 to 7.07 hrs.

Fibrinogen and α2-antiplasmin profiles reflected the perturbation of background levels as a result of administration of ocriplasmin. Fibrinogen levels generally decreased as the dose of ocriplasmin increased. The α2-antiplasmin estimates were decreased at the high ocriplasmin dose. There were no gender differences related to the ocriplasmin and α2-antiplasmin concentrations while estimates for fibrinogen were generally greater for male rats.

10-Day Dose Range Finding Study in Dogs (Study # 662293) – Dogs (1 male or female dog/group) were administered ocriplasmin by intravenous infusion at doses of 10, 12, 15, and 20 mg/kg/day for 10 days. Blood samples were taken 0.5 and 4 hrs after completion of the 1-hr infusion (Days 1 and 10). Ocriplasmin concentrations were analyzed *via* a validated ELISA assay. The results are presented on pages 26 and 27 of Dr. Ellis' review (see Appendix). Briefly, plasma concentrations of ocriplasmin tended to be lower (~2-fold) at 4 hrs post-infusion compared with the values determined at 30 min post-infusion. Conversely, α2-antiplasmin activity was higher (2 to 5-fold) at 4 hrs post-infusion. The investigators theorized that the increase in α2-antiplasmin activity at 4 hrs post-infusion was an indication that the level of this enzyme was rebounding as ocriplasmin and the ocriplasmin- α2-antiplasmin complexes were being removed from the circulation.

14-Day Toxicity Study Including Toxicokinetics in Beagle Dogs (Study # 662314) - Dogs (3 sex/group) were administered ocriplasmin by intravenous infusion at doses of 0, 2, 7, and 10 mg/kg/day every other day for 14 days. Blood samples were taken from all animals on Day 1 (0.5-6 hrs post dose) and Day 13 (0.5-24 hrs postdose) for determination of plasma concentrations of ocriplasmin, fibrinogen and α2-antiplasmin. Concentrations of ocriplasmin were analyzed *via* a validated ELISA assay. The results are presented on pages 29 and 30 of Dr. Ellis' review (see Appendix). Briefly, the systemic exposure to ocriplasmin generally increased with dose. Accumulation with

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repeated dosing was not observed up to 7 mg/kg. The sample number of values was too low at the highest dose group for an adequate assessment. Exposure (expressed as AUC) was higher (1.2 to 5.7-fold) in males than females, correlating with higher clearance in females. The T_{max} and $t_{1/2}$ ranged from 0.5-4.5 hrs and 0.92-3.68 hrs, respectively. The $t_{1/2}$ was slightly longer in females.

Perturbations of α2-antiplasmin (Table 5) and fibrinogen levels (Table 6) were associated with ocriplasmin treatment. On Day 1, the plasma activity of α2-antiplasmin decreased with ocriplasmin dose. On Day 13, α2-antiplasmin activity showed recovery, but it was still below control levels. Infusion of ocriplasmin was associated with a dose-dependent decrease in plasma fibrinogen levels on Day 1. The fibrinogen levels showed recovery on Day 13, but the levels were still below control levels.

Table 5: Mean Parameter Estimates for α2-Antiplasmin on Days 1 and 13 after IV Infusion of Ocriplasmin in Dogs (Study # 662314)

Gender	*Dose Level ($mg.kg^{-1}.day^{-1}$)*	*AUC(0.5-t) on Day 1 (%activity.h)*	*AUC(0.5-t) on Day 13 (%activity.h)*	*C_{max}(obs) on Day 1 %activity*	*C_{max}(obs) on Day 13 %activity*
Male	*0*	1031	3656	344.2	173.9
	2	1004	3393	301.0	152.9
	7	515.2	2798	139.1	146.1
	10	-	2787	122.5	155.9
Female	*0*	920.7	3806	290.1	193.8
	2	838.6	3281	232.9	147.8
	7	477.7	2664	152.4	142.9
	10	-	2915	132.3	156.8

'-' indicates no reliable estimates were calculable

Reference ID: 3198420

Table 6: Mean Parameter Estimates for Fibrinogen on Days 1 and 13 after IV Infusion of Ocriplasmin in Dogs (Study # 662314)

Gender	Dose Level (mg. $kg^{-1}.day^{-1}$)	AUC(0.5-t) on Day 1 ($mg.h.dl^{-1}$)	AUC(0.5-t) on Day 13 ($mg.h.dl^{-1}$)	C_{max}(obs) on Day 1 ($mg.dl^{-1}$)	C_{max}(obs) on Day 13 ($mg.dl^{-1}$)
Male	0	923.5	4068	178.0	185.5
	2	805.1	3477	157.0	164.0
	7	599.8	1831	117.8	116.3
	10	-	1873	88.50	136.8
Female	0	882.2	3410	170.5	156.5
	2	833.6	3823	158.3	173.5
	7	563.7	2180	121.0	134.0
	10	-	-	81.67	135.8

'-' indicates no reliable estimates were calculable

6 GENERAL TOXICOLOGY

6.1 SINGLE-DOSE TOXICITY

The following studies were previously reviewed by Dr. Amy Ellis with the initial IND submission. The review is attached in the Appendix.

- A Single-Dose Intravitreal Toxicity Study with or without Vitrectomy (with an 8-Week Observation Period) in Dutch-Belted Rabbits (Study # 500046)
- A Single-Dose Intravitreal Toxicity Study of Inactivated Microplasmin for Injection (with a 2-Week Observation Period) in Dutch-Belted Rabbits (Study # 500210)
- A Single Intravitreal Toxicity Study (with an 8-Week Observation Period) in Dutch-Belted Rabbits (Study # 57541)
- A Pilot Single Intravitreal Toxicity Study (with a 1-Week Observation Period) in Dutch- Belted Rabbits (Study # 57540)
- A Single Intravitreal Injection Toxicity Study (with an 8-Week Observation Period) of (b) (4) Microplasmin for Injection in the Cynomolgus Monkey (Study # 57544)
- A Pilot Single Intravitreal Injection Toxicity Study (with a 1-Week Observation Period) in Cynomolgus Monkeys (Study # 57543)

The following studies were previously reviewed by Dr. Maryam Rafie-Kolpin with the initial IND submission. The review is attached in the Appendix.

Reference ID: 3198420

- A Single-Dose Intravitreal Toxicity Study of [redacted] (b) (4) Microplasmin for Injection in the Göttingen Mini-Pig Followed by an 8-Week Observation Period (Study # 500209)
- An Intravitreal Bridging Study of Two Formulations of [redacted] (b) (4) Microplasmin for Injection in Göttingen Mini-Pigs with an 8-Week Observation Period (Study # 501082)
- Recombinant Human Microplasmin Acute Intravenous Toxicity Test in Rats (Study # 501684)

REPEAT-DOSE TOXICITY

The following studies were previously reviewed by Dr. Amy Ellis or Dr. Maryam Rafie-Kolpin with the initial IND submission. The reviews are attached in the Appendix.

- Recombinant Human Microplasmin Intravenous Infusion Maximum Tolerated Dose Study in Dogs (Study # 662293)
- Microplasmin 14-Day Intravenous Infusion Toxicity Study in Dogs (Study # 662314)
- Microplasmin 14-Day Intravenous Infusion Toxicity Study in Rats (Study # 21858 or 662911)

The following additional studies were submitted subsequently and not previously reviewed.

Study title: A Multiple Dose Intravitreal Toxicity Study of Microplasmin in the Cynomolgus Monkey with a 12-Week Observation Period

Study no.:	570221
Study report location:	Module 4
Conducting laboratory and location:	[redacted] (b) (4)
Date of study initiation:	March 3, 2008
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	Microplasmin, lot # PD07034, 85.4% pure by SDS-PAGE, 84% by reverse-phase HPLC, 85.9% by size-exclusion HPLC

Key Study Findings

- Ocular changes included lens subluxation, transient inflammation, miosis, iridodenesis (quivering of the iris), and vitreal opacities (expected pharmacological effect due to vitreous liquefaction) observed at both microplasmin doses.

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- The incidence and severity of the subluxation increased with repeated dosing. It was observed in only 7 animals and of slight severity on Day 27 after the first dose. After the second dose on Day 28, however, it was observed in all treated eyes with severity of slight to severe.
- Changes to ERG waveform amplitudes and implicit times were noted at both doses. There were signs of recovery at both doses.
- Group 3 (75 µg/eye) was originally planned to receive 3 doses (Day 1, 28, and 58). Because of the lens subluxation in all treated eyes (attributed to the cumulative proteolytic activity of the repeated injections of the test article on the lens zonules) and inflammation (exhibited by anterior chamber inflammation, uveitis, miosis and hazy fundus view), the third dose was not administered.
- Histopathology findings after two intravitreal doses included vitreous liquefaction, degeneration/disruption of the hyaloideocapsular ligament (with loss of ciliary zonular fibers), lens degeneration, mononuclear cell infiltration of the vitreous, and vacuolation of the retinal inner nuclear cell layer. Eye enlargement, optic disk cupping with gliosis, single cell necrosis and edema, iris and ciliary body atrophy, anterior chamber angle closure, and atrophy of the retinal neuronal cell layer were consistent with the development of glaucoma.
- A NOAEL was not established in this study.

Methods

Doses:	0, 75, and 125 µg/eye, unilateral, corresponding to 0, 41, and 68 µg/mL, based on the vitreous volume (1.83mL)
Frequency of dosing:	Day 1 and Day 28
Route of administration:	Intravitreal
Dose volume:	50 µL
Formulation/Vehicle:	0.9% NaCL
Species/Strain:	Cynomolgus monkeys
Number/Sex/Group:	6 males/group
Age:	2-3 yrs old
Weight:	2.2-3.0 kg
Satellite groups:	None
Unique study design:	The test article was administered to the right eye and control article to the left eye. Group 1 (75 µg) and Group 2 (125 µg) were administered the test article on Days 1 and 28. Group 3, also treated with 75 µg/eye, was originally planned to receive 3 doses (Day 1, 28, and 58). However, due to treatment related effects, Group 3 monkeys did not receive the Day 58 dose. All monkeys were sacrificed on Day 85.
Deviation from study protocol:	None with an impact on the interpretation of the data

Observations and Results

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Mortality (Daily): None

Clinical Signs (Daily): Pupil constriction in the treated eye was noted in all animals for several days following the intravitreal injection. The authors noted that the miosis was most likely associated with the inflammation that improved over time (see below).

Body Weights (Weekly): All groups had similar weights, which suggests there were no test article-related effects. However, in absence of a vehicle treated group, it is not possible to clearly determine is there was a test-article effect.

Feed Consumption (Daily): The data was reported as reduced appetite. Three animals in each group showed reduced appetite. The data, as presented, was not very informative to allow determination of a quantitative effect. In addition, there was no vehicle-control group for comparison.

Ophthalmoscopy [Pre-treatment, and on Days 6, 27, 34, (Day 33 for animals # 105, 106, 205, 216, 305 and 306), 41, 57, 70 and 83]: No significant lesions were noted in the left eye (control article). Lens subluxation was observed in the microplasmin treated eyes in 7/18_ animals after one ITV injection and in all animals following a second ITV injection. All lenses remained subluxated until the end of the study. The incidence and severity per animal is shown in Table 7.

Table 7: Incidence/Severity of Subluxated Lens in Monkeys after a Single or Second Intravitreal Dose (Study # 570221)

Animal Number	Day 6	Day 27*	Day 33/34	Day 41	Day 57	Day 70	Day 83
101			Slight	Moderate	Moderate	Moderate	Moderate
102			Slight	Moderate	Moderate	Moderate	Moderate
103			Slight	Slight	Slight	Slight	Slight
104			Slight	Slight	Slight	Slight	Slight
105			Slight-Moderate	Moderate	Moderate	Moderate	Moderate
106		Slight	Slight-Moderate	Moderate	Moderate	Moderate	Moderate
201			Very Slight	Slight	Slight	Slight	Slight
202			Slight-Moderate	Slight-Moderate	Moderate	Moderate	Moderate
203		Slight	Moderate	Moderate	Moderate	Moderate	Moderate
204		Slight	Severe	Severe	Severe	Severe	Severe
205	Slight	Slight	Moderate	Severe	Severe	Severe	Severe
216			Slight	Slight	Slight	Slight	Slight
301			Slight-Moderate	Slight-Moderate	Moderate	Moderate	Moderate
312		Slight	Slight	Slight	Slight	Slight	Slight
303		Very Slight	Slight	Slight	Slight	Slight	Slight
304			Moderate-Severe	Moderate	Moderate	Moderate	Moderate
305			Moderate-Severe	Moderate	Severe	Severe	Severe
306		Very Slight	Slight	Moderate	Moderate	Moderate	Moderate

* Second dose administered on Day 28

Animal #s 201-205 and 216 received 125µg/eye; all remaining animals received 75 µg/eye

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This finding suggested a lack of pulling effect from the damaged lens zonules (see histopathology findings below) creating rounding of the lens, increasing its refractive power (myopia). Iridodenesis was noted on pre-mydriatic exam in most animals with lens subluxation, as expected due to the lack of support from the lens. The iridodenesis was not observed in lenses not subluxated.

In addition to those described above, other findings observed are listed in the tables below. Transient signs of anterior uveitis observed at the beginning of the study (very slight to moderate) were subsequently graded as very slight to slight at the completion of the study. The combination of the uveitis and the incomplete pupil dilation made the fundus view hazy for several animals. Anterior chamber flare was not observed by study completion. The lenticular changes described as opacities were suspected by the authors to be secondary to the chronic lens subluxation rather than as a direct effect of ocriplasmin. Vitreal opacities (band-like) were noted in most eyes and were interpreted as an exaggerated pharmacodynamic effect of ocriplasmin (change in the physical nature of the vitreous).

Table 8: Summary of Group 1 and 3 (75 µg/eye) Ophthalmology Observations in Monkeys after a Single or Second Intravitreal Dose (Study # 570221)

Observation	Occasion (Day)						
	6	27*	33/34	41	57	70	83
Anterior chamber cells	1	1	10	10	10	7	4
Anterior chamber flare and/or flare cells	11	-	4	1	-	-	-
Fundus view limited and/or hazy	10	5	8	8	6	4	4
Pupil middilated postmydriatic	12	3	8	8	6	5	2
Retina/choroid pigment variation (incl. depigmentation and/or hyperpigmentation)	7	-	-	-	1	1	1
Vitreal opacities (band-like and/or focal and/or multifocal and/or diffuse)	5	12	12	12	12	12	11
Posterior capsule lens opacity (focal)	-	-	-	-	-	-	1
Anterior capsule lens opacity (focal and/or multifocal and/or pigmented multifocal)	-	-	1	1	4	6	7

* Second dose administered on Day 28

Reference ID: 3198420

Table 9: Summary of Group 2 (125 µg/eye) Ophthalmology Observations in Monkeys after a Single or Second Intravitreal Dose (Study # 570221)

Observation	Occasion (Day)						
	6	27*	33/34	41	57	70	83
Anterior chamber cells	2	-	6	6	5	4	4
Anterior chamber flare and/or flare cells	4	-	2	2	-	-	-
Fundus view limited and/or hazy	5	2	5	4	4	4	4
Pupil middilated postmydriatic	5	-	5	5	4	2	2
Retina/choroid pigment variation (hyperpigmentation, multifocal and/or focal)	3	2	-	-	-	-	-
Vitreal opacities (band-like and/or multifocal)	2	6	6	6	6	6	6
Anterior capsule lens opacity (focal and/or multifocal)	1	1	1	1	1	2	4
Pupil dyscoria (abnormal shape of pupil)	-	-	1	3	3	1	1
Enlarged globe (buphthalmos)	-	-	-	-	-	1	1

* Second dose administered on Day 28

Tonometry [Day 1 postdose and Days 6, 27, 34, (Day 33 for animal #105, 106, 205, 216, 305 and 306), 41, 57, 70 and 83]: All animals (control and test-article treated) showed an increase in IOP immediately after the intravitreal injection. This increase reversed before the next scheduled measurement. Two high-dose animals showed increases in IOP at later timepoints, after the second injection. Animal # 204 had increased IOP on Day 33/34 that persisted until study completion, with the maximum reading of 52 mmHg in the treated eye compared to 15 mmHg noted on Day 41. Animal # 205 showed an increased IOP on Day 41. The increased IOP for this animal also persisted until study termination, with the highest IOP noted on Day 83 (46 mmHg compared to 22 mm Hg in the control eye).

Electroretinography (Pretreatment and on Days 7, 35, 63 and 84): Adverse effects on the retina were apparent at both doses on Day 7 and/or Day 35 (reductions in photopic/scotopic a- and/or b-wave amplitudes; increases in scotopic b-wave implicit times). By Day 63 and/or Day 84, signs of recovery (improvements of wave forms and implicit times) were evident across groups (except for 1/12 animals at 75 µg/eye and 2/6 animals at 125 µg/eye group as described below).

For low dose animal # 303, retinal detachment was observed during the ophthalmology evaluations and confirmed by histopathology. This detachment and retinal changes prior to the detachment were detected in the ERG waveforms. Scotopic b-wave amplitude at -30dB could not be detected, and scotopic a-wave and/or b-wave amplitudes were greatly reduced compared to the remaining animals. By Day 84, scotopic a-wave and/or b-wave amplitudes could not be detected. Photopic changes were generally detected, but were markedly diminished compared with pre-treatment values.

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High dose animals # 204 and 205 were noted during ophthalmology evaluation with an increased IOP and severe subluxated lens. Enlarged globe (buphthalmos) was noted during ophthalmology evaluations for animal # 204 and for both animals at macroscopic examination. These changes coincided with ERG waveform changes, as scotopic a-wave and/or b-wave amplitudes were diminished or could not be detected from Day 35.

Gross Pathology (Day 85; full necropsy): Loose abnormal iris movement, loose lens, watery (liquified) vitreous and globe enlargement were considered related to ocriplasmin treatment. The loose abnormal iris movement (termed iridodenesis, clinically) was noted at necropsy and was associated with lens subluxation. The table below presents the incidence of these findings.

Table 10: Incidence of Ocriplasmin-Related Macroscopic Findings in Monkeys after Two Intravitreal Doses 28 Days Apart (Study # 570221)

	Males					
Tissue/Finding	Right Eye-Treated			Left Eye-Control		
Dose (µg/eye)	75	125	75	0	0	0
Group	1	2	3	1	2	3
Number of animals examined	6	6	6	6	6	6
Eye						
Loose, lens/abnormal iris movement	2	5	4	—	—	—
Watery (liquified), vitreous	6	6	5	2	1	2
Enlargement	—	2	—	—	—	—

	DOSE GROUP:	**1**	**2**	**3**
ORGAN/FINDING	**ANIMALS EXAMINED:**	**6**	**6**	**6**

Histopathology (Eyes only)

Adequate Battery: Yes, given the weight of evidence supporting significant systemic exposure is unlikely after two ITV doses up to 125 µg given 28 days apart.

Peer Review: No

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Histological Findings: Table 11 shows the microscopic findings in the eye considered related to ocriplasmin. These included degeneration/disruption of the hyaloideocapsular ligament (minimal to severe), lens degeneration (minimal to slight), mononuclear cell infiltration of the vitreous (minimal to slight) and vacuolation of the retinal inner nuclear cell layer (minimal to severe).

Table 11: Incidence and Severity of Ocriplasmin-related Histopathological Findings in Monkeys after Two Intravitreal Doses 28 Days Apart (Study # 570221)

Tissue/Finding		Males					
		Right Eye-Treated			Left Eye-Control		
	Dose (µg/eye)	75	125	75	0	0	0
	Group	1	2	3	1	2	3
Eye	Number examined	6	6	6	6	6	6
Degeneration/disruption: hyaloideocapsular ligament	Total Number affected	5	6	6	0	0	0
	Minimal	0	1	0	—	—	—
	Slight	1	2	0	—	—	—
	Moderate	4	2	2	—	—	—
	Marked	—	1	3	—	—	—
	Severe	—	—	1	—	—	—
Degeneration: lens							
	Total Number affected	6	6	6	2	3	0
	Minimal	1	1	0	2	3	—
	Slight	5	5	6	—	—	—
Infiltration: mononuclear cell (vitreous)							
	Total Number affected	2	6	6	0	0	0
	Minimal	2	6	5	—	—	—
	Slight	—	—	1	—	—	—
Vacuolation: retina							
	Total Number affected	0	2	2	0	0	0
	Minimal	—	1	1	—	—	—
	Slight	—	1	0	—	—	—
	Severe	—	—	1	—	—	—

The degeneration/disruption of the hyaloideocapsular ligament was accompanied by loss of the ciliary zonular fibers and/or presence of vitreous in the posterior and/or anterior chamber of the eye. The lens degeneration in the treated eyes (right) was present anteriorly and/or posteriorly, whereas in the control eyes (left) the lens degeneration was only present posteriorly. The authors noted the lens degeneration in the treated eyes could possibly be secondary to lens subluxation.

Changes in the eye considered by the authors as indicative of buphthalmos (moderate optic papilla cupping with gliosis, single cell necrosis and edema of the optic nerve; slight to marked iris and ciliary body atrophy; marked to severe filtration angle closure; and slight atrophy of the retinal neuronal cell layer) occurred in 2/6 monkeys given 125 µg microplasmin. These two monkeys (# 204 and 205) showed increased

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IOP until study termination and severe lens subluxation. The buphthalmos corresponds to the macroscopic finding of eye enlargement.

One eye given 75 µg microplasmin (#303) had marked retinal detachment with concurrent severe retinal vacuolation and hypertrophy of the retinal pigment epithelium, as well as other microscopic findings of lens degeneration, marked degeneration/disruption of the hyaloideocapsular ligament and slight mononuclear cell infiltration of the vitreous body.

Other single incidence findings consisted of single cell necrosis of the retinal outer nuclear cell layer (minimal) together with vacuolation of the inner nuclear cell layer (minimal) in one eye given 75 µg microplasmin, focal chronic inflammation of the iris with proliferation of pigmented cells in one eye given 75 µg microplasmin (slight) and posterior synechia in one eye given 125 µg microplasmin.

The incidence of these findings is shown in the table below. As noted above, the control (left) eye only showed degeneration of the lens in 2 and 3 animals from Groups 1 and 2, respectively. The dilation of the perivascular space (optic nerve) was observed in the left (control) and right (ocriplasmin-treated eye in one animal from Group 1. This finding was, therefore, considered unrelated to ocriplasmin treatment.

Table 12: Incidence of Ocriplasmin-Related Microscopic Findings in Monkeys after Two Intravitreal Doses 28 Days Apart (Study # 570221)

DOSE GROUP		**1**	**2**	**3**
NUMBER OF ANIMALS EXAMINED		**6**	**6**	**6**
EYE, RIGHT	EXAMIN:	6	6	6
- Degeneration: lens		6	6	6
- Degeneration/disruption: hyaloideocapsular ligament		5	6	6
- Fold/rosette: retina		1	1	-
- Necrosis: single cell		-	-	1
- Dilatation: perivascular space		1	-	-
- Vacuolation: retina		-	2	2
- Closed: filtration angle		-	2	-
- Atrophy: ciliary body		-	2	-

Table 12 (Cont.)

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DOSE GROUP NUMBER OF ANIMALS EXAMINED		1 6	2 6	3 6
EYE, RIGHT	CONT'D.	6	6	6
- Atrophy: iris		-	2	-
- Cupping: optic papilla		-	2	-
- Atrophy: retina		-	2	-
- Synechia		-	1	-
- Detachment: retina		-	-	1
- Inflammation: chronic		-	-	1
- Infiltration: mononuclear cell		2	6	6

Dosing Solution Analysis: Analysis was performed for the formulations used to treat Group 1 and 3. All dose formulations were within 5.3-12.0% of the theoretical targeted concentration (1.5 mg/mL).

The ocriplasmin drug substance has been manufactured (b) (4) (b) (4) during the course of development. Non-clinical, Phase 1 and Phase 2 studies were conducted using ocriplasmin drug substance (b) (4) Ocriplasmin drug substance (b) (4) was used for the manufacture of drug product which was subsequently used in Phase 2 and Phase 3 clinical trials. The following non-clinical study was a bridging study (b) (4)

Study title: A Single Dose Intravitreal Toxicity Study of Microplasmin in the Cynomolgus Monkey with a 4-Week Observation Period

Study no.: 570256
Study report location: Module 4
Conducting laboratory and location: (b) (4)
Date of study initiation: July 31, 2008
GLP compliance: Yes
QA statement: Yes
Drug, lot #, and % purity: Ocriplasmin:
Batch # PD07034, 85.4% pure by SDS-PAGE,

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84% by reverse-phase HPLC, 85.9% by size-exclusion HPLC; Batch M-PLA-P08-FOR/1.875 (also known as P-08)

The % purity for batch P-08 was not specified in the study report. However, the certificate of analysis was submitted under Clinical Study # TG-MV-003: >95% pure by SDS-PAGE.

Key Study Findings

- Lens subluxation was observed in 2 of 6 eyes that received 125 µg/eye of the initial batch, # PD07034 (Group 4). There was no similar effect on eyes that received 125 µg/eye of the new batch, # P08 (Group 2).
- Transient miosis and uveitis were observed in all groups.
- One Group 4 female showed iridodenesis.
- A more liquefied vitreous humor was observed in the eyes receiving either of the test articles. This is consistent with the pharmacological action.
- No treatment related histopathological changes were observed.
- Based on the finding of lens subluxations and higher incidence and duration of miosis, the new batch was associated with less adverse effects compared to the initial batch.
- A NOAEL was not determined in this study.

Methods

Doses:	Group 1: 25 µg/eye batch # P08 Group 2: 125 µg/eye batch # P08 Group 3: 25 µg/eye batch # PD07034 Group 4: 125 µg/eye batch # PD07034
Frequency of dosing:	Single dose
Route of administration:	Intravitreal
Dose volume:	50 µL
Formulation/Vehicle:	0.9% NaCl
Species/Strain:	Cynomolgus monkeys (*Macaca fascicularis*)
Number/Sex/Group:	3
Age:	2.5-4 years old
Weight:	2.2-3.9 kg
Satellite groups:	None
Unique study design:	This bridging study compared the test article formulation already utilized in prior clinical trials ((b) (4) Batch # PD07034), to the test article formulation intended for use in future clinical trials (liquid formulation; Batch # P08).

The test article was administered to the right eye and control article to the left eye.

All monkeys were sacrificed on Day 28.

Deviation from study protocol: None with an impact in the interpretation of the data

Observations and Results

Mortality (Twice daily): None

Clinical Signs (Twice daily for signs of ill health or reactions to treatment; weekly detailed physical examination): Constricted pupil in the ocriplasmin-treated eye was noted in all Group 3 and 4 animals (both treated with the initial batch) on the weekly observations made on Days 7, 14, and 21.

Body Weights (Weekly): In males and females, all four treatment groups had similar weights, which suggests there were no test article-related effects. However, in absence of a vehicle treated group, it is not possible to clearly determine if there was a test-article effect.

Feed Consumption (Daily by visual inspection): The data was reported as reduced appetite. Reduced appetite was observed in 1-3 females at both doses of both ocriplasmin batches. The data as presented was not very informative to allow determination of a quantitative effect. In addition, there was no vehicle-control group for comparison.

Ophthalmoscopy (Pre-treatment, and on Days 2, 7, 15 and 28): No significant lesions were noted in the left eye (control article).

At the pre-mydriatic examination on Day 2, all ocriplasmin-treated eyes (right) showed very slight to slight miosis. All but one right eye still had miosis pre-mydriatic on Day 7. On Day 15, 4 eyes in Group 1, 1 eye in Group 2, 5 eyes in Group 3 and 3 eyes in Group 4 had a very slight degree of miosis pre-mydriatic. One Group 4 female (# 453) showed iridodenesis on Days 15 and 28. The pupils were normal in all other animals on Day 28.

Post-mydriatic, there was a higher incidence of eyes with pupil mid-dilated in eyes treated with the initial batch compared to those treated with the new batch (*i.e.* 1, 2, 3, and 3 for Groups 1, 2, 3, and 4, respectively). The time to recover was also longer in the eyes treated with the initial batch (Day 15 for Groups 1 and 2 vs. Day 28 for Group 3 and 4).

Uveitis (slight to moderate anterior chamber cells) was observed in all groups and was transient (completely resolved by Day 28). Lens subluxations were noted in two of six Group 4 treated eyes (125 µg/eye initial batch). A similar effect was not

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observed in eyes that received 125 µg/eye of the new batch (Group 2). In Group 4 female #453, lens subluxation was noted on Day 7 (graded slight). This finding persisted until the end of the study with an increase in severity to moderate on Day 15 and 28. Group 4 male # 403 showed very slight lens subluxation on Day 28. In this second animal, a change in the refraction state was noted on Day 15 suggesting weakening of the lens zonules. Band-like vitreal opacities were noted in most eyes starting on Day 7 for all groups except for Group 4 (observed as early as Day 2) that persisted throughout the study. This finding is an expected pharmacological response of the test article (change in the physical nature of the vitreous).

Tonometry (Pre-treatment, and on Days 2, 7, 15 and 28): No test article-related effect was apparent.

Gross Pathology (Day 28): The vitreous humor of the treated (right) eyes was observed to be of a watery (liquefied) consistency. This correlated well with the pharmacological action of the test articles. No major difference in the incidence of this change was demonstrated between the 2 formulations or between dose levels. A similar observation was also made in a small number of control eyes (left). A rationale for the presence of watery vitreous in the control eyes was not given. The incidence of findings is summarized in table 13.

Table 13: Incidence of Treatment-Related Macroscopic Findings in Monkeys after a Single ITV Dose of Two Ocriplasmin Formulations (Study # 570256)

Tissue/Finding	Sex	Male				Female			
	Group	1	2	3	4	1	2	3	4
	Number of animals examined	3	3	3	3	3	3	3	3
Treated (right) eye									
Vitreous humour watery (liquefied)		2	3	3	3	1	3	2	3
Control (left) eye									
Vitreous humour watery (liquefied)		-	-	2	1	-	-	-	-

Group 1: 25 µg/eye batch # P08; Group 2: 125 µg/eye batch # P08; Group 3: 25 µg/eye batch # PD07034; Group 4: 125 µg/eye batch # PD07034

Histopathology (Eyes only):

Adequate Battery: Yes, given the objective of the study was to compare the ocular toxicity of two ocriplasmin clinical batches.

Peer Review: No

Histological Findings: Focal retinal degeneration was noted in one Group 3 male at, or near, the injection site. Given that this was the only incidence, this finding was considered to be a consequence of the injection procedure and not related to the test article.

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Dosing Solution Analysis: Analysis was performed for the formulations used to treat Group 1 and 3. Both dose formulations were within -2.6 to -1.8% of the theoretical targeted concentration (0.5 mg/mL). The test article as provided was used for dosing Group 2 (2.5 mg/mL) and Group 4 (2.5 mg/mL).

The following range-finding IV study conducted in rats was submitted with the NDA.

Recombinant Human Microplasmin 7 Day Intravenous Dose Range Finding Study in Rats(Study # 662288) - This study was conducted to select the highest dose for Study # 21858 (see Dr. Rafie-Kolpin review in the Appendix) - Sprague-Dawley rats (6/sex/group) received ocriplasmin at dose levels of 0, 10, 20, and 40 mg/kg/day for up to 7 days. The test article or vehicle solution was administered into the lateral tail vein *via* an initial IV loading dose over 15 min followed by a 1-hr IV infusion.

At 40 mg/kg/day, animals were sacrificed due to the severity of their clinical condition within 1-4 days of study initiation. Ocriplasmin was associated with adverse clinical observations (discolored/scabbed tails at all doses; hunched/pale appearance, subdued behavior, liquid feces, labored respiration, prostration, unkempt coat and cold to touch at 40 mg/kg/day) and decreased body weights (≥20 mg/kg/day). There were hematology (dose-depended decrease in hemoglobin, red blood cell count, and hematocrit at all doses; increased reticulocytes and WBC at ≥20 mg/kg/day) and gross necropsy changes (reddening of several internal organs at ≥20 mg/kg/day; reddening of the skin/subcutis at 40 mg/kg/day; reddening of the thymus at ≥10 mg/kg/day; reddening/scabbing a the injection site at all doses) considered related to the thrombolytic nature of microplasmin. From the results obtained on this study, the highest dose level used on a subsequent 14-day toxicity study was 10 mg/kg/day.

7 GENETIC TOXICOLOGY

Genetic toxicology studies have not been conducted with ocriplasmin. These are not generally required for biologic products.

8 CARCINOGENICITY

Animal carcinogenicity studies have not been conducted with ocriplasmin. Carcinogenicity studies with microplasmin were not considered necessary as the applicant was not seeking a clinical indication entailing chronic use.

9 REPRODUCTIVE AND DEVELOPMENTAL TOXICOLOGY

Animal reproduction studies have not been conducted with ocriplasmin. Reproductive and developmental toxicity studies were not conducted because the weight of evidence supports that significant systemic exposure is not expected in humans following a single intravitreal injection of 125 µg ocriplasmin (see Section 5).

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10 SPECIAL TOXICOLOGY STUDIES

Toxicology Study of Microplasmin and *Pichia Pastoris* Yeast Expression System Products in the Rat (Study # R02-TX-002) - This was an observational study (based on abnormal behavior) to investigate toxic effects of microplasmin or residual co-purified components of the *Pichia pastoris* yeast expression system. Female Wistar rats (6/group) received 15 mg/kg of ocriplasmin (batches Tox-E-µPLA-P03 or Tox-E-µPLA-P05), *Pitchia pastoris* extract (batch L-µPLA-P-PL-02), or solvent (b) (4) citric acid, (b) (4) mannitol in saline) at pH 7.0 or pH 3.2. Half of a total volume of 1.5 mL was administered as a slow IV bolus (over 12 min) and the other half infused IV over 1 hr. These infusions were repeated every 24 hrs during 3 days while behavior was scored.

Ocriplasmin caused abnormal behavior on Days 1 and 2 (reduced reactions, shaking, limited use of the left front paw, laying down, irregular breathing, teeth gritting, stretching, diarrhea, etc). No abnormalities were observed on Day 3. Extract of *Pichia Pastoris* yeast expression system and both solvents did not cause any abnormal behavior. The applicant concluded the results suggest that the adverse events observed after 15 mg/kg ocriplasmin were not related to the vehicle or residual co-purified *Pichia pastoris* components, but rather to the supra-pharmacological doses of ocriplasmin. The reviewer believes that due to the limited evaluation conducted, the data support but is not definitive to conclude that contaminants resulting from the yeast expression system may not have adverse effects.

Immunogenicity: The applicant stated that during the non-clinical program, the immune response was monitored in rats and dogs after IV administration of ocriplasmin using validated assays for rat and dog IgGs against ocriplasmin. However, the only data submitted with the BLA was the validation study report summarized below. Ocular immunogenicity was not evaluated in the animal studies. Except for the monkey study where 2 intravitreal doses were administered, all pivotal studies used a single dose. A strong immune response is not expected after a single ocular dose.

Validation of ELISA Methodology to Detect Dog and Rat IgG Antibodies to Microplasmin (Study # 767995) - The primary objective of the study was to validate the Elisa method developed to detect antibodies to microplasmin. Two serum samples from animals treated with the high dose (10 mg/kg every other day) in the 14-day IV repeated-dose study in dogs (Study # 662314) and rats (Study # 662288) were analyzed. The samples were diluted 1:100 to 1:6400. In dogs, one of the two samples showed a positive anti-drug antibody response at dilutions down to 1:1600. Neither of the two samples from the high-dose rats showed a positive response.

11 INTEGRATED SUMMARY AND SAFETY EVALUATION

A range of ocular findings were observed in nonclinical ocular toxicity studies of a single-dose administration to rabbits, monkeys, and minipigs, and repeated-dose (2

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doses 28-days apart) in monkeys. The rabbit was the most sensitive species to ocriplasmin-induced ophthalmologic changes, followed by the monkey. The minipigs showed less sensitivity. After a single intravitreal injection, most findings were reversible except for lens subluxation. Narrowing of retinal vessel with associated retinal atrophy was specific to the rabbit. A second intravitreal administration of ocriplasmin (28-days apart) in monkeys was associated with an increase incidence of lens subluxation, sustained increases in IOP, and a series of adverse microscopic findings in the eye. The lens subluxation was associated with degeneration/disruption of the hyaloideocapsular ligament observed microscopically, accompanied by loss of the ciliary zonular fibers. The observed lens subluxation is considered a consequence of the proteolytic activity of ocriplasmin. In monkeys and minipigs, vitreous gel breakdown was reported, an expected pharmacological action of the test article.

The applicant hypothesized that the increased sensitivity of the rabbit retina, and the monkey to a lesser extent, may be related to the smaller vitreous volume resulting in the delivery of high concentration bubble closer to the retina in the rabbit. The minipig eye more closely resembles the human eye in size and showed the least sensitivity. The reviewer agrees it is plausible that anatomical differences in the vitreous volume may have contributed to species differences in sensitivity to the toxic effects of ocriplasmin in the retina.

The adverse findings observed are described in more detail below:

Narrowing of retinal vessels: The intravitreal injection of ocriplasmin caused narrowing of the retinal vessels in Dutch-Belted rabbits at doses as low as 2.5 µg/eye. The finding was observed on Day 28 at 2.5 µg/eye, and as early as Day 2 postdose at ≥50 µg/eye. The finding was reversible by Day 56 at 2.5 µg/eye, but persisted through Day 56 at doses ≥50 µg/eye, although the severity had decreased indicating partial recovery. Narrowing of the retinal vessels was not observed in cynomolgus monkeys or minipigs at doses up to 125 µg/eye

Lens subluxation: This finding was observed in all 3 species (rabbits, minipigs, and monkeys). In rabbits, it was observed at ≥ 50 µg/eye as early as Day 14 postdose. In minipigs, lens subluxation was observed in one animal at 125 µg/eye on Day 28 postdose. In monkeys, lens subluxation was observed with low incidence on Day 6 postdose at 125 ug/eye and with increased incidence at ≥75 µg/eye on Day 27 postdose. Five to six days after a second injection (28 days apart), lens subluxation was observed in all eyes at ≥75 µg/eye. In monkeys, degeneration/disruption of the hyaloideocapsular ligament was observed microscopically, and it was accompanied by loss of the ciliary zonular fibers.

Loose lens and iridodenesis: Consistent with the observation of lens subluxation and loss of ciliary zonular fibers, loose lens and iridodenesis were observed in monkeys administered two intravitreal doses ≥75 µg/eye 28-days apart.

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Changes in IOP: These changes were observed in rabbits and monkeys. In rabbits, elevated IOP was observed in animals with and without lens subluxation. In some high dose (200 µg) animals the IOP remained elevated through the end of study (8 weeks), however reversed on Day 7 in the low dose animals (62.5 µg). In one of the high dose animals, cupping of the optic nerve (secondary to elevated IOP) was noted. In monkeys, reductions in IOP were observed at ≥25 µg/eye in one study, which resolved by Day 27. In another study, increased IOP was observed in 2 monkeys receiving two 125 µg/eye doses (28 days apart) that also had severe lens subluxation. These 2 monkeys also showed cupping of the optic nerve. The IOP increase in these two animals persisted through study termination.

Inflammation: Dose-related inflammation including uveitis was observed in both rabbits and monkeys at all doses (≥2.5 µg/eye and ≥1.5 µg/eye, respectively), but the manifestations of inflammation (aqueous flare, presence of vitreous and/or aqueous cells, and/or foveal pigment changes) were transient if adequate recovery time was allowed. In monkeys, the observed inflammation was associated with incomplete pupillary dilation or pupil constriction (miosis).

ERG Changes: This finding was observed in rabbits and monkeys. The changes consisted of decreases in scotopic/photopic a- and b-wave amplitudes (both species) and increases in scotopic a- and b-wave implicit time (monkeys). ERG responses were diminished within 2 days of microplasmin injection at doses ≥50 µg/eye in rabbits and ≥20 µg/eye monkeys. At ≤50 µg/eye, there was substantial recovery of ERG responses by Days 7-14. At higher doses (≥62.5 µg/eye), the majority of the rabbits had ERG responses close to baseline by the end of an 8-week observation period. Similarly in monkeys, this effect was reversed within an 8-week recovery period with substantial recovery occurring in many animals during the first few weeks. In monkeys administered a second intravitreal injection (28-days apart) at dose levels of 75 and 125 µg/eye, signs of recovery were evident across groups during an 84-day observation period, except for one low-dose animal with retinal detachment and two high-dose animals with severe subluxated lens.

Retinal Atrophy and Other Microscopic Findings: In rabbits, single intravitreal doses of ≥ 50 µg/eye were associated with minimal to moderate retinal atrophy (thinning, or in more severe cases, an absence of the rod and cone layer and outer nuclear layer of the retina). This may have been related to a reduced blood supply due to the more persistent narrowing of the retinal vessels caused by these doses of ocriplasmin.

Accumulation of macrophages and eosinophilic material was observed in the vitreous body and/or anterior chamber in both rabbits (≥ 50 µg/eye; minimal to moderate) and monkeys (20 and 200 µg/eye; minimal). The eosinophilic material was believed to be either test article or fibrin exudate.

One monkey administered a single dose of 200 µg/eye developed a hyphema several days after injection and also exhibited moderate inflammation and slight retinal atrophy of the photoreceptor and outer nuclear layers. It was uncertain as to whether

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these changes were directly related to test article, secondary to inflammation caused by the test article, or to trauma that occurred during the observation period.

In addition to the findings noted above, a number of microscopic findings were observed in monkeys administered 2 doses (28-days apart) of microplasmin and sacrificed after a 12-week observation period. The main findings include degeneration/disruption of the hyaloideocapsular ligament (minimal to severe), lens degeneration (minimal to slight), and mononuclear cell infiltration of the vitreous (minimal to slight) in most monkeys at 75 and 125 µg/eye, and vacuolation of the retinal inner nuclear cell layer (minimal to severe) in two monkeys each at 75 and 125 µg/eye. One eye given 75 µg microplasmin had marked retinal detachment with concurrent severe retinal vacuolation and hypertrophy of the retinal pigment epithelium. Changes in the eye that were indicative of buphthalmos (moderate optic papilla cupping with gliosis, single cell necrosis and edema; slight to marked iris and ciliary body atrophy; marked to severe filtration angle closure; and slight atrophy of the retinal neuronal cell layer) occurred in the two monkeys given two doses of 125 µg/eye microplasmin that showed increased IOP until study termination and severe lens subluxation.

The applicant made the statement that pathological changes related to intraocular hemorrhage were observed in rabbits and monkeys, and it remains unclear if this hemorrhage is related to the injection procedure itself or a pharmacologic effect of ocriplasmin. The reviewer believes the applicant refers to the following observations. In vitrectomized rabbits, vitreous hemorrhage was observed with higher incidence in ocriplasmin treated eyes (1/4, 4/4, and 2/4 at 0, 300, and 600 µg/ye, respectively). As noted above, one monkey showed a red eye and a hyphema at 200 µg/eye. Because the vitrectomy surgical procedure could also lead to vitreous hemorrhage, and the incidence of only one in monkeys, the reviewer agrees that it is difficult to definitively attribute these findings to ocriplasmin.

The applicant provided the following table summarizing the exposure margins for a single intravitreal clinical dose of 125 µg and assuming a vitreous volume of 4.36 mL in humans. The reviewer agrees with the selected lowest doses at which findings were observed in the animals after a single intravitreal dose, except for the dose at which inflammation was observed in monkeys, i.e., 1.5 µg/eye (the nominal dose was 5 µg/eye), instead of 25 µg/eye.

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Table 14: Exposure Margins for Ocriplasmin 125 µg Single Clinical Dose Based on the Lowest Dose at which Findings Occurred in Animals Receiving a Single Dose

Species	Inflammation[a]	ERG Changes	Lens Subluxation	Gross Pathology[b]	Histological Retinal Changes[b]
Lowest concentration of observation (µg/mL)[c]					
Rabbit	**2.3 µg/mL**[d]; 2.5 µg/eye	**45 µg/mL**; 50 µg/eye	**45 µg/mL**; 50 µg/eye	**45 µg/mL**; 50 µg/eye	**45 µg/mL**; 50 µg/eye
Ratio of animal to human concentration	<0.1	1.55	1.55	1.55	1.55
Incidence	3/6 eyes	5/12 eyes	2/12 eyes	7/12 eyes	2/12 eyes
Cynomolgus monkey	**14 µg/mL**[e]; 25 µg/eye	**11 µg/mL**[f]; 20 µg/eye	**41 µg/mL**; 75 µg/eye	**108 µg/mL**; 200 µg/eye	**>108 µg/mL**; >200 µg/eye
Ratio of animal to human concentration	0.48	0.38	1.41	3.72	>3.72
Incidence	14/18 eyes	2/3 eyes	4/12 eyes	1/3 eyes	0/3 eyes
Mini-pig	**>61 µg/mL**; >125 µg/eye	**>61 µg/mL**; >125 µg/eye	**61µg/mL**; 125 µg/eye	**>61 µg/mL**; >125 µg/eye	**>61 µg/mL**; >125 µg/eye
Ratio of animal to human concentration	>2.10	>2.10	2.10	>2.10	>2.10
Incidence	0/3 eyes	0/3 eyes	1/3 eyes	0/3 eyes	0/3 eyes

Abbreviation: ERG, electroretinogram.
[a]Clinical signs of inflammation such as cyclitis, iritis, and uveitis.
[b]Pathological changes such as retinal atrophy or accumulation of macrophages in the vitreous.
[c]Data are represented in concentration per mL vitreous to allow comparison with the human dose of 125 µg/eye, corresponding to 29 µg/mL.
[d]A slight and transient infiltration of vitreous cells was observed at Day 7 by ophthalmoscopy.
[e]Inflammatory cells were detected in the anterior chamber by ophthalmoscopy on Days 2 and 7, but were no longer present at Day 15.
[f]ERG changes induced by a dose of 20 µg/eye (equivalent to 11 µg/mL) showed evidence of recovery by the last day of observation (Day 8). ERG changes induced by a dose of 25 or 125 µg/eye (equivalent to 14 or 68 µg/mL vitreous, respectively) had recovered fully by Day 55.
Data on file, ThromboGenics.

The exposure margins (0.1-1.5-fold) for the findings of inflammation, ERG changes, IOP changes (not included in the table above), and lens subluxation observed in rabbits and monkeys are low. A more favorable safety margin (3.7-fold) was observed for the retinal changes observed in the monkey after a single intravitreal dose. However, except for lens subluxation, the nonclinical findings were reversible after administration of a single intravitreal dose. Based on the limited data available (one study in monkeys), a second ITV dose has the potential to cause more severe (e.g., increased incidence of lens subluxation) and prolonged ocular adverse effects (e.g., ERG changes took longer to recover). Lens subluxation was observed in one pediatric patient. A case of lens instability was observed in an adult patient. The proposed label includes a warning for the potential of lens subluxation to occur.

Safety Pharmacology studies in dogs showed a significant decrease in blood pressure, a slight increased QT and QTcV intervals, and P-wave amplitude, and a slight decrease in tidal volume. Except for P-wave amplitude, all findings showed a trend toward recovery. The exposure margin at the NOEL of 1.5 mg/kg is >130-fold the estimated systemic concentration of 46 ng/mL in humans after a single intravitreal dose, indicating low concern for similar effects in humans. In addition, no effects were

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observed in ECG parameters after a 14-day repeat-dose toxicology study in dogs at IV doses up to 10 mg/kg every other day.

In IV systemic toxicology studies in rats and dogs, the targets identified were consistent with the thrombolytic activity of microplasmin. In a dose-ranging study in rats, there were mortalities at ≥40 mg/kg/day, hematology (dose-depended decrease in hemoglobin, red blood cell count, and hematocrit at ≥10 mg/kg/day; increased reticulocytes and WBC at ≥20 mg/kg/day) and gross necropsy changes (reddening of several internal organs at ≥20 mg/kg/day; reddening of the skin/subcutis at 40 mg/kg/day; reddening of the thymus at ≥10 mg/kg/day; reddening/scabbing a the injection site at ≥10 mg/kg/day). In a follow-up study in rats, no adverse effects were observed at a dose of 10 mg/kg/day administered every other day. This dose was then selected as the NOEL. In dogs, the main findings included a transient increase in clotting time at ≥10 mg/kg/day and mortalities at ≥15 mg/kg/day. Necropsy revealed the mortalities in dogs were also associated with internal hemorrhages/reddening in several organs. The NOEL in the dog was 2 mg/kg every other day. Based on C_{max} in rats (10188 ng/mL) and dogs (31050 ng/mL), respectively, these NOELs are 220- and 675-fold the estimated systemic concentration of 46 ng/mL in humans after an intravitreal clinical dose of 125 µg. Therefore, the nonclinical data provides support to conclude that systemic toxicity of microplasmin is unlikely following a single 125 µg intravitreal injection in humans.

Additional support for the systemic safety of ocriplasmin, at the proposed human dose, is provided by the following observations:

- If ocriplasmin was completely systemically absorbed after intravitreal injection, the amount (46 ng/mL) would be miniscule compared to the amount of plasminogen in the human blood (200 µg/mL).

- If systemic bioavailability of the intraocular dose is 100% (i.e., 4.6 nmol), there would be sufficient α2-antiplasmin present in the blood to neutralize all ocriplasmin, based on the normal plasma concentration of the serine protease inhibitor α2-antiplasmin (1 nmol/mL of plasma),

12 APPENDIX/ATTACHMENTS

Initial IND review, IND 100,370 – Dr. Amy Ellis
Initial IND review, IND 100,370 – Dr. Maryam Rafie-Kolpin

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2.6 PHARMACOLOGY/TOXICOLOGY REVIEW

2.6.1 INTRODUCTION AND DRUG HISTORY

BBIND number: 100370
Review number: 1
Sequence number/date/type of submission: 000 / 12-OCT-2006 / original
Information to sponsor: Yes (transmitted on 11/7/06 by M. Puglisi, Project Manager)
Sponsor and/or agent: ThromboGenics, Inc. (New York, NY)
Manufacturer for drug substances: (b) (4)

Reviewer name: Amy L. Ellis
Division name: Anti-Infective and Ophthalmology Products
Review completion date: 12/1/06

Drug:

Trade name: none
Generic name: recombinant human microplasmin
Code names: none
Chemical names: none
CAS registry number: not provided
Molecular formula/molecular weight: 249 amino acid enzyme / 27,229 Da

Relevant INDs/NDAs/DMFs: PIND (b) (4)

Drug class: Microplasmin is a protease derived from yeast (*Pichia pastoris*) using recombinant DNA techniques. It is a truncated version of the human enzyme, plasmin.

Intended clinical population: The initial clinical population will be patients scheduled to undergo a planned pars plana vitrectomy. Microplasmin will be administered to these patients with the intent of inducing PVD, facilitating vitrectomy. (b) (4)

Clinical formulation:

Each vial contains:

(b) (4) microplasmin
(b) (4) mannitol
(b) (4) mg citric acid

The vial contents are diluted with (b) (4) ml of sterile 0.9% saline prior to injection, providing concentrations of 2.5 mg/ml microplasmin, (b) (4) mg/ml mannitol, and (b) (4) mg/ml citric acid.

Route of administration: Intravitreal

Proposed clinical protocol:

A Multicenter, Randomized, Placebo-Controlled, Double-Masked, Parallel-Group, Dose-Ranging Clinical Trial of Intravitreal Microplasmin in Patients Undergoing Surgical Vitrectomy: The MIVI III (Microplasmin for Vitreous Injection III) Trial (Study No. TG-MV-003)

This Phase IIb trial will evaluate the safety and efficacy of intravitreal injections of microplasmin given 7 days before pars plana vitrectomy. Patients (n=30/arm) will receive placebo, 25, 75, or 125 µg of microplasmin into the midvitreous. The primary efficacy endpoint will be total PVD induction without induction of retinal hole or retinal detachment, with minimal vitrector suction (< 30 sec at 40 mmHG) at the beginning of vitrectomy. Safety endpoints will include postinjection and postoperative complications including worsening visual acuity, worsening macular edema, vitreous hemorrhage, retinal tear or detachment, other reasons for re-operation, inflammation, alteration of intraocular pressure, and cataract formation. Fluorescein angiography will be used to assess leakage from vessels. Before study drug is administered, all patients will undergo a complete ophthalmologic examination including optical coherence tomography (OCT), ultrasound (A- and B-scans), fundic photography, and fluorescein angiography. Follow-up examinations (may not include all of the pre-dose assessments at each visit) will be performed 3 and 7 days after injection, then on postoperative days 1, 7, 14, 28, 90, and 180.

Inclusion Criteria:

1. Male or female patients aged ≥ 18 years with nonproliferative vitreoretinal disease without evidence of a complete macular PVD in the study eye on biomicroscopy, OCT, or B-scan who are suitable candidates for conventional 2-port or 3-port pars plana vitrectomy.

2. BCVA 20/40 or worse in the study eye.

3. BCVA 20/400 or better in the non-study eye.

4. Willing and able to provide informed consent prior to undergoing any study-related procedures.

Exclusion Criteria:

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1. Evidence of fibrocellular proliferation characterized by whitish epimacular tissue (surface wrinkling is not an exclusion criterion).

2. Evidence of complete macular PVD on biomicroscopy, OCT, or B-scan prior to planned study drug injection.

3. Patients with vitreous hemorrhage that precludes either of the following: visualization of the posterior pole by visual inspection OR adequate assessment of the macula by either OCT and/or fluorescein angiogram in the study eye.

4. Patients with rhegmatogenous retinal detachment, proliferative vitreoretinopathy (PVR), or retinal degenerative changes associated with increased risk of retinal detachment in the study eye. Such retinal degenerative changes include lattice degeneration or cystic retinal tufts. Thorough retinal examination with scleral depression should be performed in all patients to rule out these changes.

5. Patients with high myopia (axial length > 26.0 mm on A-scan ultrasound) or aphakia in the study eye.

6. Patients with history of rhegmatogenous retinal detachment in the non-study eye.

7. Patients who are considered likely to require intraocular surgery in the study eye for any reason other than the planned vitrectomy in the coming 3 months.

8. Patients who have had ocular surgery in the study eye in the prior 3 months.

9. Patients who have had a vitrectomy in the study eye at any time.

10. Patients with glaucoma that is not controlled with topical medication or that is associated with severe visual field loss.

11. Intravitreal injection of any drug or laser photocoagulation in the study eye in the previous 30 days.

12. Patients who are pregnant or of child-bearing potential not utilizing an acceptable form of contraception. Acceptable methods of birth control include intrauterine device, oral, implanted, or injected contraceptives, and barrier methods with spermicide.

13. Patients who in the investigator's view will not complete all visits and investigations.

14. Patients who have participated in an investigational drug study within the past 30 days.

15. Patients with a life expectancy less than 6 months.

16. Patients who have previously participated in this trial.

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Previous clinical experience: Human subjects have received intravenous and intravitreal doses of microplasmin. All of these subjects received drug during European clinical trials.

The sponsor has completed a Phase II study with intravitreal microplasmin given to subjects scheduled to undergo pars plana vitrectomy (PPV) for vitreomacular traction maculopathy. Ten subjects per group received one of the following microplasmin treatments: 25 µg 1 hr before PPV, 25 µg 24 hrs before PPV, 25 µg 7 days before PPV, 50 µg 24 hrs before PPV, 75 µg 24 hrs before PPV, or 125 µg 24 hrs before PPV. The sponsor reports that preliminary data from this study suggest that the treatment was well-tolerated with most adverse events related to the PPV procedure itself, as opposed to the microplasmin.

In a Phase I ascending dose tolerance study of intravenous microplasmin, healthy male volunteers received 1.5 mg/kg infused over 15 minutes without significant adverse effects. When 2 mg/kg was given over 15 minutes, 1/6 subjects experienced an anaphylactoid reaction that included urticaria and itching, but no laryngeal swelling or cardiovascular or pulmonary complications. In a second part of this study, subjects received 1 mg/kg microplasmin over 15 minutes, then received 1 hour infusions of 1, 2, 3, or 4 mg/kg (6 subjects per dose group). Infusion site reactions were seen in 2 patients at 2 mg/kg, 1 at 4 mg/kg and 3 at 5 mg/kg. In the high dose patients, the infusion site reactions included hypoesthesia, paresthesia, and/or pain at the infusion site. Urticaria was observed in 2 of these patients and the 1 hour infusion was discontinued in both because the investigator judged that they were having an “allergic-type” reaction. C5a was increased and total complement activity was decreased in both of these patients and in the patient from part 1 of this study that received 2 mg/kg over 15 minutes. C3 and C4 levels measured within 8 hours of dosing were reported to be normal. The sponsor hypothesizes that free microplasmin can activate C5 to C5a, but free microplasmin will only be circulating when α2-antiplasmin activity is almost completely depleted. The inhibition of α2-antiplasmin activity was monitored in all patients. It was inhibited in a dose-related manner, with 84% inhibition measured after a 2 mg/kg 15 minute infusion (part 1 of the study) and 99% inhibition in the 4 mg/kg group (part 2 of the study). Drug-induced elevation of microplasmin antibodies was not observed, according to the sponsor. Although 2 patients had alterations in ECG during dosing, the changes were consistent with changes in adrenergic tone and were not necessarily related to drug treatment. One of these subjects had received placebo during part 3 of the study (“older” men received 1 mg/kg microplasmin over 15 minutes followed by an additional 1 mg/kg over an hour) and the other subject received 3 mg/kg during part 2 of the study. The doses of microplasmin used in this Phase I intravenous trial are far in excess of what is proposed to be administered intravitreally in the proposed clinical trial.

Additionally, there are 3 ongoing European Phase II clinical trials with microplasmin. The first is for acute peripheral artery occlusive disease using an intra-arterial route of administration (18 subjects treated to date with up to 3.6 mg/kg). The other 2 trials are for post acute ischemic stroke- one using an intravenous route of

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administration (14 patients treated to date with up to 4 mg/kg) and the other using an intra-arterial route of administration (newly initiated, no subjects treated yet).

Disclaimer: Tabular and graphical information are constructed by the reviewer unless cited otherwise.

Studies reviewed within this submission:

The safety pharmacology studies, intravenous study in rats, and intravitreal studies in pigs were reviewed by Dr. Maryam Rafie-Kolpin and filed separately to DFS.

The following studies were reviewed by Dr. Amy Ellis:

Single Dose Toxicity Studies:

A Single-Dose Intravitreal Toxicity Study With or Without Vitrectomy (with an 8-Week Observation Period) in Dutch-Belted Rabbits (Study No. 500046)

A Single-Dose Intravitreal Toxicity Study of Inactivated Microplasmin for Injection (with a 2-Week Observation Period) in Dutch-Belted Rabbits (Study No. 500210)

A Single Intravitreal Toxicity Study (with an 8-Week Observation Period) in Dutch-Belted Rabbits (Study No. 57541)

A Pilot Single Intravitreal Toxicity Study (with a 1-Week Observation Period) in Dutch-Belted Rabbits (Study No. 57540)

A Single Intravitreal Injection Toxicity Study (with an 8-Week Observation Period) of (b) (4) **Microplasmin for Injection in the Cynomolgus Monkey** (Study No. 57544)

A Pilot Single Intravitreal Injection Toxicity Study (with a 1-Week Observation Period) in Cynomolgus Monkeys (Study No. 57543)

Repeat Dose Toxicity Studies:

Recombinant Human Microplasmin Intravenous Infusion Maximum Tolerated Dose Study in Dogs (Study No. 662293)

Microplasmin 14 Day Intravenous Infusion Toxicity Study in Dogs (Study No. 662314)

Study not reviewed within this submission: None2.6.2PHARMACOLOGY

2.6.2.1 Brief summary

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Mechanisms of action and drug activities related to proposed indication: Microplasmin is a serine protease. It degrades fibrinogen and fibrin. The sponsor hopes that this action will induce a non-traumatic PVD to facilitate vitrectomy. If microplasmin reaches the systemic circulation (unlikely to occur at a significant level following intravitreal injection), it reacts with circulating α2-antiplasmin and α2-macroglobulin and can perturb the clotting cascade.

2.6.2.4 Safety pharmacology

Reviewed by Dr. Maryam Rafie-Kolpin.

2.6.4 PHARMACOKINETICS/TOXICOKINETICS

2.6.4.1 Brief summary

PK/TK reports that were submitted with toxicity studies will be reviewed together with the parent study under the Toxicology section. The TK data collected in the repeat dose intravenous toxicity studies in rats and dogs were highly variable.

The sponsor did not conduct any specific studies to determine the absorption, distribution, metabolism, or excretion of microplasmin after intravitreal or intravenous dosing.

2.6.4.2 Methods of Analysis

The submission did not describe the methods used to measure plasma levels of microplasmin or α2-antiplasmin. This information has been requested from the sponsor.

2.6.6 TOXICOLOGY

2.6.6.1 Overall toxicology summary

General toxicology: Intravenous doses of microplasmin of up to 10 mg/kg/day given for 14 days were not associated with histopathologic, hematologic, or clinical chemistry changes in dogs or rats. Half of the dose was administered over 15 minutes and the remainder was infused over 1 hour. Clinical signs including agitation, vocalization, salivation and retching or vomiting were observed in some dogs from the 7 and 10 mg/kg dose groups despite the lack of change in microscopic or clinical chemistry/hematology parameters. No clinical signs were observed in rats. Of note, a dog given 10 mg/kg/day in a 10-day pilot study had occasional increases in clotting

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time. This pilot study showed that doses ≥15 mg/kg/day were not tolerated. Dogs given microplasmin at 15 or 20 mg/kg/day were sacrificed early for humane reasons after 4 and 2 days of dosing, respectively. Agitation and vomiting were among the clinical signs observed. Necropsy revealed internal hemorrhages (e.g., of the esophagus, stomach, duodenum, colon, liver) in the 15 and 20 mg/kg dogs that were likely exacerbated by the thrombolytic activity of microplasmin.

In an acute intravenous toxicity study in rats, doses of ≥40 mg/kg were fatal to the majority of the animals. Subdued behavior, staggering, labored breathing and prostration were observed before death. Microscopic analysis was not performed on the tissues from these animals, but gross necropsy revealed redness in the mesenteric lymph nodes, thymus, ileum, cecum and stomach in the premature decedents. These findings are likely due to internal hemorrhaging in the rats caused by perturbation of the clotting cascade by microplasmin.

Intravitreal toxicology: Single intravitreal microplasmin doses of up to 125 µg/eye to cynomolgus monkeys were not associated with any permanent ocular histopathologic changes. Dose-related but transient signs of inflammation (anterior uveitis, aqueous flare, presence of aqueous and vitreous cells, foveal pigment changes) were observed during ophthalmologic exams in most microplasmin-treated eyes. ERG responses showed dose-related reductions following microplasmin treatment, but recovered completely by the end of the 55 day post injection observation period (in all but one animal with a vitreous hemorrhage). In Göttingen minipigs, a single intravitreal 125 µg dose of microplasmin was not associated with ocular histopathologic changes, inflammation, or changes in ERG or IOP. However, a slight lens subluxation was observed 28 days after treatment in 1 of 3 minipigs that received 125 µg/eye microplasmin. Dynamic light scattering performed on the minipigs indicated dose-related changes in the vitreal structure likely due to the protease activity of microplasmin. Transient dose-related inflammation including uveitis (aqueous flare, presence of vitreal cells) was observed in Dutch-Belted rabbits after single intravitreal microplasmin injections ≥ 2.5 µg/eye. Narrowing of the retinal vessels, which was more persistent at doses ≥ 50 µg/eye, was also observed in the rabbits. These doses were also associated with a dose-related retinal atrophy (thinning, or in more severe cases, an absence of the rod and cone layer and outer nuclear layer of the retina) which may have resulted from a reduction in blood supply due to the narrowed retinal vessels. ERG responses were diminished in rabbits within the first 2 days following injection, but substantial recovery occurred in most animals within an 8-week observation period at doses up to 200 µg/eye. Lens subluxation was observed in some studies in a few rabbits at doses ≥ 50 µg/eye. In one study, it was associated with an increase in IOP; however, the investigators felt that it was not clear whether the lens subluxation was a direct consequence of elevated IOP or whether direct damage to the zonules may have occurred.

Genetic toxicology: Genetic toxicology studies have not been conducted with microplasmin. In general, these studies are not needed for biologic drug products.

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Reproductive toxicology: Reproductive toxicology studies have not been conducted with microplasmin. They may not be necessary to support development of an intravitreal drug product if the sponsor can demonstrate that significant levels of microplasmin do not reach the circulation after intravitreal injection. A teratology study may be indicated if limited absorption occurs.

2.6.6.2 Single-dose toxicity

A Single-Dose Intravitreal Toxicity Study With or Without Vitrectomy (with an 8-Week Observation Period) in Dutch-Belted Rabbits

Key study findings: Intravitreal injection of 2.5 µg/eye of microplasmin to Dutch-Belted rabbits was associated with mild, transient uveitis and a transient narrowing of the retinal vessels, but no permanent ophthalmologic or microscopic changes in the eye were observed. More persistent narrowing of the retinal vessels was observed at 50 µg/eye, with the effect still seen on Day 56 in 3/6 eyes. ERG changes associated with the narrowed retinal vessels were resolved by Day 14. Lens subluxation occurred in 2/6 eyes at 50 µg. The vitrectomy procedure left about half of the microplasmin eyes in these groups unsuitable for evaluation due to surgical trauma. Narrowing of the retinal vessels was also observed in animals treated with 62.5, 300, and 600 µg/eye and was believed to be related to microplasmin treatment. Other microscopic findings in this dose group were difficult to attribute to microplasmin and were more likely related to vitrectomy. Of note, there were 2 eyes that received 600 µg of microplasmin before vitrectomy that did not have any posterior ocular histopathologic changes that appeared drug-related.

Study no.: 500046

Conducting laboratory and location: (b) (4)

Date of study initiation: 4/22/03
GLP compliance: US GLPs were followed, except for the dose solution analysis. It is noted that the method used to analyze the dosing solutions (determination of protein content) is not specific. The sponsor is assuming that that there is no significant breakdown of microplasmin over the time frame that the dosing solutions are used (albeit a short time frame in the case of single intravitreal injections). They mention that the presence of (b) (4) in the (b) (4) vehicle contributes a small amount of protein to the dosing solutions, but the sponsor believes that they can easily account for it by using (b) (4) as the assay blank and subtracting background.
QA report: yes (x) no ( )
Drugs, lot #, % purity: Microplasmin (Lot No. Tox-L-µPLA-P06), > 90% purity

Methods

Doses: Rabbits received vehicle injections into the left eye and microplasmin injections into the right eye. The higher dose groups, 62.5, 300, and 600 µg/eye underwent vitrectomy one hour after injection. The lower dose groups, 2.5 and

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50 µg/eye did not undergo vitrectomy.
Species/strain: Dutch-Belted rabbits
Number/sex/group (main study): 4 males/vitrectomy group, 6 males/group with no vitrectomy
Route, formulation, volume: Intravitreal administration at a dose volume of 50 µl per eye. Microplasmin and its placebo (citric acid/mannitol present in the same amount as in the microplasmin drug product) (b) (4)
Satellite groups used for toxicokinetics: Not done
Age: 5-6 months old
Weight: 1.8-2.4 kg
Sampling times for TK: n/a
Unique study design or methodology: Vitrectomies (depending on group assignment) were performed on microplasmin-treated eyes about 2 hours after injection. Half of the rabbits also had vitrectomies performed on control eyes. These procedures followed those performed on treated eyes. BSS+ was used as the replacement fluid for the vitreous humor. Animals were sacrificed at the end of the 56 day observation period. Postmortem evaluations focused on the eyes, although the rabbits did undergo an external gross examination.

Results:

Mortality/Clinical Signs: Rabbits were observed twice daily for viability and clinical signs. The animals underwent detailed physical examinations on the day before dosing, weekly during the study, and just before sacrifice. All animals survived until scheduled sacrifice. There were no clinical signs of toxicity attributed to the test articles.

Body weights: Animals were weighed prior to dosing and weekly thereafter. There were no treatment-related effects.

Food consumption: Recorded daily. Treatment-related changes in food consumption were not observed.

Water consumption: Not measured.

Ophthalmoscopy: Ophthalmic examinations consisting of ophthalmoscopy, tonometry, and ERG were performed prior to the initiation of dosing, and on Days 2, 7, 14, 28, and 56. Fundic photographs were taken for each eye prior to treatment and repeated as considered necessary by the veterinary ophthalmologist. Eyes were also examined immediately following injection via indirect ophthalmoscopy and slit lamp biomicroscopy.

There were no changes in intraocular pressure that appeared related to microplasmin in any treatment group.

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Dose-related narrowing of the retinal vessels was observed beginning on Day 2 in all non-vitrectomized eyes treated with microplasmin (2.5 or 50 µg/eye). In the lower dose group, the change was observed on Day 28, but it was resolved by Day 56. In the higher dose group, the attenuated retinal vessels persisted through Day 28 in all of the rabbits and through Day 56 in 3/6. Subluxation of the lens was seen beginning on Day 14 in 2 of these 50 µg rabbits. Vitreous cells (associated with mild uveitis) were observed on Days 7, 14, and 28 in half of the 2.5 µg rabbits and all of the 50 µg rabbits, but they were no longer present by Day 56. Transient, minimal changes observed in some non-vitrectomized rabbits included vitreous hemorrhage, vitreous opacities, aqueous flare and conjunctival swelling. These were likely related to the injection process as they were seen in control as well as microplasmin-treated eyes. Conjunctival congestion was observed in most eyes from each dose group, including control eyes, for a few days after injection. It resolved within a week. No changes in ERG were observed at 2.5 µg/eye. Scotopic b-wave amplitude was diminished in 3/6 50 µg eyes on Day 2, with similar effects on photopic b-waves and a-wave amplitudes. This effect on ERG was less on Day 7, and present in only one of the animals on Day 14. By Day 28, the ERG was back to baseline.

One to two animals in each treatment group each had at least one eye that experienced severe trauma related to vitrectomy and was excluded from evaluation (leaving only about half of the microplasmin-treated eyes in these groups available for evaluation). Signs of this trauma included partial or complete retinal detachment with or without retinal tears and a large amount of hemolyzed blood in the vitreous. Cataracts were present in one eye. Retinal vessel narrowing was observed in all microplasmin/ vitrectomy groups. Although it did not appear dose-related in the vitrectomized microplasmin-treated animals and it was present in a control eye, this attenuation of the retinal vessels is likely to be related to drug based on observations in non-vitrectomized microplasmin-treated rabbits. It appeared similar to the findings from the 50 µg non-vitrectomized group, above. Vitreous cells were seen in most microplasmin-treated eyes and 2 control eyes in the vitrectomized animals, but they were not present by the end of the observation period. Transient iritis was observed in most vitrectomized eyes; signs associated with this condition included aqueous flare, fibrin clot, incomplete mydriasis, iris bombé, and anterior capsule pigment. Other transient or mild findings associated with the surgical procedure included conjunctival hyperemia or edema, hemorrhage (various locations), corneal edema, decreased pupillary response, vitreous opacity, blurry fundus, focal cataract or scar, or interior retinal detachment. Excluding the animals that experienced severe surgical trauma, reductions in the scotopic b-wave amplitude were observed on Day 2 in one 300 µg eye (resolved by Day 7) and on Day 7 in one 600 µg eye (resolved by Day 14. The 300 µg eye also had a reduction in photopic a-wave amplitude on Day 7 that was resolved by Day 14.

Hematology/Clinical chemistry: Not done.

Urinalysis: Not done.

Organ weights: Not done.

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Histopathology: Adequate Battery: Performed on eyes and optic nerves only-acceptable for this study.

Peer review: yes ( ), no (x)

Microscopic changes seen in eyes from all treatment groups that appeared related to the injection procedure included focal retinal and lens degeneration. Minimal retinal degeneration/atrophy was observed in 2/6 eyes treated with 50 µg microplasmin (no vitrectomy). No microplasmin-related histopathologic changes were seen in the 2.5 µg group.

Two eyes that were injected with 600 µg of microplasmin before vitrectomy did not have any drug-induced posterior ocular histopathologic changes. When retinal degeneration was observed in the vitrectomized groups, it ranged from moderate to massive. Mild to moderate retinal detachment was also observed. These retinal findings were difficult to attribute to drug treatment because they were also present in 2/4 control vitrectomy eyes. Minimal to slight mixed cell infiltration of the vitreous body in some vitrectomized eyes also appeared related to the surgical procedure, as it was not dose-related and was present in a control eye.

A Single-Dose Intravitreal Toxicity Study of Inactivated Microplasmin for Injection (with a 2-Week Observation Period) in Dutch-Belted Rabbits

Key study findings: A single 50 µg injection of microplasmin to rabbit eyes was associated with mild to moderate narrowing of the retinal vessels persisting through 14 days of postinjection observation. No signs of retinal damage were observed microscopically following microplasmin injection. Narrowing of retinal vessels was observed in 1/3 rabbit eyes injected with 280 µg of human plasmin. Minimal to slight focal retinal atrophy was observed in all eyes treated with human plasmin. Signs of transient inflammation including mild uveitis and mild iritis were observed in several microplasmin-treated eyes. Iritis was more severe in eyes treated with human plasmin. ERG changes (reduction in scotopic b-wave) observed in microplasmin-treated eyes appeared to be reversible.

Study no.: 500210

Conducting laboratory and location: (b) (4)

Date of study initiation: 6/16/03
GLP compliance: no
QA report: yes ( ) no (x)
Drugs, lot #, % purity: Microplasmin (Lot No. Tox-L-µPLA-P06); Plasmin from human plasma (Lot No. 043K0741); % purity was not provided for either compound

Methods

Doses: The treatment groups were as follows:

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Group 1- 50 µg microplasmin (right eye), placebo (left eye)
Group 2- 50 µg microplasmin (right eye), 50 µg inactivated microplasmin (left eye), aprotinin was used as the inactivation agent
Group 3- 280 µg human plasmin (right eye), BSS+ (left eye)
Species/strain: Dutch-Belted rabbits
Number/sex/group (main study): 3 males/group
Route, formulation, volume: Intravitreal administration at a dose volume of 50 µl per eye. Microplasmin and its placebo (citric acid/mannitol present in the same amount as in the microplasmin drug product) (b) (4)

Satellite groups used for toxicokinetics: Not done
Age: 5-6 months old
Weight: 1.7-2.1 kg
Sampling times for TK: n/a
Unique study design or methodology: Animals were sacrificed on Day 15 after a single intravitreal injection on Day 1. Postmortem evaluations focused on the eyes, although the internal organs of the rabbits were examined grossly.

Results:

Mortality/Clinical Signs: Rabbits were observed twice daily for viability and clinical signs. All animals survived until scheduled sacrifice. There were no clinical signs of toxicity attributed to the test articles. Conjunctival swelling and redness of skin adjacent to the eye observed in one Group 1 animal were attributed to the injection procedure.

Body weights: Animals were weighed prior to dosing and weekly thereafter. There were no treatment-related effects.

Food consumption: Recorded daily. Treatment-related changes in food consumption were not observed.

Water consumption: Not measured.

Ophthalmoscopy: Ophthalmic examinations consisting of ophthalmoscopy, tonometry, and ERG were performed prior to the initiation of dosing, and on Days 2, 7, and 14. Fundic photographs were taken for each eye prior to treatment and repeated as considered necessary by the veterinary ophthalmologist. Eyes were also examined immediately following injection via indirect ophthalmoscopy and slit lamp biomicroscopy.

Mild to moderate (primarily the former) narrowing of the retinal vessels was observed on Day 2 in 3/6 eyes treated with 50 µg microplasmin. All microplasmin-treated eyes had narrowed retinal vessels on Days 7 and 14. This effect was observed in 1/3 eyes treated with 280 µg human plasmin, but not in eyes treated with placebo, BSS+, or inactivated microplasmin.

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Signs of inflammation, including a mild, transient uveitis was observed in microplasmin-treated eyes. Inflammation was more severe in eyes treated with human plasmin. Punctate vitreal opacities (PVOs, slight to severe) were observed only on Day 2 in 1 placebo eye, 3/6 microplasmin eyes, and 2/3 human plasmin eyes. The PVOs were gone by Day 7. Slight numbers of vitreous cells were seen in 5/6 microplasmin eyes on Day 7 and in all microplasmin eyes on Day 14. Moderate to severe numbers of vitreous cells were seen in 3/3 human plasmin eyes on Days 7 and 14. Mild iritis (slight to moderate aqueous flare) was observed on Day 2 in 1/3 placebo eyes, 1/3 inactivated microplasmin eyes, and 4/6 microplasmin eyes. More severe iritis was observed in the eyes treated with human plasmin. Additional signs in the human plasmin eyes included congestion of the iris (3/3), incomplete mydriasis (3/3), and blurry fundus (2/3). In the placebo-, inactivated microplasmin-, and microplasmin-treated eyes, signs of inflammation were gone by Day 7. Iritis was still present in 2/3 human plasmin eyes on Day 7, with blurry fundus still observed in 3/3 of these eyes on Day 7 and 2/3 of these eyes on Day 14. On Day 7, focal retinal hemorrhages were observed in 2/3 eyes treated with human plasmin. This finding was still present in one of these eyes on Day 14. A transient reduction in intraocular pressure was observed in human plasmin-treated eyes on Day 7, consistent with the severity of iritis seen with this treatment.

Microplasmin treatment was associated with ERG changes. On Day 2, severe reduction of the scotopic b-wave was observed in 2/6 microplasmin eyes, with slight reduction observed in an additional 2/6 rabbits. The b-wave amplitude was also reduced in 4/6 eyes treated with microplasmin compared to the contralateral eye (2 compared to placebo control and 2 compared to inactivated microplasmin). By Day 7, most of the ERG changes were no longer evident with the exception that one microplasmin-treated eye still had a “generally slightly diminished” ERG on Days 7 and 14. An elevation in b-wave amplitude was seen in 2 microplasmin eyes and 1 human plasmin eye on Day 7, but this observation is of uncertain biological significance.

Hematology/Clinical chemistry: Not done.

Urinalysis: Not done.

Organ weights: Not done.

Histopathology: Adequate Battery: Performed on eyes and optic nerves only-acceptable for this study.

Peer review: yes ( ), no (x)

Compound-related changes were observed in the vitreous body. A minimal amount of eosinophilic material was observed in 4/6 microplasmin-treated eyes, with a minimal infiltration of mononuclear cells seen in 2 of these eyes. Injection of human plasmin was associated with minimal to slight focal retinal atrophy, a minimal amount of eosinophilic material, and slight infiltration of mononuclear cells in 3/3 eyes. In 1/3 rabbit eyes treated with human plasmin, the mononuclear cell infiltration extended into

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the optic nerve. Minimal to slight focal retinal atrophy was observed in all eyes treated with human plasmin.

A Single Intravitreal Toxicity Study (with an 8-Week Observation Period) in Dutch-Belted Rabbits

Key study findings: Single intravitreal microplasmin doses of 62.5, 125, or 200 µg/eye to Dutch-Belted rabbits were associated with a narrowing of the retinal vessels leading to dose-related retinal atrophy in most animals. ERG responses were diminished in most animals, with signs of recovery apparent in the majority by the end of a 58-day observation period. Lens subluxation was observed in 1/6 low dose eyes and 3/6 high dose eyes. Although it was observed only in eyes that demonstrated elevated IOP, the report stated that it was not clear whether it was a direct consequence of elevated IOP or whether direct damage to the zonules may have occurred. Transient signs of inflammation (iritis, presence of vitreous cells) were observed all microplasmin-treated eyes. A NOAEL was not found in this study.

Study no.: 57541

Conducting laboratory and location: (b) (4)

Date of study initiation: 11/5/02
GLP compliance: US GLPs were followed, except for the dose solution analysis.
QA report: yes (x) no ( )
Drugs, lot #, % purity: Microplasmin (Lot No. Tox-L-µPLA-P06), > 90% purity

Methods

Doses: Rabbits received vehicle injections into the left eye and microplasmin injections into the right eye. Dose groups were 62.5, 125, and 200 µg/eye.
Species/strain: Dutch-Belted rabbits
Number/sex/group (main study): 6 females/group
Route, formulation, volume: Intravitreal administration at a dose volume of 50 µl per eye. Microplasmin and its placebo (citric acid/mannitol present in the same amount as in the microplasmin drug product) (b) (4)
Satellite groups used for toxicokinetics: Not done
Age: 5-6 months old
Weight: 2.1-2.9 kg
Sampling times for TK: n/a
Unique study design or methodology: Animals were sacrificed at the end of the 58 day observation period. Postmortem evaluations focused on the eyes, although the rabbits did undergo gross external and internal examinations.

Results:

Reference ID: 3198420

Mortality/Clinical Signs: Rabbits were observed twice daily for viability and clinical signs. The animals underwent detailed physical examinations before dosing, weekly during the study, and just before sacrifice. All animals survived until scheduled sacrifice. There were no clinical signs of systemic toxicity attributed to the test articles.

Body weights: Animals were weighed on the day prior to dosing and weekly thereafter. There were no treatment-related effects.

Food consumption: Recorded daily. Treatment-related changes in food consumption were not observed.

Water consumption: Not measured.

Ophthalmoscopy: Ophthalmic examinations consisting of ophthalmoscopy, tonometry, and ERG were performed prior to the initiation of dosing, and on Days 2, 7, 20 (ERG only), 21/22 (ophthalmology/tonometry only), 28, 42, and 58. Fundic photographs were taken for each eye prior to treatment and repeated as considered necessary by the veterinary ophthalmologist. Eyes were also examined immediately following injection via indirect ophthalmoscopy and slit lamp biomicroscopy.

Slight to severe narrowing of the retinal vessels was observed in all microplasmin-treated eyes and 1 control eye beginning on Day 2 and persisting in these eyes through Day 28. Severity did not correlate with dose, though it did decrease with time. By Day 42, the incidence of the lesion decreased with slight narrowing observed in 4/6 microplasmin-treated eyes in each of the 62.5 and 125 µg dose groups, 3/6 200 µg eyes, and the 1 control eye. On Day 58, slightly narrowed retinal vessels were observed in the 1 control eye, 2/6 low dose eyes, 3/6 mid dose eyes, and 4/6 high dose eyes. On Day 28, extensive retinal degeneration (though patchy) was seen in one high dose eye and this lesion persisted throughout the rest of the study.

Lens subluxation was observed in 3/6 high dose eyes (first seen on Days 21,22, or 28) and 1/6 low dose eyes (first seen on Day 42). Elevated intraocular pressure was observed on several occasions in all of these eyes. IOP was elevated for the entire observation period in these high dose rabbits, but it was observed only until Day 7 in the low dose rabbit. In one of the high dose animals, cupping of the optic nerve (secondary to elevated IOP) was noted. The report states that it is unknown whether increased IOP was directly responsible for lens subluxation, or whether primary damage to the zonules may have occurred.

Signs of inflammation manifested by the presence of slight to severe numbers of vitreous cells and mild iritis (accompanied by a slight to moderate aqueous flare) were observed in most eyes that were treated with microplasmin. Vitreous cells were first observed on Day 2 in 3 drug-treated rabbits and 1 control. By Day 7, they were present in all microplasmin-treated eyes, but only 2 controls. The number of vitreous cells generally correlated with dose. Vitreous cells were still present on Day 28, but they

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were gone in all but 5 drug-treated eyes on Day 42 and were no longer observed at all by Day 58. Iritis was seen in 15/18 microplasmin-treated eyes on Day 2, compared with 2/18 control eyes. It resolved by Day 7 in all but one drug-treated eye where it was no longer observed on Day 22.

Transient slight to severe swelling of the conjunctiva was observed in 15/18 microplasmin-treated eyes and 4/18 control eyes. All swelling resolved by Day 7.

Scotopic and photopic ERGs were severely diminished in eyes from all microplasmin treatment groups. Some recovery was observed in most animals by Day 7, but there were individuals in each group that experienced some diminution of ERG response throughout the post dose observation period. The majority of animals had ERG responses close to baseline by Day 58.

Hematology/Clinical chemistry: Not done.

Urinalysis: Not done.

Organ weights: Not done.

Histopathology: Adequate Battery: Performed on eyes and optic nerves only-acceptable for this study.
Peer review: yes ( ), no (x)

Retinal atrophy was observed in 5/6 low dose rabbits, 4/6 at the mid dose, and 5/6 at the high dose. The pathologist noted a thinning, or in more severe cases, an absence of the rod and cone layer and outer nuclear layer of the retina. Retinal atrophy was dose related, graded as minimal at the low dose, minimal to slight at the mid dose, and minimal to moderate at the high dose. One high dose rabbit also had eosinophilic material present in the vitreous body that was believed to be a manifestation of fibrin exudation.

Focal retinal or lens degeneration was seen in a few eyes spread throughout the treatment groups (including a control eye). These changes appeared related to the injection procedure.

A Pilot Single Intravitreal Toxicity Study (with a 1-Week Observation Period) in Dutch-Belted Rabbits

Key study findings: Single intravitreal microplasmin doses of 200 or 885 µg/eye to Dutch-Belted rabbits were associated with a narrowing of the retinal vessels leading to retinal atrophy. Scotopic and photopic ERG responses were diminished following microplasmin treatment although there were signs of recovery at the end of the 1 week observation period. Signs of inflammation (iritis, presence of vitreous cells) were observed all microplasmin-treated eyes. Eosinophilic material (believed to be either test

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article or fibrin exudate) was present in the vitreous body of all microplasmin-treated eyes.

Study no.: 57540

Conducting laboratory and location: (b) (4)

Date of study initiation: 9/11/02
GLP compliance: US GLPs were followed, except for the dose solution analysis.
QA report: yes (x) no ( )
Drugs, lot #, % purity: Microplasmin (Lot No. Tox-L-µPLA-P06), > 90% purity

Methods

Doses: Rabbits received vehicle injections into the left eye and microplasmin injections into the right eye. Dose groups were 200 and 885 µg/eye.
Species/strain: Dutch-Belted rabbits
Number/sex/group (main study): 3 females/group
Route, formulation, volume: Intravitreal administration at a dose volume of 50 µl per eye. Microplasmin and its placebo (citric acid/mannitol present in the same amount as in the microplasmin drug product) (b) (4)
Satellite groups used for toxicokinetics: Not done
Age: about 5 months old
Weight: 1.8-2.1 kg
Sampling times for TK: n/a
Unique study design or methodology: Animals were sacrificed at the end of the 7 day observation period. Postmortem evaluations focused on the eyes, although the rabbits did undergo gross external and internal examinations.

Results:

Mortality/Clinical Signs: Rabbits were observed twice daily for viability and clinical signs. The animals underwent detailed physical examinations before dosing, weekly during the study, and just before sacrifice. All animals survived until scheduled sacrifice. There were no clinical signs of systemic toxicity attributed to the test articles.

Body weights: Animals were weighed on the day prior to dosing and on Day 8. There were no treatment-related effects.

Food consumption: Recorded daily. Treatment-related changes in food consumption were not observed.

Water consumption: Not measured.

Reference ID: 3198420

Ophthalmoscopy: Ophthalmic examination consisting of biomicroscopy and indirect ophthalmoscopy was done on prior to the initiation of dosing and on Days 2, 6, and 8. ERG was performed prior to the initiation of dosing, and on Days 2, 4 and 8. Eyes also underwent ophthalmic examination immediately following injection.

Narrowing of the retinal vessels was observed in 5/6 microplasmin-treated eyes beginning on Day 2 and in all microplasmin-treated eyes by Day 6. Moderate to severe numbers of vitreous cells were seen in 3 high dose eyes on Day 2 and slight to severe numbers of vitreous cells were observed in all microplasmin-treated eyes by Day 6. These changes were still observed on Day 8 with no diminution of severity. Iritis manifested by slight and moderate aqueous flare was seen in one 200 µg eye and one 885 µg eye on Day 2. Conjunctival hyperemia and episcleral congestion were transient in most eyes, but persisted in the high dose group.

Scotopic ERGs (b-wave amplitude) were severely reduced in microplasmin-treated eyes from both groups. B-waves were not measurable in some rabbits. A-wave amplitude at 0 dB was also diminished after microplasmin treatment, but increased in a-wave amplitude were observed at -30 dB (likely an artifact of the severely reduced b-wave). Implicit times tended to be prolonged for both a- and b-waves of the scotopic ERGs. Photopic ERGs were more variable after microplasmin injection. Implicit time was prolonged in some animals without any change in mean amplitude of a-waves at 1 Hz. Other animals had increased a-waves (similar to the observation made with scotopic ERGs). Mean b-wave amplitudes were reduced by 85-91% at 1 Hz in both groups of microplasmin-treated eyes and implicit times tended to be prolonged. Some eyes did not respond to stimuli. At 29 Hz, b-wave amplitude was reduced by 74-90% in the drug-treated eyes with no effect observed on implicit times. The severity of the ERG changes did not increase with dose. Signs of recovery were observed during the observation period, but recovery was not complete by the time of sacrifice.

Hematology/Clinical chemistry: Not done.

Urinalysis: Not done.

Organ weights: Not done.

Histopathology: Adequate Battery: Performed on eyes and optic nerves only-acceptable for this study.
Peer review: yes ( ), no (x)

Retinal atrophy (thinning or absence of the rod and cone layer, thinning of outer nuclear layer) was seen in all microplasmin-treated eyes. It was minimal at 200 µg/eye and minimal to slight at 885 µg/eye. An accumulation of macrophages was seen in all drug-treated eyes. Accumulation was minimal in 2/3 low dose eyes, slight in 1/3 low dose eyes and 2/3 high dose eyes, and moderate in 1/3 high dose eyes.

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Eosinophilic material present in the vitreous body of all microplasmin-treated eyes was graded minimal to slight at the low dose and slight to moderate at the high dose. It was believed to be either test article or fibrin exudate.

Focal retinal degeneration observed in some eyes (including controls) in both groups appeared related to the injection procedure.

A Single Intravitreal Injection Toxicity Study (with an 8-Week Observation Period) of [redacted] (b) (4) Microplasmin for Injection in the Cynomolgus Monkey

Key study findings: Single intravitreal microplasmin doses of up to 125 µg/eye to cynomolgus monkeys were not associated with any permanent ocular histopathologic changes. Scotopic and photopic ERG responses showed dose-related reductions following microplasmin treatment, but recovered by the end of the 55 day post injection observation period. Dose-related inflammation was observed in most microplasmin-treated eyes. At 5 µg/eye, slight to moderate numbers of vitreous cells were observed and at 25 and 125 µg/eye, foveal pigment changes were seen in addition to vitreous cells. Slight to moderate anterior uveitis was observed at the high dose manifested by aqueous flare and the presence of aqueous cells.

Study no.: 57544
Conducting laboratory and location: [redacted] (b) (4)

Date of study initiation: 1/15/03
GLP compliance: US GLPs were followed, except for the dose solution analysis.
QA report: yes (x) no ( )
Drugs, lot #, % purity: Microplasmin (Lot No. Tox-L-µPLA-P06), > 90% purity

Methods

Doses: Monkeys received vehicle injections into the left eye and microplasmin injections into the right eye. Dose groups were 5, 25, and 125 µg/eye.
Species/strain: Cynomolgus monkeys
Number/sex/group (main study): 6 males/group
Route, formulation, volume: Intravitreal administration at a dose volume of 50 µl per eye. Microplasmin and its placebo (citric acid/mannitol present in the same amount as in the microplasmin drug product) [redacted] (b) (4)
Satellite groups used for toxicokinetics: Not done
Age: 3-5 years old
Weight: 3.1-4.2 kg
Sampling times for TK: n/a
Unique study design or methodology: Animals were sacrificed at the end of the 55 day observation period. Postmortem evaluations focused on the eyes,

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although the monkeys did undergo gross external examinations. Detailed internal examinations were not performed on animals sacrificed as scheduled.

Results:

Mortality/Clinical Signs: Monkeys were observed twice daily for viability and clinical signs. The animals underwent detailed physical examinations before dosing, weekly during the study, and just before sacrifice. All animals survived until scheduled sacrifice. There were no clinical signs of systemic toxicity attributed to the test articles.

Body weights: Animals were weighed on the day prior to dosing and weekly throughout the study. There were no treatment-related effects.

Food consumption: Recorded daily. Treatment-related changes in food consumption were not observed.

Water consumption: Not measured.

Ophthalmoscopy: Ophthalmic examinations (biomicroscopy, direct and indirect ophthalmoscopy), tonometry, and ERG were done prior to the initiation of dosing and on Days 2, 6, 27, 42 and 55. Fundic photographs were taken before treatment and repeated as considered necessary by the veterinary ophthalmologist to document treatment-related changes. Eyes also underwent ophthalmic examination immediately following injection.

Slight (3 eyes) to moderate (1 eye) anterior uveitis was observed in the 125 µg eyes. Signs included aqueous flare, and slight to moderate numbers of aqueous cells. Fundi appeared normal, though blurry. In the moderately affected eye, incomplete pupillary dilation was observed, congestion was present, and IOP was too low to measure. By Day 6, uveitis had resolved or shown marked improvement.

Slight to moderate numbers of vitreous cells were seen in three 5 µg eyes, two 25 µg eyes, and one 125 µg eye on Day 2. On Day 6, small numbers of vitreous cells were seen in all microplasmin-treated animals. Foveal pigment changes were also observed in 2 animals each in the mid and high dose groups. Vitreous cells were present in fewer microplasmin-treated eyes on Days 27 and 42. They were observed in only 3 low dose eyes on Day 55. The incidence of foveal pigment changes began to decrease on Day 42, and these changes were observed in only 1 mid dose animal on Day 55.

Subconjunctival hemorrhages observed in several eyes injected with microplasmin or vehicle were attributed to the injection procedure, as was a moderate to severe vitreous hemorrhage that occurred in a mid dose monkey. The vitreous hemorrhage and the dense vitreous opacity that followed prevented visualization of the fundus from Day 6 for rest of the observation period.

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One Day 2, reduced IOP was observed in most eyes. The small reductions seen in the control and 5 µg eye groups were comparable. Reductions were generally greater in the 25 µg/eye animals (range of 0-7 mmHg) and they were greater still in the 125 µg/eye group (too low to measure in one monkey and 3-8 mmHg in the rest). IOP reductions resolved by Day 27. A transient increase IOP was observed in 1 mid dose monkey on Day 6. The relevance of this change to drug treatment is unknown.

Dose-related microplasmin-induced changes in ERG were observed. The report stated that the trends for scotopic and photopic ERGs were similar and it discussed only the data for scotopic ERGs in detail. On Day 2, scotopic a-wave amplitudes for all eyes were within baseline except for one high dose monkey which did not respond to the -10 dB stimulus. Scotopic b-wave amplitudes were not affected at 5 µg/eye. At 25 µg/eye, scotopic b-wave amplitudes were diminished in 4/6 eyes at -30 dB (the eye with vitreous hemorrhage was not responsive). At -10 and 0 dB, 3/6 25 µg eyes had diminished responses (the eye with the vitreous hemorrhage responded slightly). At 125 µg/eye, all 6 eyes had diminished scotopic b-wave amplitudes at all 3 intensities. At -30 dB, 4/6 of the eyes were not responsive and at -10 dB, 2/6 were not responsive. At 0 dB, all of the high dose eyes responded, albeit at a severely diminished capacity. On Day 6, scotopic a-wave amplitudes were slightly diminished at the higher intensities in one mid and one high dose monkey. Scotopic b-wave amplitudes were still moderately to severely diminished in most mid and high dose eyes, especially at -30 dB, but there were signs of recovery in a few of the animals in these dose groups. On Day 27, scotopic a- and b-wave amplitudes were severely diminished in one low dose animal at all 3 intensities. Two other monkeys in this group had slightly diminished a- or b-wave amplitudes at -30 dB. The reason for this delayed affect is unclear, as no ERG deficits were observed in these animals on Days 42 or 55. The report noted that the ERG data in this study were highly variable. At the mid and high doses, the incidence of animals with ERG changes was lower on Day 27 and the severity of the changes was lower in most of the affected animals. On Day 42, additional improvement was observed in the animals in these groups. On Day 55, ERGs were within baseline levels for all animals with the exception of the mid dose monkey with the vitreous hemorrhage (slightly diminished scotopic b-wave amplitude at all intensities).

Hematology/Clinical chemistry: Not done.

Urinalysis: Not done.

Organ weights: Not done.

Histopathology: Adequate Battery: Performed on eyes and optic nerves only-acceptable for this study.
Peer review: yes ( ), no (x)

There were no microscopic changes that appeared related to microplasmin. Minimal mononuclear cell infiltration was observed in a few eyes from all treatment

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groups, including controls, and, according to the study report, is often observed in monkeys of this strain and age.

A Pilot Single Intravitreal Injection Toxicity Study (with a 1-Week Observation Period) in Cynomolgus Monkeys

Key study findings: Single intravitreal microplasmin doses of 20 µg/eye to cynomolgus monkeys were not associated with microscopic ocular changes other than minimal numbers of macrophages in the vitreous. Eyes that received 200 µg also contained eosinophilic material believed to be either test article or fibrin exudate. One 200 µg eye that developed a hyphema several days after injection also exhibited slight retinal atrophy of the photoreceptor and outer nuclear layers. It was uncertain as to whether these changes were directly related to test article, secondary to inflammation caused by the test article, or to trauma that occurred during the observation period. Scotopic and photopic ERG responses were diminished following microplasmin treatment although the 20 µg group showed substantial recovery on Day 8. Slight to moderate uveitis (slight aqueous flare, presence of moderate numbers of aqueous cells) was seen on Day 2 in all 3 eyes that received 200 µg of microplasmin and 1/3 eyes that received 20 µg. On Day 8, inflammation had subsided in all eyes (with the exception of the high dose eye with hyphema) so that only small numbers of vitreous cells were present.

Study no.: 57543

Conducting laboratory and location: (b) (4)

Date of study initiation: 11/20/02
GLP compliance: US GLPs were followed, except for the dose solution analysis.
QA report: yes (x) no ( )
Drugs, lot #, % purity: Microplasmin (Lot No. Tox-L-µPLA-P06), > 90% purity
Methods

Doses: Monkeys received vehicle injections into the left eye and microplasmin injections into the right eye. Dose groups were 20, and 200 µg/eye.
Species/strain: Cynomolgus monkeys
Number/sex/group (main study): 3 males/group
Route, formulation, volume: Intravitreal administration at a dose volume of 50 µl per eye. Microplasmin and its placebo (citric acid/mannitol present in the same amount as in the microplasmin drug product) (b) (4)

Satellite groups used for toxicokinetics: Not done
Age: "adult" (not otherwise specified)
Weight: 2.3-4.6 kg
Sampling times for TK: n/a

Reference ID: 3198420

Unique study design or methodology: Animals were sacrificed at the end of the observation period on Day 8. Postmortem evaluations focused on the eyes, although the monkeys did undergo gross external examinations.

Results:

Mortality/Clinical Signs: Monkeys were observed twice daily for viability and clinical signs. The animals underwent detailed physical examinations before dosing and just before sacrifice. All animals survived until scheduled sacrifice. There were no clinical signs of systemic toxicity attributed to the test articles.

Body weights: Animals were weighed on the day prior to dosing and on the day of sacrifice. There were no treatment-related effects.

Food consumption: Recorded daily. Treatment-related changes in food consumption were not observed. One or two animals from both dose groups had reduced food consumption on Day 3, but these were believed to be related to stress or discomfort and not a direct effect of the test article.

Water consumption: Not measured.

Ophthalmoscopy: Ophthalmic examinations (biomicroscopy, direct and indirect ophthalmoscopy), tonometry, and ERG were done prior to the initiation of dosing and on Days 2 and 8. Fundic photographs were taken before treatment and repeated as considered necessary by the veterinary ophthalmologist to document treatment-related changes. Eyes also underwent ophthalmic examination immediately following injection.

Slight to moderate uveitis (slight aqueous flare, presence of moderate numbers of aqueous cells) was seen on Day 2 in all 3 eyes that received 200 µg of microplasmin and 1/3 eyes that received 20 µg. This reaction caused blurring of the fundus in the high dose animals and one could not undergo fundus evaluation due to the severity of the blurring. Pupils of eyes with uveitis would not dilate completely. A fibrin clot was present in 2/3 high dose eyes. By Day 8, the high dose eye that had the worst inflammation on Day 2 developed a hyphema. The blood precluded a complete ophthalmic evaluation of that eye and its IOP was too low to measure. Vitreous cells were observed in all of the other drug-treated eyes on Day 8, but no retinal lesions were observed and IOPs were normal.

Both doses of microplasmin had adverse effects on scotopic and photopic ERGs. On Day 2, scotopic b-wave amplitudes were reduced at intensities (-30, -10, and 0 dB) and frequencies (1 and 29 Hz). The reductions were generally more severe at 200 µg/eye. All three 200 µg eyes were unresponsive at -30 dB and 2/3 were also unresponsive at -10 dB. At 20 µg, 2/3 eyes were unresponsive at both -30 and -10 dB. Implicit times for b-waves were also reduced in the drug-treated eyes. Scotopic a-wave amplitude was reduced in 2/3 eyes that received 20 µg and in all 3 eyes that received 200 µg.

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Substantial recovery of ERG was observed on Day 8 in the 20 µg group, but not at 200 µg. The hyphema present in one of the high dose monkeys was likely to have interfered with ERG measurement, but the other 2 monkeys in this group were still experiencing significant reductions in a- and b-wave amplitudes on Day 8.

Hematology/Clinical chemistry: Not done.

Urinalysis: Not done.

Organ weights: Not done.

Histopathology: Adequate Battery: Performed on eyes and optic nerves only- acceptable for this study.
Peer review: yes ( ), no (x)

Minimal numbers of macrophages were observed in the vitreous of eyes from both microplasmin groups. Two eyes in the 200 µg group had a minimal amount of eosinophilic material (likely either test article or fibrin) in the vitreous and/or anterior chamber. The third eye from the high dose group was that with the hyphema. This eye had a moderate amount of eosinophilic material that had a fibrillar appearance in the vitreous along with a low number of neutrophils and macrophages. Slight retinal atrophy of the photoreceptor and outer nuclear layers was also observed. Multifocal moderate hemorrhages were seen in the anterior chamber and iris. The pathology report stated that the effects seen in the eye with hyphema were likely exacerbated by a trauma to the eye that occurred after treatment ("an autotraumatism").

2.6.6.3 Repeat-dose toxicity

Recombinant Human Microplasmin Intravenous Infusion Maximum Tolerated Dose Study in Dogs

Key study findings: The 10 and 12 mg/kg/day doses were considered acceptable to use for a pivotal repeat dose toxicity study in dogs. An increased clotting time was observed at both of these dose levels and after 7 days of dosing, the dog that received 10 mg/kg/day experienced sensations during infusion that caused it to "knock" its body against the sides of its pen. Doses of 15 and 20 mg/kg/day were not tolerated. Dogs that received these doses were sacrificed early for humane reasons after 4 and 2 days of dosing, respectively. Agitation and vomiting were among the clinical signs observed. Necropsy revealed internal hemorrhages (e.g., of the esophagus, stomach, duodenum, colon, liver) in the 15 and 20 mg/kg dogs that were likely exacerbated by the thrombolytic activity of microplasmin.

Study no.: 662293 ([redacted] (b) (4) Report No. 20936)

Reference ID: 3198420

Conducting laboratory and location: (b) (4)
Date of study initiation: 11/1/01
GLP compliance: not GLP
QA report: yes ( ) no (x)
Drug, lot #, % purity: Microplasmin, Batch No. TOX-E-μPLA-PO3, purity not stated

Methods

Doses: 10, 12, 15, or 20 mg/kg/day
Species/strain: Beagle dogs
Number/sex/group (main study): 1 animal at each dose level (M or F)
Route, formulation, volume: Doses were administered by intravenous infusion through a venous access port in the jugular vein. (b) (4) Half of each dose was administered over 15 minutes at a dose volume of 1 ml/kg and the other half was given over an hour at the same dose volume. Dosing occurred daily for up to 10 days.
Satellite groups used for toxicokinetics: Main study animals were used for TK.
Age: approximately 8 months old
Weight: 5.9-8.4 kg
Sampling times for TK: Blood samples for the measurement of microplasmin, fibrinogen, and α2-antiplasmin plasma levels were drawn 30 minutes and 4 hours after the end of infusion on the first and last days of dosing.
Unique study design or methodology: The first dog (male) was given 10 mg/kg of microplasmin, the second (female) was given 20 mg/kg, the third was given 15 mg/kg, and the fourth, 12 mg/kg. The second through fourth doses were chosen based on the reactions of the previous dog to its initial dose of microplasmin.

Results:

Mortality/Clinical Signs: Dogs were checked several times daily for viability and clinical signs of toxicity.

No clinical signs were observed in the 10 mg/kg dog until Day 7. On that day, the dog appeared agitated. On days 7-10, the animal “knocked” its body against the sides of its pen during infusion. When blood was collected for TK on Day 10, it took 5 minutes for the sampling site to stop bleeding.

The 20 mg/kg dog rubbed its body on the pen floor during the Day 1 infusion. Increased clotting time (7 minutes) was observed when the 30 minute TK blood sample was drawn on Day 1. On Day 2, emesis occurred 10 minutes after the start of infusion and again near the end of infusion. The dog was agitated, then subdued with pale mucous membranes and excessive salivation near the end of infusion, continuing after infusion was over. The later vomited material was yellow and red. The dog was sacrificed for humane reasons on Day 2.

On Day 1 of dosing, prolonged clotting time (over 1 hour) was noted when the 30 minute TK sample was drawn from the 15 mg/kg dog. The animal vomited after infusion

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on Days 2 and 3. The dog was agitated on Day 4, paced around its pen, and "knocked" its body against the sides of the pen during infusion. Vocalization and emesis were observed 40 minutes into the 1 hour infusion on Day 4 and the dog was subdued after infusion. It was sacrificed for humane reasons on Day 4.

The only clinical observation noted for the 12 mg/kg dog that may have been related to drug treatment was increased clotting time (15 minutes) when the 4 hour TK sample was drawn on Day 1.

Body weights: Animals were weighed weekly prior to the initiation of dosing and twice weekly during the dosing period. There were no treatment-related effects on body weight.

Food consumption: Recorded daily. During the dosing period, food was offered after infusion. Treatment-related changes in food consumption were not observed in the animals that were not sacrificed *in extremis*.

Water consumption: Not measured, available *ad libitum*.

Ophthalmoscopy: Not done.

ECGs: Not done.

Hematology/Clinical chemistry: Blood samples were collected from fasted dogs prior to the initiation of dosing and at the end of the dosing period prior to sacrifice. Treatment-related effects on hematology parameters were not observed, although several animals had prolonged clotting times following infusion of microplasmin. The blood samples collected for hematology evaluation were not collected until the day after the animals received their last dose of drug and clotting ability may have returned by that time. The only changes in clinical chemistry parameters were observed in the dogs sacrificed *in extremis*. AST was elevated in both the 15 and 20 mg/kg dogs (much higher in the latter) and ALT and triglycerides were also elevated in the 20 mg/kg dog.

Urinalysis: Not done.

Gross pathology: Hemorrhage of the duodenum, colon, abdominal wall, and esophagus occurred at 15 mg/kg. The animal also had enlarged, reddened bronchiolar and mediastinal lymph nodes. The 20 mg/kg dog exhibited bruising and edema of the distal esophagus and cardiac sphincter, diaphragmatic hemorrhage, and reddening of the stomach and lymph nodes. This animal had dark lung lobes and a dark liver hilum. These findings are consistent with the thrombolytic activity of microplasmin. The possible relationship of red cecum observed in the 10 mg/kg dog and red abdominal cavity in the 12 mg/kg animal to drug treatment is unclear. Tissues from the 10 and 12 mg/kg dogs did not undergo histopathologic analysis.

Organ weights: No treatment-related effects were apparent.

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Histopathology: Adequate Battery: The study protocol did not call for the collection of a full battery of tissues. The table in the Appendix lists the organs that were preserved and/or weighed. The only tissues examined microscopically were those from the animals that were sacrificed early. These procedures are acceptable for a nonGLP dose-setting study.

Peer review: yes ( ), no (x)

In the 15 mg/kg dog, acute hemorrhage was observed within the submucosa and muscle layers of the duodenum, colon and esophagus and between the fascial planes of the right ventral body wall. The surface of the intestinal epithelium did not exhibit any microscopic changes. An increased bleeding tendency would be plausible following microplasmin treatment.

The 20 mg/kg dog exhibited congestion and hemorrhage of the exterior distal esophageal wall, gastric serosa (near the cardiac sphincter), and within sinusoids around the central veins and portal tracts of the liver. The pathologist considered these findings to be an effect of the vomiting. As with the 15 mg/kg dog, the bleeding was likely exacerbated by the activity of the test article.

Histologic observations at the infusion sites of both animals were consistent with what would be expected after implantation of a venous access port.

Toxicokinetics: Although the protocol called for plasma fibrinogen levels to be measured, there was insufficient sample remaining to do the analysis in all but one case. Thus, no meaningful data were available for this parameter. In most dogs, microplasmin levels fell between 30 minutes and 4 hours after infusion, while the activity of α2-antiplasmin rose. It is notable that microplasmin levels are not higher in the 15 and 20 mg/kg dogs than in the 12 mg/kg dog at these time points. It is possible that a difference between these doses may have been detected if a blood sample had been drawn earlier Microplasmin binds to α2-antiplasmin and forms a complex with a half life of approximately 12 hours. The investigators theorized that the increase in α2-antiplasmin activity at the 4 hour time point was an indication that the level of this enzyme was rebounding as microplasmin and the microplasmin- α2-antiplasmin complexes were being removed from the circulation.

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Plasma Levels of Microplasmin and α2-Antiplasmin in Beagle Dogs Following Intravenous Infusion of Microplasmin

Dose (mg/kg) (M/F)	Day	Microplasmin (µg/ml)		α2-Antiplasmin (% activity)	
		30 min	4 hr	30 min	4 hr
10/M	1	16.57	7.16	7.4	33.5
	10	7.91	9.88	12.8	29.6
12/F	1	17.98	9.88	8.2	30.2
	10	43.73	22.02	14.1	27.6
15/M	1	25.20	15.10	8.7	32.0
	4	17.97*	---	14.1*	---
20/F	1	31.13	22.57	5.4	28.9

*sample drawn at 45 min, rather than 30 min after infusion

Microplasmin 14 Day Intravenous Infusion Toxicity Study in Dogs

Key study findings: Although clinical signs including agitation, vocalization, salivation and retching or vomiting were observed in some animals from the 7 and 10 mg/kg dose groups, necropsy did not reveal any gross or microscopic changes that appeared treatment-related. Adverse clinical signs were not observed at 2 mg/kg. There were no toxicologically significant changes in hematology or clinical chemistry parameters that appeared related to microplasmin in any dose group. Plasma analysis for fibrinogen levels and α2-antiplasmin activity showed that reductions in both parameters occurred in concert with microplasmin administration; these levels rebounded to near control levels by 24 hours after infusion.

Study no.: 662314 (b) (4) Report No. 21420)

Conducting laboratory and location: (b) (4)
Date of study initiation: 1/28/02
GLP compliance: OECD GLP
QA report: yes (x) no ( )
Drug, lot #, % purity: Microplasmin, Batch No. TOX-L-µPLA-PO3, > 90% purity

Methods

Doses: 0, 2, 7, or 10 mg/kg (chosen based on the results from the dose-setting study above)- analysis found the dosing solutions to be within 10% of intended concentration, though only total protein was measured- not activity
Species/strain: Beagle dogs
Number/sex/group (main study): 3/sex/dose group with an additional 1/sex/group for recovery
Route, formulation, volume: Doses were administered by intravenous infusion through a venous access port in the jugular vein. The dogs wore jackets to house the infusion system. (b) (4) Half of each dose was administered over 15 minutes at a dose volume of 1

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ml/kg and the other half was given over an hour at the same dose volume. Doses were given every other day for 14 days for a total of 7 administrations. The vehicle- treated animals received 0.9% NaCl containing mannitol and citric acid (excipients present in the microplasmin drug product) at the same levels as the dosing solution of the high dose group.
Satellite groups used for toxicokinetics: Main study animals were used for TK.
Age: approximately 6-6.5 months old
Weight: 5.5-9.1 kg
Sampling times for TK: Blood samples for the measurement of microplasmin, fibrinogen, and α2-antiplasmin plasma levels were drawn 30 minutes and 1, 2, 4, and 6 hours on Day 1 of dosing from the control, 2, and 7 mg/kg groups. For the high dose group, TK samples were drawn 4 and 6 hours after dosing. On Day 13, TK samples were drawn from all treatment groups 30 minutes and 1, 2, 4, 6, and 24 hours after dosing.
Unique study design or methodology: Main study animals were sacrificed on the day after the final infusion. Recovery animals were kept for an additional 14 days prior to sacrifice.

Results:

Mortality/Clinical Signs: Dogs were checked at least twice daily for viability and clinical signs of toxicity.

No treatment-related clinical signs were observed in the control or low dose dogs.

In the 7 mg/kg group, one male had clear, jelly-like feces containing red mucoid material on Day 3. Emesis occurred in another male from this group at the end of the 15 minute infusion on Day 13. A female dog was agitated and rubbed against the wall of the pen while vocalizing on Day 9 during the initial part of the infusion. These clinical signs were not observed 10 minutes into the 1 hour infusion on Day 9.

At 10 mg/kg, 2 males had green mucoid feces that contained red material on Day 7. Another male from this group was agitated and vocalized during infusions on Days 11 and 13. On Day 13, it was also observed rubbing against the walls and floor of the pen. Similar signs were observed in a female at 10 mg/kg during infusion on Day 13. Another female from this group experienced salivation and retching on Day 9.

Body weights: Animals were weighed weekly. The high dose dogs tended to gain less weight than the other groups, although the absolute differences were small.

Food consumption: Recorded daily. During the dosing period, food was offered after infusion. Treatment-related changes in food consumption were not observed.

Water consumption: Not measured, available *ad libitum* (except during urine collection).

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Ophthalmoscopy: Indirect ophthalmoscopy was performed prior to the initiation of dosing, near the end of the treatment period, and near the end of the recovery period. No treatment-related effects were observed.

ECGs: Recorded using Limb Lead II prior to the initiation of dosing and 1 hour after dosing on Days 1 and 13. No treatment-related effects were observed.

Hematology/Clinical chemistry: Blood samples were collected from fasted dogs twice prior to the initiation of dosing (before and after catheter implantation surgery) and at the end of the dosing and recovery periods prior to sacrifice. No treatment-related effects on hematology parameters were observed. Small increases in total protein and albumin levels were observed in the mid and high dose dogs, but their toxicological significance is uncertain.

Urinalysis: Urine specimens were obtained before the initiation of dosing and toward the end of the dosing and recovery periods. No treatment-related effects were observed.

Gross pathology: No treatment-related effects were apparent.

Organ weights: No treatment-related effects were apparent.

Histopathology: Adequate Battery: Yes, the table in the Appendix lists the organs that were preserved and/or weighed. In addition, the stifle joint was collected.
Peer review: yes ( ), no (x)

No treatment-related effects were apparent. Microscopic examination of tissues at the infusion sites of animals from all dose groups did not reveal differences between the groups.

Toxicokinetics: The methods used by the investigators to measure the concentration (or activity) of microplasmin, fibrinogen, or α2-antiplasmin in plasma samples were not provided in the report. Systemic exposure to microplasmin generally increased with dose. Exposure (expressed as AUC) was higher in males than females, consistent with the higher clearance rate and greater volume of distribution in females. All control samples with the exception of 2 (1 male at 30 minutes on Day 13 and 1 female 1 hr on Day 1) were below the limit of quantitation for microplasmin. It is noted that the 2 control samples above the LOQ had microplasmin levels far below even the lowest concentrations measured in the drug-treated groups (≥45-fold difference). TK parameters for microplasmin could only be calculated for the high dose group on Day 13 due to sparse sampling on Day 1. Estimates of microplasmin half life were approximately 1-2 hours at the low and mid dose, with half life tending to be longer in females (despite their higher clearance rate). At the high dose, half life was estimated at 3.7-3.9 hours. The values for clearance and volume of distribution for microplasmin varied widely even within the same dose group, so their reliability is questionable.

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Perturbations of α2-antiplasmin and fibrinogen levels were associated with microplasmin treatment. At the 30 minute time point (the first measurement taken after infusion of microplasmin), the plasma activity level of α2-antiplasmin was at its lowest. The Day 13 data showed that the activity of this protein rebounded to control levels (or beyond) by 24 hours after the last infusion. Infusion of microplasmin was associated with a decrease in plasma fibrinogen levels. The decrease was greater between 2 and 7 mg/kg than between 7 and 10 mg/kg. For the first 4 hours after infusion of 7 or 10 mg/kg on Day 13, the blood of several animals did not clot during the time set in the assay, so an accurate measurement could not be obtained. By 6 hours after infusion, the fibrinogen levels began to rebound and they were not far below controls by 24 hours.

Microplasmin Plasma TK Parameters after IV Infusion

Dose (mg/kg)	**Day**	**$AUC_{0-\infty}$** (µg•hr/ml)		**Cmax** (µg/ml)		**Cl** (ml/kg·hr)		**Vd** (ml/kg)	
		M	F	M	F	M	F	M	F
2	1	15.6	10.5	10.1	5.3	160.7	211.1	225.3	408.3
	13	15.9	11.0	12.0	5.1	152.5	191.6	219.9	344.5
7	1	159.0	36.0	61.8	25.0	50.1	235.9	65.7	592.8
	13	184.9	32.3	66.7	26.4	45.2	259.0	70.4	711.0
10	1	---	---	---	---	---	---	---	---
	13	115.1	135.4	20.5	41.6	88.2	74.0	481.5	381.0

2.6.6.9 Discussion and Conclusions

The rabbit was more sensitive to microplasmin-induced ophthalmologic changes than cynomolgus monkeys or minipigs, but the latter 2 species are thought to be more clinically relevant ocular models. The intravitreal injection of microplasmin caused narrowing of the retinal vessels in Dutch-Belted rabbits at doses as low as 2.5 µg/eye, but this was not observed in cynomolgus monkeys or minipigs at doses up to 125 µg/eye. Dose-related inflammation including uveitis was observed in both rabbits and monkeys, but the manifestations of inflammation (aqueous flare, presence of vitreous and/or aqueous cells, and/or foveal pigment changes) were transient if adequate recovery time was allowed. ERG responses were diminished within 2 days of microplasmin injection in rabbits and monkeys. This effect was reversed in the monkeys within an 8-week recovery period with substantial recovery occurring in many animals during the first few weeks. In rabbits, single intravitreal doses of $\geq$ 50 µg/eye were associated with retinal atrophy (thinning, or in more severe cases, an absence of the rod and cone layer and outer nuclear layer of the retina). This may have been related to a reduced blood supply due to the more persistent narrowing of the retinal vessels caused by these doses of microplasmin. There was substantial recovery of ERG responses in rabbits at the end of an 8-week observation period, but recovery was not complete in some of the higher dose animals.

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Systemic toxicity of microplasmin is unlikely following an intravitreal injection. Significant systemic exposure is not expected following the low (microgram) intravitreal doses that are planned for the clinical trial. Intravenous doses of up to 10 mg/kg/day for up to 14 days produced no microscopic changes or changes in clinical chemistry parameters in dogs and rats. A 10 mg/kg dose did transiently prolong clotting time in some dogs. Doses of ≥ 15 mg/kg caused internal hemorrhaging, likely due to the thrombolytic activity of microplasmin.

OVERALL CONCLUSIONS AND RECOMMENDATIONS

Summary: The pharmacologist has no objection to the initiation of the proposed clinical trial of microplasmin in patients undergoing surgical vitrectomy. The intravitreal data from minipigs and cynomolgus monkeys suggest that the clinical doses (25, 75, or 125 µg/eye) should be reasonably safe. Additionally, there is European clinical experience with single intravitreal doses up to 125 µg/eye. Transient inflammation following the administration of drugs via the intravitreal route is not uncommon and the animal data indicate that inflammation would likely be transient if it occurs in humans. A diminution of ERG response associated with inflammatory changes in animals would also be anticipated to be reversible in humans. Although dose-related narrowing of the retinal vessels was observed in rabbits and appeared to be associated with a dose-related retinal atrophy in this species, the effect was not observed in minipigs or monkeys given doses of microplasmin up to 125 µg/eye. Slight retinal degeneration was observed in one monkey eye that received 200 µg of microplasmin, but this eye also developed a hyphema following trauma that occurred after microplasmin treatment during the observation period, so some changes observed in this animal were difficult to attribute solely to microplasmin treatment. The vitreal volumes of humans, cynomolgus monkeys and minipigs are estimated to be approximately 4, 2-3, and 3.5 ml, respectively. Thus, the 125 µg doses of microplasmin administered to the monkeys and minipigs are comparable (or about 12.5-50% greater) than the highest dose planned for the clinical trial.

Internal comments: The comparability of the different batches of microplasmin used in the nonclinical studies with the drug to be used in clinical trials is uncertain, according to the Chemistry reviewers. They have requested information from the sponsor to permit a more comprehensive comparison between the batches.

External comments (sent to sponsor on 11/7/06 by M. Puglisi): Your method for determining the concentration of microplasmin in your dosing solutions for the nonclinical studies appears to be inadequate. Simply measuring the protein content of the dosing solutions is nonspecific and does not provide information about the stability of microplasmin in the dosing solutions. You should use a method of analysis specific for microplasmin that will demonstrate its stability in the dosing solutions used in your studies. Additionally, you did not provide adequate information regarding the purity of the microplasmin that you used in the nonclinical studies. Although you provided TK data for repeat dose studies conducted in dogs and rats, you did not provide the

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methods of analysis that you used to measure the levels of microplasmin or α2-antiplasmin in the plasma samples. Please provide these methods with appropriate validation.

Signatures:

Reviewer Signature _____

Team Leader Signature_____ Concurrence Yes ___ No ___

Histopathology Inventory

Study	662293	662314
Species	Dog	Dog
Adrenals	X*	X*
Aorta		X
Bone Marrow		X[a]
Bone		
Brain	X*	X*
Cecum		X
Cervix		
Colon		X
Duodenum		X
Epididymis		X*
Esophagus		X
Eye		X
Fallopian tube		
Gall bladder		
Gross lesions		X
Harderian gland		
Heart	X*	X*
Ileum		X
Injection site	X	X
Jejunum		X
Kidneys	X*	X*
Lachrymal gland		
Larynx		X
Liver	X*	X*
Lungs	X*	X*
Lymph nodes,		

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cervical		
Lymph nodes mandibular		X
Lymph nodes, mesenteric		X
Mammary Gland		X
Nasal cavity		
Optic nerves		
Ovaries	X*	X*
Pancreas	X*	X*
Parathyroid		X*
Peripheral nerve		
Pharynx		
Pituitary		X*
Prostate		X*
Rectum		X
Salivary gland		X*
Sciatic nerve		X
Seminal vesicles		
Skeletal muscle		X
Skin		X
Spinal cord		X
Spleen	X*	X*
Sternum		X
Stomach		X
Testes	X*	X*
Thymus	X*	X*
Thyroid		X*
Tongue		X
Trachea		X
Urinary bladder		X
Uterus	X*	X*
Vagina	X	X
Zymbal gland		

X, tissue collected
*, organ weight obtained
[a], smears of rib bone marrow were taken

Reference ID: 3198420

PHARMACOLOGY/TOXICOLOGY SAFETY REVIEW

IND Number: **100,370**
Date/Type of Submission: October 12, 2006/ IT
Information to Sponsor: Yes () No (x)
Sponsor: ThromboGenics Inc.
Reviewer Name: Maryam Rafie-Kolpin, Ph.D.
Division Name: Division of Anti-Infective and Ophthalmology Products

Drug: Recombinant Human (rh) Microplasmin

Relevant INDs/NDAs/DMFs: None

Drug Class: Thrombolytic agent

Indication: (b) (4)

Route of Administration: Intravitreal injection

Disclaimer: Sponsor's materials have been used in this IND review.

Studies reviewed within this submission: This is a review of safety pharmacology, single dose and repeat dose toxicity studies conducted in Göttingen mini-pigs and rats. Other single and repeat dose studies submitted to this IND are reviewed by Dr. Amy Elis. Furthermore, background information concerning this drug can be found in her review.

Safety pharmacology

Neurological effects:
Study title: Evaluation of the Effect of Microplasmin on the Modified Irwin Screen Test in the Rat ([redacted] (b) (4) Study # 257003)
This study was conducted by [redacted] (b) (4) in accordance with the GLP regulations.
Microplasmin was administered intravenously to 4 groups of 5 male Sprague-Dawley rats at 0 mg/kg (vehicle), 2 mg/kg, 6 mg/kg and 10 mg/kg. The positive control group received an oral dose of 30 mg/kg of caffeine. Behavioral and clinical observations were recorded for all animals predose, and at 15, and 30 min, 1, 2, and 6 h post-dose. Animals were examined at 24 h if treatment-related changes were observed at 6 h. In general, increased startle response, difficult handling, tachypnea, diarrhea, or increased alertness was observed. Grooming sessions was noted in all groups. A decrease in the exploratory activity at 6 h post dose was also reported. These behavioral changes did not follow a dose-response relationship.
Treatment of animals with caffeine resulted in an increase in startle response, vocalization during handling, an increase in exploratory activity and increased alertness. Statistical analyses were performed on pain response and locomotor activity. This analysis indicated that there were no differences between caffeine-treated animals and vehicle or Microplasmin-treated animals. Sponsor explained that the lack of differences between positive control group and vehicle or test article treated groups was caused by the administration of caffeine to non-fasting animals in the caffeine-treated group. Lack of appropriate positive control renders the data inconclusive.

Cardiovascular effects:
Study title: Effect on the Cardiovascular and Respiratory System in the Anaesthetized Dog ([redacted] (b) (4)
This study was conducted by [redacted] (b) (4) This study was conducted in accordance with GLP regulations.
The purpose of this study was to evaluate the potential effects of the test article on the cardiovascular, respiratory system and hematological parameters in anesthetized beagle dogs. Four beagle dogs were used in this study. Animals were administered vehicle, 0.15 mg/kg, 1.5 mg/kg or 15 mg/kg sequentially with at least 30 min between the end and the beginning of each administration. Body weight, cardiovascular parameters (systolic and diastolic blood pressure and heart rate), electrocardiogram, respiratory parameters (respiratory rate, tidal volume and minute volume), hematological parameters and blood gases were monitored. Toxicokinetic analysis was performed using ELISA assays.
Infusion of 15 mg/kg resulted in a significant decrease in the systolic, diastolic and mean blood pressure within the first 5 min of infusion and they continued to fall. Decreases of 52.4% and 51% were observed in the systolic and diastolic blood pressures, respectively, at 20 min. The mean blood pressures fell by 51% at the end of the infusion. A decrease of 12.5% was observed in the heart rate with 15 mg/kg

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microplasmin treatment at the end of infusion compared to the pre-dose values. No significant changes in the QT interval were observed at any dose.
A decrease in the blood pH from 7.196 control value to 7.04 at 15 mg/kg dose was observed. No significant effects were observed in the respiratory rate or minute volume at any of dose levels. A 25% decrease was observed in the tidal volume with 15 mg/kg compared to the pre-dose values at 10 min after the end of infusion. Since a decrease in the blood pH was observed it was expected to see an increase in the tidal volume and/or respiratory rate to allow the blood pH to return to normal. The sponsor did not provide any explanation regarding these contradictory observations.
Significant increases in the RBC (37%), hemoglobin (42%), and hematocrit (38%) were observed in the 15 mg/kg dose. The hematological values were within the physiological range and were therefore not toxicologically significant. A toxicologically significant increase of 52% was observed in the glucose value compared to predosing value in the 15 mg/kg treatment. Plasma concentrations of microplasmin were determined using an ELISA assay. The plasma concentration of microplasmin increased with dose. The increases in the plasma concentrations were not dose-proportional. The minimum detection limit was 0.625 ng/ml in the buffer. Microplasmin concentrations are summarized in the table below.

Determined Plasma Concentrations of Microplasmin (μg/ml)

Dose (mg/kg)	0	0.15	1.5	15
Animal				
1	<LOQ	1.31	6.87	54.25
2	<LOQ	1.61	6.88	68.96
3	<LOQ	1.50	7.22	112.36
4	<LOQ	1.76	6.16	250.72

LOQ= Limit of quantitation of the assay

TOXICOLOGY

Single-dose toxicity

Study Title: A Single Dose Intravitreal Toxicity Study of (b) (4) Microplasmin for Injection in the Göttingen Mini-Pig Followed by an 8-Week Observation Period (Final study report)

Key study findings: Single intravitreal injection of Microplasmin appeared safe at 5 and 25 µg/eye. One incident of partial luxation of the lens was observed in one high dose animal on Day 28 which remained stable through Day 56. In this study, 25 µg/eye was considered the NOAEL.

Study no.: (b) (4) study # 500209

Reference ID: 3198420

Volume #, and page #: 21, and 1
Conducting laboratory and location: [redacted] (b) (4)
Date of study initiation: December 16, 2003
GLP compliance: Yes
QA report: yes (x) no ( )
Drug, lot #, and % purity: [redacted] (b) (4) TOX-L-µPLA-P06, and purity > 90%
Vehicle: [redacted] (b) (4) consisting of [redacted] (b) (4) citric acid pH 3.1 and [redacted] (b) (4) mannitol, Lot # M-µPLA-BL-01

Methods

Doses: Control, 5, 25 and 125 µg/ 50µl
Species/strain: Göttingen Mini-Pig
Number/sex/group: 3 males
Route, formulation, and volume: Intravitreal injection of 50 µl of Microplasmin in the right eye only, followed by a 56-day observation period. The left eye was treated with the vehicle.
Age: 3.5 months
Weight: 6.4-8.1 kg

Observation and Times:
Clinical signs: Twice daily
Body weights: Weekly
Food consumption: Individual food consumption was noted but not recorded unless an animal did not consume its food in its entirety.
Ophthalmoscopy: Slit lamp, indirect ophthalmoscopy, tonometry, electroretinography and dynamic light scattering (DLS) were performed. Tonometry was performed once pretreatment and on Days 2, 7, 28 and 56. Bilateral electroretinography was performed once during the pre-treatment period, on Day 2, 7, 28 or 31 and 56. DLS was performed separately for each eye of each animal.
Gross pathology: At the end of treatment or recovery period
Organ weights: Not performed
Histopathology: Eyes and optic nerves were stained with hematoxylin and eosin. For each eye a total of five sagittal sections were evaluated. The injection site was examined only when it was present in the routine sections.

Adequate Battery: yes (x), no ( )
Peer review: yes ( ), no (x)

Results:

Mortality: One high dose animal (402) died under anesthesia during ERG procedure on Day 56. The death was not considered test article-related.

Clinical signs: Animal 203 had a red placebo-treated (left) eye on Day 3.

Body weights: No test article-related effects were noted.

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Food consumption: There were no test article-related effects on food consumption.

Ophthalmoscopy: Slight to moderate subconjunctival hemorrhage and/or conjunctival hyperemia were observed in control and treated eyes. These observations were considered to be related to the injection procedure. No test article effects were observed in the low and mid dose groups. One animal in the 125 µg group had a slight displacement of the lens on Day 28 which remained unchanged on Days 41 and 56. It is not clear if the slight displacement of the lens observed in this animal is coincidental or is caused by the treatment. Tonometry and ERG results indicated that there were no test article effects on these parameters. The DLS indicated the vitreous particle size decreased slightly for the low-dose, increased slightly in the mid-dose and increased greatly for the high-dose animals. It was expected that vitreous particle size would decrease after administration of Plasminogen due to the protease activity of this product. The Sponsor speculated that the administration of Microplasmin in the early stages could have initiated vitreous gel breakdown. The increase in the dose resulted in an increase in the vitreous gel breakdown, however as vitreous molecular chains are broken down, the polyelectrolyte broken pieces form aggregates which results in the increased vitreous particle size observed. Therefore, intravitreal treatment of eyes with Microplasmin resulted in significant change in the morphology of the vitreous by cleaving vitreous structures.

Gross pathology: There were no test article-related macroscopic changes

Histopathology: Adequate Battery: yes (x), no ( )
Peer review: yes ( ), no (x)
There were no test article-related microscopic effects.

Reviewer's comments: A single intravitreal injection of Microplasmin at 5, 25 or 125 µg/eye was well tolerated in male Göttingen mini-pig. The only toxicologically significant change was observed in one animal in the 125 µg/eye group. This animal developed partial luxation of the lens on Day 28. Intravitreal treatment with Microplasmin resulted in significant morphological change in the vitreous due to breakdown of vitreous structure. The NOAEL was 25 µg/eye for this study.

Study title: **An Intravitreal Bridging Study of Two Formulations of** (b) (4) **Microplasmin for Injection in Göttingen Mini-Pigs with An 8-Week Observation Period**

Key study findings: Both formulations were well tolerated. The NOAEL was 100 µg/eye in this study.

Study no.: (b) (4) # 501082
Volume # and page #: 22, and 1
Conducting laboratory and location: (b) (4)
Date of study initiation: March 15, 2005

Reference ID: 3198420

GLP compliance: Yes
QA report: yes (x) no ( )
Drug, lot #, and % purity: Microplasmin solution for injection, M-PLA-REC-FOR/1.87-a, According to the sponsor, the Microplasmin concentrations were ≥ 90%
Vehicle: Placebo Microplasmin for injection consisting of (b) (4) citric acid pH 3.1 and (b) (4) mannitol

Methods

Doses: Placebo, 50 and 100 µg/eye in (b) (4) or citric acid pH 3.1

Species/strain: Göttingen mini-pig

Number/sex/group: 3 males/group, with the exception of Group 4 which received 4 males

Route, formulation, volume, and infusion rate: Intravitreal injection of 50 µl of the test article in either (b) (4) or in citric acid in one eye

Age: 6 months

Weight: 8.8-12.9 kg

Study design or methodology: Groups 1 and 3 received 50 or 100 µg/eye of Microplasmin (b) (4) in one eye. The fellow eye was used as the control eye. This eye received placebo Microplasmin for injection (b) (4) Animals in Group 2 and 4 received 50 or 100 µg/eye of Microplasmin in citric acid in one eye. The fellow eye was used as the control and received placebo Microplasmin in citric acid.

Observation and Times:
Clinical signs: Twice daily
Body weights: Weekly and prior to scheduled necropsy
Food consumption: Daily
Ophthalmoscopy: Animals were subjected to ocular examination consisting of slit lamp, indirect ophthalmoscopy, tonometry and electroretinography, once during the pretreatment, and on Days 2, 7, 28 and 56.
EKG: Not performed
Hematology: Not performed
Clinical chemistry: Not performed
Urinalysis: Not performed
Gross pathology: At the end of recovery period
Organ weights: Not performed
Histopathology: Eyes and optic nerves were stained with hematoxylin and eosin. For each eye a total of five sagittal sections were evaluated. The injection site was examined only when it was present in the routine sections.

Adequate Battery: yes (x), no ( )
Peer review: yes ( ), no (x)

Results:

Mortality: Animal 103 died presumably due to the anesthetic complications on Day 56 during ERG recording.

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Clinical signs: No test article-related clinical signs were noted.

Body weights: No test article-related effects on body weight were noted.

Food consumption: No test article-related changes in the food consumption were noted.

Ophthalmoscopy: Subconjunctival hemorrhage and conjunctival hyperemia were observed in control as well as the treated groups. These effects were resolved by Day 2. The sponsor concluded that these observations were as a result of the injection procedure. This conclusion appears reasonable. ERG results did not show any "group trends" indicative of a test article-related effect. Similarly, tonometry results did not indicate test article-related effects on intraocular pressure.

Gross pathology: No macroscopic test article-related effects were reported.

Histopathology: Adequate Battery: yes (x), no ( ) Only ocular tissues were examined.
Peer review: yes ( ), no (x)
No test article-related microscopic changes were noted in the eyes. Most changes observed were considered incidental or procedure driven.

Reviewer's comments: Intravitreal administration of Microplasmin at 50 or 100 µg/eye doses were well tolerated in either (b) (4) or Citric acid solution (pH 3.1) by Göttingen mini-pig. In this study, 100 µg/eye dose was considered the NOAEL.

Study title: **Recombinant Human Microplasmin Acute Intravenous Toxicity Test in Rats**

Key study findings: Treatment of animals with a single intravenous administration of 40 mg/kg Microplasmin resulted in 8 premature deaths. Rats treated with 25 mg/kg intravenously showed signs of labored breathing, and subdued behavior. At 10 mg/kg dose of Microplasmin, no signs of toxicity were noted in rats. This dose was considered NOAEL.

Study no.: (b) (4) # 501684
Volume #, and page #: 22 and 1
Conducting laboratory and location: (b) (4)
Date of study initiation: August 23, 2001
GLP compliance: Yes
QA report: yes (x) no ( )
Drug, lot #, and % purity: Microplasmin, Tox-E-µPLA-P05-A, and 91%
Vehicle: Saline

Methods

Reference ID: 3198420

Doses: 0, 2, 10, 25 and 50 mg/kg doses were administered in the dose-ranging study and 0, 10, 25 and 40 mg/kg doses were administered in the main study

Species/strain: Sprague-Dawley rats

Number/sex/group: 2 female animals/group were used in the dose-ranging study. In the main study 5/sex were used in each study group.

Route, volume, and infusion rate: Intravenous; a constant dose volume of 5 ml/min was used to administer the vehicle or the test article. The dose rate was about 0.7-1.4 ml/min.

Age: 6-7 weeks

Weight: 174-207 g

Study design or methodology: Animals in the dose-ranging study phase were observed for 7 days. Animals in the main study were observed for up to 14 days. Dosing was conducted in a step-wise staggered manner.

Observation and Times:

Clinical signs: Twice daily

Body weights: On Day 1 in the dose-ranging study and Days 1, 8 and 15 in the main study

Food consumption: Not performed

Ophthalmoscopy: Not examined

EKG: Not performed

Hematology: Not performed

Clinical chemistry: Not performed

Urinalysis: Not performed

Gross pathology: Macroscopic examination was made only in the animals in the main study. The necropsy examination consisted of an examination of the cranial, thoracic and abdominal organs and tissues *in situ*.

Organ weights: Not performed

Histopathology: specify groups examined, special stains, etc

Adequate Battery: yes ( ), no (x)—explain: Histopathology examination was not performed.

Peer review: yes ( ), no (x)

Results:

Mortality: All animals (2/2) in the 50 mg/kg treatment group in the dose range finding Phase died. In the main study, 4/5 male and 4/5 female animals were found dead in the 40 mg/kg group.

Clinical signs:

Dose-range finding phase: at 25 mg/kg, labored breathing and subdued behavior were reported 2 min after dosing. At 50 mg/kg dose, both animals were found dead 2.25 h after dosing. Staggering, labored breathing and prostration were noted in these animals prior to death.

Main study: At 25 mg/kg subdued behavior and staggering were noted 15 min after dosing. Subdued behavior and labored breathing was observed in animals treated with 40 mg/kg dose. Four males and 3 females were found dead within 4 h after dosing and

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one female was found dead approximately 6 h after administration of 40 mg/kg dose. The clinical signs in the remaining animals included labored breathing, hunched appearance and subdued behavior. All animals recovered by Day 2. The clinical signs observed in the 25 and 40 mg/kg animals are considered test article-related.

Body weights: No change in the body weight gain was noted.

Gross pathology: No abnormalities were noted in the animals in the 10 and 25 mg/kg groups. In the 40 mg/kg animals, redness in the mesenteric lymph nodes, thymus, ileum, caecum and stomach were reported. These macroscopic observations were made in the premature decedents. These findings are considered treatment related.

Reviewer's Comments: At 10 mg/kg dose of Microplasmin, no signs of toxicity were noted in rats. This dose was considered NOAEL.

Repeat-dose toxicity

Study title: Microplasmin 14 Day Intravenous Infusion Toxicity Study in Rats

Key study findings: Intravenous infusion of Microplasmin at 0, 2, 7 and 10 mg/kg for 14 days, administered every other days, was well tolerated by SD rats. The NOEL was considered to be 10 mg/kg. TK results indicated that exposure and Cmax increased with dose. The increase in the PK parameters was not dose proportional.

Study no.: (b) (4) report no. 21858
Volume #, and page #: 23 and 1
Conducting laboratory and location: (b) (4)
Date of study initiation: May 6, 2002
GLP compliance: Yes
QA report: yes (x) no ( )
Drug, lot #, and % purity: rh Microplasmin, L-µPLA-P06, ≥ 90%
Vehicle: Mannitol 30 mg/ml and citric acid 4.8 mg/ml diluted in saline so that the concentration of mannitol and citric acid would correspond to the concentrations of these components for the high dose group.

Methods

Doses: 0, 2, 7 and 10 mg/kg/day administered every other day for a total of 14 days

Species/strain: Sprague Dawley rats

Number/sex/group: 10

Route, formulation, volume, and infusion rate: Intravenous administration via an initial loading dose (5 ml/kg) over a 15 min period followed by a 1 h intravenous infusion (5ml/kg/hr) via an indwelling femoral catheter

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Satellite groups used for toxicokinetics or recovery: 5/sex. On completion of the dosing period, the satellite animals were kept for a minimal of 14 days post dose recovery period.

Age: 12-13 weeks

Weight: 360-517 g for males and 205-293 g for females

Observation and Times:

Clinical signs: Twice daily
Body weights: Weekly
Food consumption: Weekly
Ophthalmoscopy: Pretrial and towards the end of the dosing and recovery periods using an indirect ophthalmoscope.
EKG: Not performed
Hematology: Blood was collected from all animals at the end of the treatment period and from satellite animals at the end of the recovery period.
Clinical chemistry: Blood was collected from all animals at the end of the treatment period and from satellite animals at the end of the recovery period.
Urinalysis: Urine was collected from all animals at the end of the treatment period and from satellite animals at the end of the recovery period.
Gross pathology: All animals were subjected to necropsy examination consisting of complete external and internal examination to include body orifices and cranial, thoracic and abdominal organs and tissues.
Organ weights: The following organs were weighed; adrenals, brain, epididymides, heart, kidney, liver, lungs, optic nerve, ovary, parathyroids, pituitary, prostate, spleen, testes, thymus, thyroids and uterus.
Histopathology: All animals were subjected to histopathological examination.

Adequate Battery: yes (x), no ( )
Peer review: yes ( ), no (x)

Results:

Mortality: No test article-related mortalities were reported. One female animal (#42) in the control group was prematurely sacrificed on Day 6. Cause of death was explained as procedure-related mortality.

Clinical signs: One female animal in Group 1 (#42) was killed on Day 6. Signs of intermittent dragging hind limbs, pale discoloration of skin at extremities and pale discolored skin around the eyes were noted. The clinical signs observed in this animal were not considered test article-related. Therefore, there were no clinical signs observed in this study.

Body weights: No body weight changes were noted in this study.

Food consumption: No test article changes in the food consumption were observed.

Ophthalmoscopy: No test article-related ophthalmologic changes were observed.

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Hematology: No toxicologically significant changes were observed in the hematological parameters in this study.

Clinical chemistry: At 7 mg/kg/day, 5% increases were observed in total protein and albumin of males and a 6% increase was observed in the total protein value of females. Statistically significant increases were also observed in total protein (5%) and albumin (10%) male animals only, in 10 mg/kg/day group. These changes were not considered toxicologically significant since there were within the physiological range. Therefore, no test-test article changes in the clinical chemistry parameters were noted.

Urinalysis: There were no effects on urinalyses.

Gross pathology: Scabs and masses were noted at the infusion sites. This observation was made in the treated and control male and female animals. Due to lack of a dose response relationship, these lesions are considered to be procedural-related and not test article- related. No test article-related lesions were noted in the necropsy.

Organ weights: No toxicologically significant changes in the absolute and relative organ weights were observed.

Histopathology: Adequate Battery: yes (x), no ( )
Peer review: yes ( ), no (x)
In the main study animals, minimal (3/20) to severe (10/20) phlebitis of vena cava was observed in all animals at the injection site. In addition, mild to marked intimal proliferation and thrombosis of the femoral vein and the vena cava were noted. The pathologist considered these signs secondary to the infusion procedure. This conclusion appeared reasonable.
Inflammation and bronchiolo-alveolar hyperplasia was also observed in the lungs of a 7 mg/kg/day female. Necrosis with inflammation, sinusoidal leucocytosis, periportal inflammatory cell infiltration and bile duct hyperplasia were noted in the liver. Increased extramedullary hematopoiesis and lymphoid hyperplasia were noted sporadically. No dose relationship was associated with any of these findings. These observations were considered to be associated with the inflammation at the injection site by the Sponsor. Due to lack of a dose-response relationship, this reviewer does not consider these findings test article-related.
In the recovery group, inflammation (mild to moderate) of the tail and phlebitis (minimal), intimal proliferation (minimal to moderate) and thrombosis of the femoral vein and the vena cava were observed in all animal groups. This was considered catheter-related. Similar findings as those in the main study group were noted in the livers of the animals in the recovery group.

Toxicokinetics: Blood samples for the determination of plasma concentrations of Microplasmin, fibrinogen and alpha2-antiplasmin levels were collected on the first and last day of dosing. Samples were obtained at 15, and 30 min, 1 h, 2 h and 4 h. Systemic exposure to Microplasmin increased with dose. However, the increase in the exposure was not dose proportional. Systemic exposure and Cmax increased following

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repeat dosing in the mid and high dose groups. Accumulation of Microplasmin to a steady state was observed in the mid and high dose. Tmax for Microplasmin was variable and ranged from 1.5 to 5.25 h. The elimination half-time was also variable and ranged from 0.72 to 7.07 h. According to the Sponsor, no consistent trends in CL and Vd were apparent. These parameters appeared variable. The PK parameters are summarized in the table below.

Mean PK Parameters following a single iv injection of Microplasmin on Days 1 and 13

Gender	Dose level (mg/kg/day)	Day	$AUC_{(0-\infty)}$ (ng.h/ml)	$AUC_{(0-t)}$ (ng.h/ml)	Cmax (ng/ml)	Tmax (h)	T1/2 (h)	Cl (ml/h/kg)	Vd (ml/kg)
Male	2	1	NC	1817	645.1	3.25	NC	N/C	NC
		13	NC	977.8	466.8	5.25	NC	NC	NC
	7	1	19266	7783	3766	1.50	7.07	363.3	3705
		13	34419	15647	5210	1.50	5.20	203.4	1525
	10	1	NC	13063	5454	1.50	NC	NC	NC
		13	43557	24982	10214	2.25	3.15	229.6	1044
Female	2	1	2621	1831	724	1.75	2.18	763.0	2401
		13	NC	946.8	470.7	5.25	NC	NC	NC
	7	1	8699	8470	4381	2.25	0.72	804.7	830.2
		13	14839	9216	4720	1.75	2.31	471.7	1573
	10	1	37537	14810	5152	1.5	6.84	266.4	2630
		13	20030	17316	10162	1.5	0.87	499.3	625.6

NC= Estimate was not calculated

Exposure and Cmax to alpha2-antiplasmin decreased with the increase in the Microplasmin dose. The decrease in the alpha2-antiplasmin was not seen consistently for each dose. The PK parameters for alpha2-antiplasmin are summarized in the table below.

Mean PK Parameters for α2-antiplasmin on Day1 and 13

Gender	Dose Level (mg/kg/day)	Day 1		Day 13	
		AUC(0-∞) (ng.h/ml)	Cmax (mg/dl)	AUC(0-∞) (ng.h/ml)	Cmax (mg/dl)
Male	0	237.6	65.1	224.5	60.90
	2	252.9	68.0	241.3	66.10
	7	227.6	74.3	239.2	68.70
	10	197.2	69.1	172.1	60.90
Female	0	233.3	64.6	233.7	65.10
	2	247.4	68.4	245.3	67.50
	7	242.4	74.6	243.3	70.60
	10	163.6	56.1	155.8	54.80

The PK estimates for alpha2-antiplasmin were comparable between Days 1 and 13 following *iv* infusion of Microplasmin. No gender differences were observed in the systemic exposure to Microplasmin or alpha2-antiplasmin.

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Reviewer's Comments: Intravenous infusions of Microplasmin at 0, 2, 7 and 10 mg/kg for 14 days were well tolerated by SD rats. No test article-related toxicological changes were observed. Microscopic findings were attributed to the method of administration rather than test article. The NOEL was considered 10 mg/kg/day. Exposure and Cmax increased with dose but were not dose proportional on Day 1 and 13. As expected, exposure and Cmax for alpha2-antiplasmin decreased with the increase in the Microplasmin dose.

OVERALL CONCLUSIONS AND RECOMMENDATIONS

Summary: Microplasmin at 15 mg/kg resulted in a decrease in the systolic, diastolic and mean blood pressure values compared to the predose values. At this dose, the percent tidal volume change also decreased. The safety of intravitreal administration of Microplasmin was evaluated by a single intravitreal injection of Microplasmin followed by a 56-day recovery period in Göttingen mini-pig. A single intravitreal injection of Microplasmin at 5, 25 or 125 µg/eye was well tolerated in male Göttingen mini-pigs. The only toxicologically significant change was observed in one animal in the 125 µg/eye group. This animal developed partial luxation of the lens on Day 28. Microplasmin also resulted in significant morphological changes in the vitreous due to breakdown of vitreous structure. The NOAEL was 25 µg/eye for this study. A similarly designed single dose bridging study of Microplasmin in (b) (4) or citric acid (pH 3.1) indicated that both formulations were well tolerated by Göttingen mini-pig. The NOAEL was 100 µg/eye in this study.

Intravenous administration of Microplasmin at 0, 10, 25, and 40 resulted in 8 premature deaths at 40 mg/kg in Sprague Dawley rats. Treatment related microscopic findings at 40 mg/kg Microplasmin included redness in the mesenteric lymph nodes, thymus, ileum, caecum and stomach in prematurely deceased animals. Rats treated intravenously with 25 mg/kg showed signs of labored breathing, and subdued behavior. At 10 mg/kg dose of Microplasmin, no signs of toxicity were noted in rats. This dose was considered NOAEL.

In the 14-day alternate-day dosing study in rats, intravenous administration of Microplasmin at 0, 2, 7, and 10 mg/kg was well tolerated. No test article-related toxicities were noted in this study. Macroscopic and microscopic findings included masses and scabs at the infusion sites and minimal to severe phlebitis of the vena cava in all animals at the injection site, mild to marked proliferation and thrombosis of the femoral vein and of the vena cava. These findings were noted in all groups and were attributed to the method of administration rather than the test article. The NOEL was considered 10 mg/kg/day. Exposure and Cmax increased with dose but were not dose proportional on Days 1 and 13 of this study.

To determine the concentration of the test article, the sponsor measured the total protein or Microplasmin concentrations. This method did not provide any information about the specific activity of the Microplasmin used in these studies. Therefore, the method used for analysis of the test article is considered inadequate.

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The safety margin was calculated from the ration of NOAEL/NOEL in the single dose ocular study in the mini-pigs to the proposed first dose in humans.

Proposed Human Dose		NOAEL Ratio[A]
μg	**μg/kg[B]**	**Mini-Pig**
25	0.416	12

A= Ratio of animal NOAEL to human dose based on μg/kg
B= Based on a 60 kg body weight

The ratio provides a safety margin of 12 in mini-pig. The volume of human (7 ml) vitreous and pig (6 ml) are very similar. Therefore, the doses used in the nonclinical studies support the proposed clinical dose. Microplasmin has been administered intravitreally and intravenously in humans at starting doses of 25 μg and 1 mg/kg, respectively. No adverse effects were reported at these doses. The doses of Microplasmin used in the intravenous studies were far in excess of first human dose in the proposed clinical trial. The duration of the studies reviewed support the proposed single dose clinical trial. Thus, the clinical trial may proceed from the pharmacology/toxicology point of view.

Internal comments: The safety and toxicity findings were discussed with Dr. Ellis. Based on the nonclinical studies reviewed the clinical trial may proceed.
Signatures:

Reviewer Signature _____

Team Leader Signature_____Concurrence Yes ___ No ___

Reference ID: 3198420

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/s/

MARIA I RIVERA
10/02/2012

LORI E KOTCH
10/02/2012

Comments on BLA 125422 Jetrea ocriplasmin intravitreal

From A. Jacobs, AD

9/24/12

1. I concur that there are no pharm/tox approval issues for this BLA.
2. I concur with the pregnancy category C
3. I have conveyed some editorial comments to the reviewer and supervisor and they will be addressed as appropriate

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/s/

ABIGAIL C JACOBS
09/28/2012

PHARMACOLOGY/TOXICOLOGY FILING CHECKLIST FOR NDA/BLA or Supplement

BLA Number: 125422 **Applicant:** Thrombogenics, Inc **Stamp Date:** 4-17-2012

Drug Name: JETREA™ (Ocriplasmin) **BLA Type:** Priority review request

On **initial** overview of the NDA/BLA application for filing:

	Content Parameter	Yes	No	Comment
1	Is the pharmacology/toxicology section organized in accord with current regulations and guidelines for format and content in a manner to allow substantive review to begin?	X		
2	Is the pharmacology/toxicology section indexed and paginated in a manner allowing substantive review to begin?	X		
3	Is the pharmacology/toxicology section legible so that substantive review can begin?	X		
4	Are all required (*) and requested IND studies (in accord with 505 b1 and b2 including referenced literature) completed and submitted (carcinogenicity, mutagenicity, teratogenicity, effects on fertility, juvenile studies, acute and repeat dose adult animal studies, animal ADME studies, safety pharmacology, etc)?	X		
5	If the formulation to be marketed is different from the formulation used in the toxicology studies, have studies by the appropriate route been conducted with appropriate formulations? (For other than the oral route, some studies may be by routes different from the clinical route intentionally and by desire of the FDA).	X		The sponsor conducted a bridging study in monkeys comparing the toxicology of [redacted] (b) (4) liquid formulations of ocriplasmin. The initial toxicology studies were conducted [redacted] (b) (4) The sponsor utilized the liquid formulation in the Phase 2 and Phase 3 clinical trials and nonclinical bridging studies administered by intravitreal injection in monkeys and minipigs.
6	Does the route of administration used in the animal studies appear to be the same as the intended human exposure route? If not, has the applicant submitted a rationale to justify the alternative route?	X		
7	Has the applicant submitted a statement(s) that all of the pivotal pharm/tox studies have been performed in accordance with the GLP regulations (21 CFR 58) or an explanation for any significant deviations?	X		

File name: 5_Pharmacology_Toxicology Filing Checklist for NDA_BLA or Supplement 010908

PHARMACOLOGY/TOXICOLOGY FILING CHECKLIST FOR NDA/BLA or Supplement

	Content Parameter	Yes	No	Comment
8	Has the applicant submitted all special studies/data requested by the Division during pre-submission discussions?	X		
9	Are the proposed labeling sections relative to pharmacology/toxicology appropriate (including human dose multiples expressed in either mg/m2 or comparative serum/plasma levels) and in accordance with 201.57?		X	Section 8.1 and 13.2 of the label needs revision of the safety margins and/or the content of the information proposed to be included.
10	Have any impurity – etc. issues been addressed? (New toxicity studies may not be needed.)	X		Refer to product quality reviewer checklist for further information.
11	Has the applicant addressed any abuse potential issues in the submission?	X		The drug is not expected to have abuse potential.
12	If this NDA/BLA is to support a Rx to OTC switch, have all relevant studies been submitted?			Not applicable

IS THE PHARMACOLOGY/TOXICOLOGY SECTION OF THE APPLICATION FILEABLE? Yes

If the NDA/BLA is not fileable from the pharmacology/toxicology perspective, state the reasons and provide comments to be sent to the Applicant.

Please identify and list any potential review issues to be forwarded to the Applicant for the 74-day letter.

Reviewing Pharmacologist Date

Team Leader/Supervisor Date

Reference ID: 3138753

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/s/

MARIA I RIVERA
05/31/2012

LORI E KOTCH
05/31/2012