Hindawi Publishing Corporation
Journal of Biomedicine and Biotechnology
Volume 2012, Article ID 354979, 6 pages
doi:10.1155/2012/354979

Review Article

Ocriplasmin for Vitreoretinal Diseases

Irena Tsui, Carolyn K. Pan, Ehsan Rahimy, and Steven D. Schwartz

Retina Division, Jules Stein Eye Institute, University of California, Los Angeles, CA 90095, USA

Correspondence should be addressed to Irena Tsui, itsui@jsei.ucla.edu

Received 27 March 2012; Accepted 6 June 2012

Academic Editor: Lindsey A. Miles

Copyright © 2012 Irena Tsui et al. This is an open access article distributed under the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

Fibronectin and laminin are clinically relevant plasmin receptors in the eye. Located at the vitreoretinal interface, they are cleaved by ocriplasmin (Microplasmin, ThromboGenics, Iselin, NJ), a novel ophthalmic medication. A series of clinical trials to study ocriplasmin for the treatment of vitreoretinal diseases such as vitreomacular traction, macular hole, and exudative age-related macular degeneration are underway. The results are promising and may impact patient care.

1. Introduction

The vitreous occupies approximately 80% of the eye and is composed of water, collagen fibers, and hyaluronic acid [1]. In children, the vitreous is normally attached to the retinal surface and relatively innocuous. With aging, the vitreous physiologically liquefies and separates from the retina in a process called posterior vitreous detachment (PVD). The strongest points of vitreoretinal attachment are at the optic nerve, central retina (macula), blood vessels, and ora serrata.

At any age, the vitreous can be abnormally adherent to the macula, leading to sight-threatening diseases such as vitreomacular traction and macular hole [2, 3]. Vitreomacular traction is also implicated in the worsening of diabetic retinopathy and exudative age-related macular degeneration (AMD). Traditionally, diseases of the vitreoretinal interface have been treated with surgery to mechanically detach the vitreous from the retina and improve vision.

Vitreous surgery carries inherent risks such as bleeding, infection, retinal detachment, and accelerated cataract formation. Furthermore, inducing vitreous separation from the retina, particularly in the setting of an abnormal vitreoretinal interface, is among the most technically challenging and dangerous steps of vitreous surgery. Therefore, pharmacological vitreolysis has been an important research goal in ophthalmology [4].

Ocriplasmin (Microplasmin, ThromboGenics, Iselin, NJ) is a medicine that may be injected into the vitreous and administered in an office setting. It is a new technique to pharmacologically induce a posterior vitreous detachment by cleaving the extracellular matrix that adheres the vitreous to the internal limiting membrane of the retina [5–7]. The biomedical rationale and status of ocriplasmin for vitreoretinal diseases are discussed herein.

2. Plasmin Receptors in the Eye

Plasmin, the key enzyme of the fibrinolytic cascade, is also known to cleave other extracellular matrix components, specifically laminin and fibronectin [8–10]. In the eye, both molecules localize to the vitreoretinal interface, where they are postulated to play a central role in the adherence of collagen fibers between the vitreous and the internal limiting membrane (ILM) [11–17].

Early work showing the efficacy of plasmin in inducing a posterior vitreous detachment (PVD) was largely performed in rabbit eyes. Verstraeten et al. [18] initially demonstrated that intravitreal injection of plasmin followed by mechanical core vitrectomy successfully induced a PVD, as was later confirmed by histologic analysis. Hikichi et al. [19] aiming to avert the need for vitrectomy, subsequently combined intravitreal injection of plasmin with SF6 gas into rabbit eyes to successfully create a PVD without any signs of retinal toxicity. Interestingly, plasmin given alone was not sufficient to induce PVD in either study [18, 19]. In contrast to the previous reports, which investigated the intraocular effects of plasmin after only 1 week, Kim et al. [20] followed plasmin-injected rabbit eyes for 4 months, with no significant toxicity

FORMYCON-1075

observed. Additionally, they showed that plasmin alone was sufficient to produce a clean separation between the vitreous cortex and retina [20].

Gandorfer et al. [21] demonstrated that the degree of vitreoretinal separation induced by plasmin directly correlates with the concentration as well as length of exposure to the enzyme. Porcine eyes exposed to 1 unit of plasmin for 30 minutes had a dense network of residual collagen fibrils covering the ILM, while those exposed to 1 unit of plasmin for 60 minutes had only sparse collagen fibrils remaining. Furthermore, eyes treated with 2 units of plasmin for 60 minutes had a smooth retinal surface on postmortem examinations, consistent with a bare ILM. A later study by the same group was the first to duplicate these results in human cadaver eyes, and without any evidence of induced retinal damage [22].

Li et al. [23] separately investigated administration of intravitreal plasmin injections in human cadaver eyes. Using electron microscopy, they observed progressively less evidence of vitreous collagen fibers on the retinal surface with increasing doses of plasmin administered (1, 2, and 3 units) without producing morphological changes or acute toxicity to the inner retina. Through immunocytochemical labeling techniques, they were also able to demonstrate that treatment with plasmin dramatically decreased the density of fibronectin and laminin at the ILM. Uemura et al. [24] additionally confirmed through Western blot analyses that fibronectin and laminin were degraded by plasmin to several fragments of lower molecular weight in the ILMs collected from patients with macular holes or cystoid macular edema who underwent vitrectomy.

Cleavage of fibronectin and laminin may actually offer only a partial explanation to the molecular basis of pharmacologic vitreous detachment. Given that laminin and fibronectin are present at other ocular tissues beyond the vitreoretinal interface, such as the lens, ciliary body, retinal vessels, and lamina cribrosa [14], how is it that intravitreal plasmin injection can induce a PVD without adversely affecting these other structures? The answer may lie in plasmin's additional ability to activate endogenous matrix metalloproteinases (MMP), namely MMP-2 (gelatinase A), which normally reside within the vitreous in their proenzyme state [25–30]. Due to its affinity for various collagens, notably basement membrane type IV, activation of MMP-2 by exogenous plasmin likely contributes to the formation of PVD [26].

Beyond creating a PVD, Brown et al. [27] showed that experimentally injected active MMP-2-cleaved bovine vitreous collagen and concluded that MMP-2 activity could be considered a potential mechanism for the vitreous liquefaction seen in aging as well as various pathologic states. Animal studies suggest that plasmin, likely through activation of MMP-2, may also liquefy the vitreous and be of particular benefit as an adjunct to small-gauge vitrectomy systems. This, in turn, may facilitate both easier and increased vitreous removal during vitrectomy, while shortening duration of surgery. Staubach et al. [31] measured a greater reduction in the wet weight of enucleated porcine eyes injected with plasmin compared with controls once the vitreous was removed by core vitrectomy. Corroborating these findings, Hermel et al. [32] observed a 27% increase in rate of vitreous removal through a 25-gauge cutting system in rabbit eyes injected with plasmin as compared with no injection.

Clinically, autologous plasmin has been utilized as an adjunct to vitrectomy in numerous patient cohorts. Given the robust vitreoretinal adhesion in pediatric patients, Trese and colleagues investigated the utility of plasmin-assisted vitrectomy in the repair of traumatic macular holes [33, 34], stage 5 retinopathy of prematurity [35], and complicated X-linked retinoschisis [36], reporting successful anatomic outcomes in all groups. The use of plasmin-assisted vitrectomy to treat stage 3 full-thickness macular holes has revealed higher rates of spontaneous PVD noted intraoperatively in conjunction with reduction in overall surgery time [24, 37–39]. Other investigators employing preoperative plasmin in cases of tractional diabetic macular edema found higher incidences of spontaneous PVD at the time of surgery [40, 41], less suction required to create a PVD when needed [42], and improved postoperative visual outcomes compared with controls [41]. Hirata et al. [43] observed that plasmin pretreatment in patients with proliferative diabetic retinopathy resulted in significantly less surgical time and a decreased risk for iatrogenic retinal breaks.

Unfortunately, autologous plasmin has several shortcomings which limit its feasibility for routine clinical practice. First, it is not readily available, and the process to obtain it is time-consuming and expensive. Autologous plasminogen must be harvested from the patient's own blood, then converted by streptokinase to plasmin *in vitro* prior to use. Second, this procedure must be done immediately before surgery as plasmin is exceedingly unstable and rapidly inactivates itself via autolysis and binding to α2-antiplasmin.

Recent advances in pharmaceutical drug development led to the discovery of ocriplasmin (Microplasmin), a recombinant product of only the catalytic domain of human plasmin [44]. Distinct advantages of ocriplasmin over plasmin include. (1) it is approximately one-fourth the size of plasmin (22-kDa versus 88-kDa) which is thought to facilitate greater penetration of vitreous and epiretinal tissues; (2) generation by recombinant techniques ensures product sterility and eliminates the risk of microbial contamination associated with blood derivatives; (3) when commercially available, it will allow investigators to avoid the rigorous preparation of autologous plasmin; (4) it is more stable than plasmin which simplifies storage and timing of administration [45].

Intravitreal ocriplasmin has been evaluated in several preclinical studies utilizing porcine, rat, rabbit, feline, and human cadaver eyes as the experimental model [46–48]. Gandorfer et al. initially reported a dose- and time-dependent cleavage between the posterior hyaloid and the ILM created by ocriplasmin without any adverse effects on retinal structure, in both human cadaver and feline eyes. Doses greater than or equal to 125.0 μg (equivalent to 2 units of plasmin (Sigma-Aldrich, Poole, United Kingdom)) produced a complete PVD with bare ILM in the human eyes, as demonstrated by electron microscopy.

De Smet et al. [49] confirmed these findings in a porcine eye model, observing that microplasmin caused vitreolysis

and PVD in a dose- and time-dependent fashion. The minimal effective dose also appeared to be 125 μg [49]. Sakuma et al. [50] corroborated these findings as well in rabbit eyes using doses of microplasmin ranging from 12.5 to 250 μg. They, too, found that 125 μg of microplasmin or greater successfully induced a complete PVD, while lower doses only induced a partial PVD. In all treated eyes, there was a temporary reduction in the a- and b-wave amplitudes on electroretinography, which recovered by 14 days after injection all groups except the 250 μg treatment group. In this higher dose faction, while the b-wave eventually recovered, a-wave alterations persisted at 90 days. A mild, transient vitreous haze was also noted within the first day after injection in this and other studies [12, 50, 51].

Most recently, Chen et al. [12] were able to show through immunofluorescence histochemistry that intravitreal microplasmin degraded fibronectin and laminin not only at the vitreoretinal interface, but also at the level of the photoreceptor layer in the outer retina of rats. Theoretically, the smaller molecular weight of ocriplasmin facilitates deeper penetration of retinal tissue but it is uncertain if this holds true in humans.

3. Clinical Trials with Ocriplasmin

In 2004, a series of clinical trials sponsored by ThromboGenics were initiated and collectively called Microplasmin for IntraVitreous Injection- Tractional Release without Surgical Treatment (MIVI-TRUST). To date, there are 14 studies involving intravitreal administration of ocriplasmin (ClinicalTrials.gov). Of these, 9 are included in the MIVI series. Results of the first three clinical trials (MIVI-I, MIVI-IIT, and MIVI-III) are published [7, 18, 19].

In each of these clinical trials, all patients with prior vitreous surgery and/or history of retinal detachments were excluded. Adverse events were also recorded and none of these studies have thus far shown an increased rate of retinal detachment, a known complication of posterior vitreous detachment, after ocriplasmin therapy.

3.1. MIVI-I: A Dose-Escalation Clinical Trial of Intravitreal Microplasmin in Patients Undergoing Surgical Vitrectomy for Vitreomacular Traction Maculopathy. MIVI-I was a Phase I/II safety study with dose escalation (25–125 micrograms) and increasing exposure time (1 hour–1 week) [7]. Sixty patients were enrolled in 6 successive cohorts. All patients had vitreomacular traction (VMT) maculopathy for which vitrectomy was indicated, including macular edema associated with VMT, stage II-III macular hole of <6 months duration since symptom onset, demonstration of vitreomacular adhesion (VMA) based on preoperative optic coherence tomography (OCT), or an OCT finding of posterior hyaloid membrane inserting onto the macula but with some area of clear separation visible between the retina and the posterior hyaloid. Results demonstrated that intravitreal ocriplasmin was well tolerated and capable of inducing a pharmacologic PVD in some patients.

3.2. MIVI-II: A Randomized, Sham-Injection-Controlled, Double-Masked, Ascending-Dose, Dose-Range-Finding Trial of Microplasmin Intravitreal Injection for Nonsurgical PVD Induction for Treatment of Diabetic Macular Edema. MIVI-II was a Phase II trial evaluating PVD induction in patients with diabetic macular edema (DME) 14 days after injection of intravitreal ocriplasmin versus sham. Disease status and safety at 6 months were also evaluated. The study was completed in 2010; however, results have not yet been published at the time of this paper.

3.3. MIVI-IIT: A Randomized, Sham-Injection-Controlled, Double-Masked, Ascending-Dose, Dose-Range-Finding Trial of Microplasmin Intravitreal Injection for Nonsurgical PVD Induction for Treatment of Vitreomacular Traction. MIVI-IIT was a randomized, double-masked Phase II trial with a control sham injection [18]. Sixty patients were enrolled in 4 cohorts. Patients in each of the cohorts were randomized to active treatment or sham injection. In the first 3 cohorts, increasing doses of ocriplasmin (75, 125, and 175 micrograms) were administered. In the fourth cohort, patients received 125 micrograms of intravitreal ocriplasmin monthly until the VMA was released, up to a total of 3 doses.

The first 3 cohorts had a nonsurgical resolution of VMA in 8, 25, 44, and 27% of the patients who received sham, 75, 125, and 175 micrograms of ocriplasmin, respectively. In the fourth cohort, ocriplasmin caused a PVD in 58% of patients at one month after the last treatment.

The MIVI-IIT trial provides support for the potential use of ocriplasmin in the nonsurgical treatment of VMA.

3.4. MIVI-III: 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. MIVI–III evaluated the safety and efficacy of a preoperative intravitreal injection of ocriplasmin in patients already scheduled for vitreous surgery [19]. One hundred twenty-five patients scheduled for pars plana vitrectomy (PPV) for the treatment of either VMT or macular hole were enrolled in this Phase II placebo-controlled double-masked dose-ranging clinical trial. A single intravitreal injection of ocriplasmin (25, 75, or 125 micrograms) or placebo was administered 7 days prior to PPV. The presence or absence of PVD at baseline, injection day, operative day, and postinjection day 90 and 180 were evaluated.

Rates of PVD observed at the time of surgery were 10, 14, 18, and 31% in the placebo, 25-, 75-, and 125-microgram ocriplasmin groups, respectively. The rates of resolution of VMT precluding the need for PPV at day 35 were 3, 10, 15, and 31% for the placebo, 25-, 75-, and 125-microgram ocriplasmin groups, respectively. At day 180, these rates were 3%, 7%, 15%, and 28%. At both day 35 and day 180, the rates of canceled vitrectomy in the 125-microgram ocriplasmin group were statistically significant when compared to the placebo group ($P < 0.01$ and $P = 0.01$, resp.).

MIVI-III concluded that ocriplasmin injection at a dose of 125 micrograms led to a greater likelihood of induction

and progression of PVD than placebo injection. This study also suggested that patients receiving ocriplasmin were more likely to not require vitrectomy surgery and that further trials were warranted.

3.5. MIVI-5: A Randomized, Sham-Injection-Controlled, Double-Masked, Multicenter Trial of Ocriplasmin Intravitreal Injection for Treatment of Focal Vitreomacular Adhesion in Subjects with Exudative Age-Related Macular Degeneration (AMD). Exudative AMD is a serious cause of blindness in elderly patients, and current standard of care includes monthly intravitreal antivascular endothelial growth factor (VEGF) injections [52]. Vitreomacular traction is thought to exacerbate AMD by exerting tractional forces on the macula, hypothetically stimulating abnormal blood vessel growth.

MIVI-5 is an ongoing clinical trial evaluating the safety and efficacy of intravitreal ocriplasmin in patients diagnosed with exudative AMD with focal VMA. Patients enrolled in this study have active subfoveal choroidal neovascular membrane and have received at least 3 antiangiogenic intravitreal injections, with evidence of focal VMA on OCT. Patients who have previously received more than 9 antiangiogenic intravitreal injections are excluded.

The primary outcome measure is the proportion of patients with release of focal VMA by day 28 as determined by a masked central reading center. MIVI-5 started in early 2010 and completion is anticipated in late 2012.

3.6. MIVI-TRUST (TG-MV-006): A Randomized, Placebo-Controlled, Double-Masked, Multicenter Trial of Microplasmin Intravitreal Injection for Nonsurgical Treatment of Focal Vitreomacular Adhesion and MIVI-TRUST (TG-MV-007): A Randomized, Placebo-Controlled, Double-Masked, Multicenter Trial of Microplasmin Intravitreal Injection for Nonsurgical Treatment of Focal Vitreomacular Adhesion. MIVI-TRUST TG-MV-006 and TG-MV-007 are both Phase III clinical trials evaluating the safety and efficacy of a 125-microgram dose of intravitreal ocriplasmin in patients with focal VMA. The primary outcome measure was the nonsurgical resolution of focal VMA at postinjection day 28. Both studies began late 2008 and were completed in 2010. Final published results from these 2 studies are not yet available.

3.7. MIVI-8: An Open-Label, Single-Centre Trial of Microplasmin Intravitreal Injection for Nonsurgical Treatment of Focal Vitreomacular Adhesion. MIVI-8 is a Phase II clinical trial assessing the safety and efficacy of 125-microgram ocriplasmin administered as an intravitreal injection in patients with focal VMA. Primary outcome measures include full ophthalmologic examination at baseline, postinjection days 7, 14, 28, and months 3 and 6. A secondary outcome is the proportion of patients with nonsurgical resolution of focal VMA at study visits other than the 28-day postinjection visit. MIVI-8 completed in April 2011 and published results are currently pending.

3.8. MIVI-10: An Open-Label, Ascending-Exposure-Time, Single-Center Trial to Evaluate the Pharmacokinetic Properties of Ocriplasmin (Generic Name of the Molecule Microplasmin) Intravitreal Injection in Subjects Scheduled for Primary Pars Plana Vitrectomy. The purpose of MIVI-10 is to evaluate the pharmacokinetic properties of intravitreal ocriplasmin when administered at different time points prior to planned PPV. In this Phase II clinical trial, 38 patients undergoing primary PPV received an intravitreal injection of 125-microgram ocriplasmin 5 minutes to 7 days prior to surgery. Ocriplasmin activity levels in the vitreous samples were evaluated. The study completed in early 2011 and final results have not yet been published.

3.9. Non-MIVI Trials. In addition to the MIVI-TRUST trials, there are 4 other trials that are currently evaluating the use of intravitreal ocriplasmin(ClinicalTrials.gov). A single-center, placebo-controlled Phase II clinical trial is actively enrolling patients to assess the efficacy of a high-dose (1.875 milligram) intravitreal injection of ocriplasmin in the treatment of focal VMA in patients with exudative AMD. Besides improving AMD by eliminating vitreomacular traction, ocriplasmin may also affect the pharmacokinetics and efficacy of anti-VEGF agents. A study in rabbits showed that bevacizumab (an anti-VEGF agent) in combination with ocriplasmin facilitated the penetration of bevacizumab into the retina [53]. The secondary endpoint of this clinical trial involving AMD and ocriplasmin is a decrease in subsequently required anti-VEGF injections.

Another study recruiting patients is Ocriplasmin for Treatment of Symptomatic Vitreomacular Adhesion Including Macular Hole (OASIS), a Phase II clinical trial evaluating the treatment of symptomatic vitreomacular adhesion including macular hole with a single 125-microgram intravitreal injection of ocriplasmin.

Additionally, the Microplasmin Intravitreal Administration in Participants with Uveitic Macular Edema is an ongoing Phase I/II trial investigating the safety and potential efficacy of intravitreal ocriplasmin as a possible treatment for macular edema secondary to uveitis. This study was initiated in 2010 and is anticipated to conclude in early 2012.

Finally, the Microplasmin in Children (MIC) Trial is also recruiting patients to assess the safety and efficacy of intravitreal ocriplasmin as an adjunct to conventional vitrectomy for the treatment of pediatric patients under 16 years of age. The vitreous in children is denser and more adherent to the retina as compared with that in adults, and the safety and efficacy profile of ocriplasmin may differ in children and adults. This Phase II, placebo-controlled, double-masked trial will evaluate a 175-microgram dose of ocriplasmin in pediatric patients undergoing a standard 2-port or 3-port PPV. Any child diagnosed with Stage 1, 2, 3, or 5 retinopathy of prematurity (ROP) at the time of the surgery is excluded.

In summary, the safety and efficacy of ocriplasmin for vitreoretinal diseases are being systematically evaluated in over a dozen clinical trials. The medicine is meant to help some patients avoid surgery or at least make vitreous surgery safer in others.

4. Federal Drug Administration Approval

In December 2011, a Federal Drug Administration (FDA) application was submitted for the use of ocriplasmin 2.5 mg/mL in adults. It was withdrawn and resubmitted for Priority Review in April 2012. If FDA approved, future applications for ocriplasmin would include its use in more common vitreomacular diseases such as diabetic retinopathy and vein occlusions.

Acknowledgment

Dr. S. D. Schwartz receives research support from Thrombo-Genics.

References

[1] J. Sebag and E. A. Balazs, "Morphology and ultrastructure of human vitreous fibers," *Investigative Ophthalmology and Visual Science*, vol. 30, no. 8, pp. 1867–1871, 1989.

[2] N. Yamada and S. Kishi, "Tomographic features and surgical outcomes of vitreomacular traction syndrome," *American Journal of Ophthalmology*, vol. 139, no. 1, pp. 112–117, 2005.

[3] L. K. Chang, H. F. Fine, R. F. Spaide, H. Koizumi, and H. E. Grossniklaus, "Ultrastructural correlation of spectral-domain optical coherence tomographic findings in vitreomacular traction syndrome," *American Journal of Ophthalmology*, vol. 146, no. 1, pp. 121–127, 2008.

[4] A. Gandorfer, "Enzymatic vitreous disruption," *Eye*, vol. 22, no. 10, pp. 1273–1277, 2008.

[5] P. Udaondo, M. Diaz-Llopis, S. Garcia-Delpech, D. Salom, and J. F. Arevalo, "Microplasmin for vitreomacular traction," *Ophthalmology*, vol. 117, no. 9, pp. 1859–1860, 2010.

[6] A. Gandorfer, "Objective of pharmacologic vitreolysis," *Developments in Ophthalmology*, vol. 44, pp. 1–6, 2009.

[7] M. D. de Smet, A. Gandorfer, P. Stalmans et al., "Microplasmin intravitreal administration in patients with vitreomacular traction scheduled for vitrectomy: the MIVI I trial," *Ophthalmology*, vol. 116, no. 7, pp. 1349–1355.e2, 2009.

[8] L. A. Liotta, R. H. Goldfarb, and R. Brundage, "Effect of plasminogen activator (urokinase), plasmin, and thrombin on glycoprotein and collagenous components of basement membrane," *Cancer Research*, vol. 41, no. 11, part 1, pp. 4629–4636, 1981.

[9] M. Hermel, W. Dailey, and M. K. Hartzer, "Efficacy of plasmin, microplasmin, and streptokinase-plasmin complex for the in vitro degradation of fibronectin and laminin- implications for vitreoretinal surgery," *Current Eye Research*, vol. 35, no. 5, pp. 419–424, 2010.

[10] B. Papp, T. Kovacs, and I. Lerant, "Conditions of formation of the heparin-fibronectin-collagen complex and the effect of plasmin," *Biochimica et Biophysica Acta*, vol. 925, no. 3, pp. 241–247, 1987.

[11] X. Li, X. Shi, and J. Fan, "Posterior vitreous detachment with plasmin in the isolated human eye," *Graefe's Archive for Clinical and Experimental Ophthalmology*, vol. 240, no. 1, pp. 56–62, 2002.

[12] W. Chen, W. Mo, K. Sun, X. Huang, Y. L. Zhang, and H. Y. Song, "Microplasmin degrades fibronectin and laminin at vitreoretinal interface and outer retina during enzymatic vitrectomy," *Current Eye Research*, vol. 34, no. 12, pp. 1057–1064, 2009.

[13] A. Uemura, M. Nakamura, S. Kachi et al., "Effect of plasmin on laminin and fibronectin during plasmin-assisted vitrectomy," *Archives of Ophthalmology*, vol. 123, no. 2, pp. 209–213, 2005.

[14] T. Kohno, N. Sorgente, and T. Ishibashi, "Immunofluorescent studies of fibronectin and laminin in the human eye," *Investigative Ophthalmology and Visual Science*, vol. 28, no. 3, pp. 506–514, 1987.

[15] T. Kohno, N. Sorgente, R. Patterson, and S. J. Ryan, "Fibronectin and laminin distribution in bovine eye," *Japanese Journal of Ophthalmology*, vol. 27, no. 3, pp. 496–505, 1983.

[16] S. R. Russell, J. D. Shepherd, and G. S. Hageman, "Distribution of glycoconjugates in the human retinal internal limiting membrane," *Investigative Ophthalmology and Visual Science*, vol. 32, no. 7, pp. 1986–1995, 1991.

[17] T. Kohno, N. Sorgente, R. Goodnight, and S. J. Ryan, "Alterations in the distribution of fibronectin and laminin in the diabetic human eye," *Investigative Ophthalmology and Visual Science*, vol. 28, no. 3, pp. 515–521, 1987.

[18] T. C. Verstraeten, C. Chapman, M. Hartzer, B. S. Winkler, M. T. Trese, and G. A. Williams, "Pharmacologic induction of posterior vitreous detachment in the rabbit," *Archives of Ophthalmology*, vol. 111, no. 6, pp. 849–854, 1993.

[19] T. Hikichi, N. Yanagiya, M. Kado, J. Akiba, and A. Yoshida, "Posterior vitreous detachment induced by injection of plasmin and sulfur hexafluoride in the rabbit vitreous," *Retina*, vol. 19, no. 1, pp. 55–58, 1999.

[20] N. J. Kim, H. G. Yu, Y. S. Yu, and H. Chung, "Long-term effect of plasmin on the vitreolysis in rabbit eyes," *Korean Journal of Ophthalmology*, vol. 18, no. 1, pp. 35–40, 2004.

[21] A. Gandorfer, E. Putz, U. Welge-Lüßen, M. Grüterich, M. Ulbig, and A. Kampik, "Ultrastructure of the vitreoretinal interface following plasmin assisted vitrectomy," *British Journal of Ophthalmology*, vol. 85, no. 1, pp. 6–10, 2001.

[22] A. Gandorfer, S. Priglinger, K. Schebitz et al., "Vitreoretinal morphology of plasmin-treated human eyes," *American Journal of Ophthalmology*, vol. 133, no. 1, pp. 156–159, 2002.

[23] X. Li, X. Shi, and J. Fan, "Posterior vitreous detachment with plasmin in the isolated human eye," *Graefe's Archive for Clinical and Experimental Ophthalmology*, vol. 240, no. 1, pp. 56–62, 2002.

[24] A. Uemura, M. Nakamura, S. Kachi et al., "Effect of plasmin on laminin and fibronectin during plasmin-assisted vitrectomy," *Archives of Ophthalmology*, vol. 123, no. 2, pp. 209–213, 2005.

[25] J. J. Plantner, A. Smine, and T. A. Quinn, "Matrix metalloproteinases and metalloproteinase inhibitors in human interphotoreceptor matrix and vitreous," *Current Eye Research*, vol. 17, no. 2, pp. 132–140, 1998.

[26] A. Takano, A. Hirata, Y. Inomata et al., "Intravitreal plasmin injection activates endogenous matrix metalloproteinase-2 in rabbit and human vitreous," *American Journal of Ophthalmology*, vol. 140, no. 4, pp. 654–660, 2005.

[27] D. J. Brown, P. Bishop, H. Hamdi, and M. C. Kenney, "Cleavage of structural components of mammalian vitreous by endogenous matrix metalloproteinase-2," *Current Eye Research*, vol. 15, no. 4, pp. 439–445, 1996.

[28] S. Monea, K. Lehti, J. Keski-Oja, and P. Mignatti, "Plasmin activates pro-matrix metalloproteinase-2 with a membrane-type 1 matrix metalloproteinase-dependent mechanism," *Journal of Cellular Physiology*, vol. 192, no. 2, pp. 160–170, 2002.

[29] A. Gandorfer and A. Kampik, "Intravitreal plasmin injection activates endogenous matrix metalloproteinase-2 in rabbit

and human vitreous," *American Journal of Ophthalmology*, vol. 141, no. 4, pp. 784–785, 2006.

[30] J. M. Sivak and M. E. Fini, "MMPs in the eye: emerging roles for matrix metalloproteinases in ocular physiology," *Progress in Retinal and Eye Research*, vol. 21, no. 1, pp. 1–14, 2002.

[31] F. Staubach, V. Nober, and P. Janknecht, "Enzyme-assisted vitrectomy in enucleated pig eyes: a comparison of hyaluronidase, chondroitinase, and plasmin," *Current Eye Research*, vol. 29, no. 4-5, pp. 261–268, 2004.

[32] M. Hermel, J. Prenner, M. Alabdulrazzak, W. Dailey, and M. Hartzer, "Effect of intravitreal plasmin on vitreous removal through a 25-gauge cutting system in the rabbit in vivo," *Graefe's Archive for Clinical and Experimental Ophthalmology*, vol. 247, no. 3, pp. 331–334, 2009.

[33] W. C. Wu, K. A. Drenser, M. T. Trese, G. A. Williams, and A. Capone, "Pediatric traumatic macular hole: results of autologous plasmin enzyme-assisted vitrectomy," *American Journal of Ophthalmology*, vol. 144, no. 5, pp. 668–672, 2007.

[34] A. R. Margherio, R. R. Margherio, M. Hartzer, M. T. Trese, G. A. Williams, and P. J. Ferrone, "Plasmin enzyme-assisted vitrectomy in traumatic pediatric macular holes," *Ophthalmology*, vol. 105, no. 9, pp. 1617–1620, 1998.

[35] W. C. Wu, K. A. Drenser, M. Lai, A. Capone, and M. T. Trese, "Plasmin enzyme-assisted vitrectomy for primary and reoperated eyes with stage 5 retinopathy of prematurity," *Retina*, vol. 28, no. 3, supplement, pp. S75–S80, 2008.

[36] W. C. Wu, K. A. Drenser, A. Capone, G. A. Williams, and M. T. Trese, "Plasmin enzyme-assisted vitreoretinal surgery in congenital X-linked retinoschisis: surgical techniques based on a new classification system," *Retina*, vol. 27, no. 8, pp. 1079–1085, 2007.

[37] M. T. Trese, G. A. Williams, and M. K. Hartzer, "A new approach to stage 3 macular holes," *Ophthalmology*, vol. 107, no. 8, pp. 1607–1611, 2000.

[38] T. Sakuma, M. Tanaka, J. Inoue, A. Mizota, M. Souri, and A. Ichinose, "Efficacy of autologous plasmin for idiopathic macular hole surgery," *European Journal of Ophthalmology*, vol. 15, no. 6, pp. 787–794, 2005.

[39] S. Rizzo, G. Pellegrini, F. Benocci, C. Belting, U. Baicchi, and M. Vispi, "Autologous plasmin for pharmacologic vitreolysis prepared 1 hour before surgery," *Retina*, vol. 26, no. 7, pp. 792–796, 2006.

[40] T. Sakuma, M. Tanaka, J. Inoue, A. Mizota, M. Souri, and A. Ichinose, "Use of autologous plasmin during vitrectomy for diabetic maculopathy," *European Journal of Ophthalmology*, vol. 16, no. 1, pp. 138–140, 2006.

[41] C. Azzolini, A. D'Angelo, G. Maestranzi et al., "Intrasurgical plasmin enzyme in diabetic macular edema," *American Journal of Ophthalmology*, vol. 138, no. 4, pp. 560–566, 2004.

[42] T. Asami, H. Terasaki, S. Kachi et al., "Ultrastructure of internal limiting membrane removed during plasmin-assisted vitrectomy from eyes with diabetic macular edema," *Ophthalmology*, vol. 111, no. 2, pp. 231–237, 2004.

[43] A. Hirata, A. Takano, Y. Inomata, N. Yonemura, N. Sagara, and H. Tanihara, "Plasmin-assisted vitrectomy for management of proliferative membrane in proliferative diabetic retinopathy: a pilot study," *Retina*, vol. 27, no. 8, pp. 1074–1078, 2007.

[44] H. L. Wu, G. Y. Shi, and M. L. Bender, "Preparation and purification of microplasmin," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 84, no. 23, pp. 8292–8295, 1987.

[45] E. W. Schneider and M. W. Johnson, "Emerging nonsurgical methods for the treatment of vitreomacular adhesion: a review," *Clinical Ophthalmology*, vol. 5, no. 1, pp. 1151–1165, 2011.

[46] A. Gandorfer, M. Rohleder, C. Sethi et al., "Posterior vitreous detachment induced by microplasmin," *Investigative Ophthalmology and Visual Science*, vol. 45, no. 2, pp. 641–647, 2004.

[47] T. Sakuma, M. Tanaka, A. Mizota, J. Inoue, and S. Pakola, "Safety of in vivo pharmacologic vitreolysis with recombinant microplasmin in rabbit eyes," *Investigative Ophthalmology and Visual Science*, vol. 46, no. 9, pp. 3295–3299, 2005.

[48] M. D. De Smet, C. Valmaggia, J. Zarranz-Ventura, and B. Willekens, "Microplasmin: ex vivo characterization of its activity in porcine vitreous," *Investigative Ophthalmology and Visual Science*, vol. 50, no. 2, pp. 814–819, 2009.

[49] M. D. De Smet, C. Valmaggia, J. Zarranz-Ventura, and B. Willekens, "Microplasmin: ex vivo characterization of its activity in porcine vitreous," *Investigative Ophthalmology and Visual Science*, vol. 50, no. 2, pp. 814–819, 2009.

[50] T. Sakuma, M. Tanaka, A. Mizota, J. Inoue, and S. Pakola, "Safety of in vivo pharmacologic vitreolysis with recombinant microplasmin in rabbit eyes," *Investigative Ophthalmology and Visual Science*, vol. 46, no. 9, pp. 3295–3299, 2005.

[51] W. Chen, X. Huang, X. W. Ma, W. Mo, W. J. Wang, and H. Y. Song, "Enzymatic vitreolysis with recombinant microplasminogen and tissue plasminogen activator," *Eye*, vol. 22, no. 2, pp. 300–307, 2008.

[52] D. F. Martin, M. G. Maguire, G. S. Ying, J. E. Grunwald, S. L. Fine, and G. J. Jaffe, "Ranibizumab and bevacizumab for neovascular age-related macular degeneration," *The New England Journal of Medicine*, vol. 364, no. 20, pp. 1897–1908, 2011.

[53] D. T. Goldenberg, F. J. Giblin, M. Cheng et al., "Posterior vitreous detachment with microplasmin alters the retinal penetration of intravitreal bevacizumab (Avastin) in rabbit eyes," *Retina*, vol. 31, no. 2, pp. 393–400, 2011.