

Pharmacologic Vitreolysis— Premise and Promise of the First Decade

The term pharmacologic vitreolysis first appeared in the world's literature one decade ago in the pages of the journal *Retina*.¹ The coining of this term formalized a new field and was predicated on a premise and a promise. The premise was that we now understood enough about the molecular organization, structure, and physiology of vitreous and its role in retinal disorders, and that this knowledge could be used to enhance vitreoretinal surgery with pharmacologic adjuncts. The promise was that this novel form of therapy could one day supplant surgery by mitigating the pathophysiology of vitreous at the earliest stages of disease and also improve ocular physiology and metabolism. Although there have been many interesting developments during the first decade, most recently the results of MIVI III (Microplasmin Intravitreal Injection III, a Phase IIb multicenter, randomized, controlled clinical trial evaluating microplasmin, which showed the release of vitreo-macular traction without surgical intervention in 30% of patients), too few of these objectives have been met to date. There are important lessons to be learned in considering why there has not been more rapid progress.

Vitreous is believed to play an important role in ocular health and disease.^{2,3} Posterior vitreous detachment (PVD) is characterized by gel liquefaction (synchisis) and vitreoretinal dehiscence with collapse (syneresis) of the posterior vitreous away from the retina.^{2,3} It is plausible that much like the phenomenon of apoptosis, PVD is preprogrammed to happen, because PVD occurs in the overwhelming majority of individuals. Furthermore, it is currently believed that in a variety of diseases, such as diabetic retinopathy^{4,5} and age-related macular degeneration,^{6,7} complete PVD protects against more advanced stages of disease. In a minority of cases, however, liquefaction occurs without sufficient dehiscence at the vitreoretinal interface to allow for clean separation of the posterior vitreous cortex from the retina. This is known as "anomalous PVD."⁸ The sequelae of anomalous PVD vary depending on where the gel is most liquefied and

where the posterior vitreous cortex is most firmly adherent to the retina. The clinical setting is also extremely important insofar as anomalous PVD may have very different effects in an individual with diabetes as compared with a nondiabetic patient, or in the setting of a genetic predisposition to certain forms of age-related macular degeneration. Thus, the interplay of these multiple factors is an important consideration. Also important is whether the posterior vitreous cortex that remains adherent to the retina is full or partial thickness, the latter a consequence of splitting in the posterior vitreous cortex, known as vitreoschisis.⁹ Histopathologic studies¹⁰ have shown that vitreoschisis is present in 80% of eyes with proliferative diabetic retinopathy. Clinical investigations¹¹ using combined optical coherence tomography and scanning laser ophthalmoscopy have detected vitreoschisis in half of the eyes with macular holes and macular pucker. The various permutations of anomalous PVD are diagrammed in Figure 1. As can be appreciated, seemingly disparate diseases can in fact be perceived as different consequences of the same initiating event, i.e., anomalous PVD.

Our enhanced understanding of the role of vitreous in retinal disorders has led investigators to use pharmacologic vitreolysis in diseases such as diabetic retinopathy,¹² macular holes,¹³ retinopathy of prematurity¹⁴ and even congenital retinoschisis.¹⁵ Since initial attempts all used enzymes as adjuncts to surgery, the term "enzymatic vitreolysis" prevailed in the early literature.^{16,17} However, in 1998, the term "pharmacologic vitreolysis" was proposed,¹ so that vitreolytic agents could be grouped according to their mechanism of action as either "enzymatic" or "nonenzymatic." Furthermore, it was proposed that these agents could be grouped, as either nonspecific agents, such as tissue plasminogen activator,¹⁸ plasmin,^{19,20} microplasmin,^{21,22} and nattokinase²³; or substrate-specific agents, such as chondroitinase,^{24,25} dispase,^{26,27} and hyaluronidase.^{28,29} Although this perspective does have intellectual appeal on theoretical grounds, the original classification system seems to have little relevance today, because other than two nonenzymatic approaches (urea/vitreosolve³⁰ and Arginine-Glycine-Aspartate (RGD) peptides³¹), the overwhelming ma-

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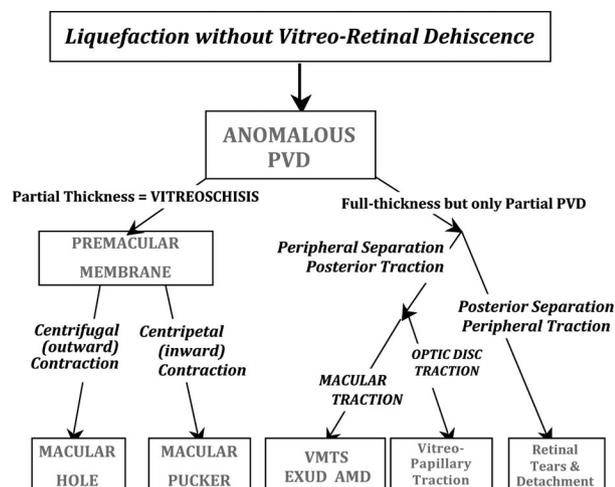


Fig. 1. Schematic diagram of anomalous posterior vitreous detachment. PVD, posterior vitreous detachment; VMTS, vitreomacular traction syndrome; Exud AMD, exudative age-related macular degeneration.

majority of drugs that have been tested during the first decade are enzymes. It would seem, therefore, that in the next decade of pharmacologic vitreolysis an alternative classification might be more useful. Rather than basing this new classification system on the properties of the agents themselves, grouping could be organized on the basis of the biologic effects of the different agents, in particular whether they induce liquefaction (“liquefactants”) or whether they induce dehiscence at the vitreoretinal interface (“interfactants”). Table 1 lists the different pharmacologic vitreolysis agents currently in development classified on the basis of biologic activity.

Another important consideration for the future of pharmacologic vitreolysis research and development is that when implemented clinically, pharmacologic vitreolysis will be performed on the eyes with abnormal vitreous. This is not only true for all the ophthalmic conditions identified in Figure 1, but also sys-

temic condition such as diabetes. It is known, for example, that diabetes induces significant biochemical^{32–35} and structural³⁶ effects on vitreous, resulting in diabetic vitreopathy,³⁷ termed by some diabetic vitreoretinopathy.³⁸ Because diabetic vitreous is so different from normal vitreous, studies on the effects of pharmacologic vitreolysis on normal vitreous may fail to result in effective implementation of this therapy in pathologic conditions. This consideration might partly explain why hyaluronidase (developed as Vitrase, ISTA Pharmaceuticals, Irvine, CA) failed in Phase III Food and Drug Administration clinical trials for treating vitreous hemorrhage in diabetic retinopathy. No preclinical studies were done using Vitrase on diabetic vitreous. Although this agent did clear vitreous hemorrhage in 32.9% of cases, the saline control also cleared the blood in 25.5% of cases.³⁹ Another explanation for the failure of Vitrase relates to the fact that hyaluronidase is not an interfactant, only a liquefactant (Table 1). Thus, although hyaluronidase will liquefy gel vitreous, it will not induce vitreoretinal dehiscence. In proliferative diabetic retinopathy, this will result in persistent traction on neovascularization with subsequent recurrent hemorrhages and vision loss. Previous studies have found similarly disappointing results with hyaluronidase.^{28,29}

A notable exception to the foregoing precedent is the study of Zhiliang et al, published in this issue of *Retina*. These investigators studied the effects of pharmacologic vitreolysis in diabetic rats. The results showed that hyaluronidase alone did not induce PVD in any of the 10 subjects tested, confirming previous studies.^{28,29} Plasmin alone induced only a partial PVD in 10 of 10 subjects (100%). Although previous investigations using plasmin^{12,14,15,29} and microplasmin^{13,21} claimed to induce total PVD, these experiments were performed in nondiabetic vitreous. Pilot clinical studies^{12,40} in diabetic eyes without controls found that plasmin was effective as an adjunct to surgery for diabetic vitreoretinopathy, but there were no studies using plasmin in diabetic eyes without surgery. Thus, further work needs to be undertaken with diabetic eyes using plasmin in a nonsurgical setting. This is important, because past studies^{4,5} have shown that a partially detached vitreous carries the worst prognosis for progressive diabetic retinopathy. The results of the studies presented herein suggest that plasmin alone would not only fail to achieve effective pharmacologic vitreolysis in diabetic vitreopathy, but might even worsen the prognosis by inducing a partial (anomalous) PVD. Interestingly, the combination of plasmin and hyaluronidase in the studies by Zhiliang et al induced a total PVD in 8 of 10 eyes. Of course, these studies were in an animal model of diabetic

Table 1. Pharmacologic Vitreolysis Classification Based on Biologic Activity

Liquefactants (agents that liquefy the gel vitreous)
Nonspecific: tPA, plasmin, microplasmin, nattokinase, vitreosolve*
Substrate specific: chondroitinase, hyaluronidase
Interfactants (agents that alter the vitreoretinal interface)
Nonspecific: tpa, plasmin, microplasmin, nattokinase, vitreosolve*
Substrate specific: dispase, chondroitinase, RGD-peptides*

tPA, plasmin, microplasmin, nattokinase, and vitreosolve are believed to be both liquefactants and interfactants.

*Nonenzymatic agents.

tPA, tissue plasminogen activator.

vitreopathy and the results need to be corroborated in humans.

Zhiliang et al are to be congratulated for having brought to reality the previously proposed^{29,41} concept of combination pharmacologic vitreolysis, because combining plasmin with hyaluronidase was indeed effective in achieving a total PVD in 80% of cases. Thus, not only did these investigators appropriately study pharmacologic vitreolysis in a diabetic model, but they also used combination therapy to attain a therapeutic outcome that neither agent alone could achieve. It is likely that in these studies the liquefactive hyaluronidase induced gel liquefaction whereas the interfactant properties of plasmin induced sufficient dehiscence at the vitreoretinal interface to induce total PVD in a much higher percentage of cases than has been observed to date in any clinical trials. Future clinical trial protocols might thus benefit from incorporating combination therapy whose value was previously suggested in theory⁴¹ and has been proven in practice in nondiabetic vitreous²⁹ and now in experimental models of diabetic vitreopathy. Including outcome measures of ocular physiology and metabolism would also test the hypothesis that pharmacologic vitreolysis can induce salubrious effects on ocular metabolism.^{42,43}

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References

- Sebag J. Pharmacologic vitreolysis. *Retina* 1998;18:1–3.
- Sebag J. *The Vitreous—Structure, Function, and Pathobiology*. New York, NY: Springer-Verlag; 1989.
- Sebag J, Yee KMP. Vitreous—from biochemistry to clinical relevance. In: William T, Edward AJ, eds. *Duane's Foundations of Clinical Ophthalmology*. Vol. 1. Philadelphia, PA: Lippincott Williams & Wilkins; 2007:chap 16.
- Jalkh A, Takahashi M, Topilow HW, et al. Prognostic value of vitreous findings in diabetic retinopathy. *Arch Ophthalmol* 1982;100:432–434.
- Akiba J, Arzabe CW, Trempe CL. Posterior vitreous detachment and neovascularization in diabetic retinopathy. *Ophthalmology* 1990;97:889–891.
- Krebs I, Brannath W, Glittenberg K, Zeiler F, Sebag J, Binder S. Posterior vitreo-macular adhesion: a potential risk factor for exudative age-related macular degeneration. *Am J Ophthalmol* 2007;144:741–746.
- Robison C, Krebs I, Binder S, et al. Vitreo-macular adhesion in active and end-stage age-related macular degeneration. *Am J Ophthalmol*. 2009 Mar 26 [Epub ahead of print].
- Sebag J. Anomalous posterior vitreous detachment: a unifying concept in vitreo-retinal disease. *Graefes Arch Clin Exp Ophthalmol* 2004;242:690–698.
- Sebag J. Vitreoschisis. *Graefes Arch Clin Exp Ophthalmol* 2008;246:329–332.
- Schwartz SD, Alexander R, Hiscott P, Gregor ZJ. Recognition of vitreoschisis in proliferative diabetic retinopathy. *Ophthalmology* 1996;103:323–328.
- Sebag J, Gupta P, Rosen R, Garcia P, Sadun AA. Macular holes and macular pucker: the role of vitreoschisis as imaged by optical coherence tomography-scanning laser ophthalmoscopy. *Trans Am Ophthalmol Soc* 2007;105:121–131.
- Williams JG, Trese MT, Williams GA, Hartzler MK. Autologous plasmin enzyme in the surgical management of diabetic retinopathy. *Ophthalmology* 2001;108:1902–1905.
- Sakuma T, Tanaka M, Inoue M, Mizota A, Souri M, Ichinose A. Efficacy of autologous plasmin for idiopathic macular hole surgery. *Eur J Ophthalmol* 2005;15:787–794.
- Wu WC, Drenser KA, Lai M, Capone A, Trese M. Plasmin enzyme-assisted vitrectomy for primary and reoperated eyes with stage 5 retinopathy of prematurity. *Retina* 2008;28(3 suppl):S75–S80.
- Wu WC, Drenser KA, Capone A, Williams GA, Trese MT. Plasmin enzyme-assisted vitreoretinal surgery in congenital X-linked retinoschisis: surgical techniques based on a new classification system. *Retina* 2007;27:1079–1085.
- Lariukhina GM, Ziangirova GG. Experimental enzymatic vitreolysis. *Vestn Oftalmol* 1977;6:77–79.
- Zagórski Z. Effect of enzymatic vitreolysis on the absorption of experimental vitreous hemorrhage. Preliminary report. *Klin Oczna* 1983;85:197–199.
- Hesse L, Nebeling B, Schroeder B, Heller G, Kroll P. Induction of posterior vitreous detachment in rabbits by intravitreal injection of tissue plasminogen activator following cryopexy. *Exp Eye Res* 2000;70:31–39.
- Verstraeten TC, Chapman C, Hartzler M, Winkler BS, Trese MT, Williams GA. Pharmacologic induction of posterior vitreous detachment in the rabbit. *Arch Ophthalmol* 1993;111:849–854.
- Uemura A, Nakamura M, Kachi S, et al. Effect of plasmin on laminin and fibronectin during plasmin-assisted vitrectomy. *Arch Ophthalmol* 2005;123:209–213.
- Sakuma T, Tanaka M, Mizota A, Inoue J, Pakola S. Safety of in vivo pharmacologic vitreolysis with recombinant microplasmin in rabbit eyes. *Invest Ophthalmol Vis Sci* 2005;46:3295–3299.
- Sebag J, Ansari RR, Suh KI. Pharmacologic vitreolysis with microplasmin increases vitreous diffusion coefficients. *Graefes Arch Clin Exp Ophthalmol* 2007;245:576–580.
- Takano A, Hirata A, Ogasawara K, et al. Posterior vitreous detachment induced by nattokinase (subtilisin NAT): a novel enzyme for pharmacologic vitreolysis. *Invest Ophthalmol Vis Sci* 2006;47:2075–2079.
- Hageman GS, Russell SR. Chondroitinase-mediated disinsertion of the primate vitreous body. *Invest Ophthalmol Vis Sci* 1994;35:1260.
- Bishop PN, McLeod D, Reardon A. Effects of hyaluronan lyase, hyaluronidase, and chondroitin ABC lyase on mammalian vitreous gel. *Invest Ophthalmol Vis Sci* 1999;40:2173–2178.
- Tezel TH, Del Priore LV, Kaplan HJ. Posterior vitreous detachment with dispase. *Retina* 1998;18:7–15.
- Wang F, Wang Z, Sun X, Wang F, Xu X, Zhang X. Safety and efficacy of dispase and plasmin in pharmacologic vitreolysis. *Invest Ophthalmol Vis Sci* 2004;45:3286–3290.
- Hikichi T, Kado M, Yoshida A. Intravitreal injection of hyal-

- uronidase cannot induce posterior vitreous detachment in the rabbit. *Retina* 2000;20:195–198.
29. Wang ZL, Zhang X, Xu X, Sun XD, Wang F. PVD following plasmin but not hyaluronidase: implications for combination pharmacologic vitreolysis therapy. *Retina* 2005;25:38–43.
 30. Nickerson CS. Engineering the Mechanical Properties of Ocular Tissues. Thesis, Doctor of Philosophy, California Institute of Technology, 2006.
 31. Oliveira LB, Meyer CH, Kumar J, et al. RGD peptide-assisted vitrectomy to facilitate induction of a posterior vitreous detachment: a new principle in pharmacologic vitreolysis. *Curr Eye Res* 2002;25:333–340.
 32. Shires TK, Faeth JA, Pulido JS. Nonenzymatic glycosylation of vitreous proteins in vitro and in the streptozotocin-treated diabetic rat. *Retina* 1990;10:153–158.
 33. Sebag J, Buckingham B, Charles MA, Reiser K. Biochemical abnormalities in vitreous of humans with proliferative diabetic retinopathy. *Arch Ophthalmol* 1992;110:1472–1476.
 34. Sebag J, Nie S, Reiser K, Charles MA, Yu NT. Raman spectroscopy of human vitreous in proliferative diabetic retinopathy. *Invest Ophthalmol Vis Sci* 1994;35:2976–2980.
 35. Stitt AW, Moore JE, Sharkey JA, et al. Advanced glycation end products in vitreous: structural and functional implications for diabetic vitreopathy. *Invest Ophthalmol Vis Sci* 1998;39:2517–2523.
 36. Sebag J. Abnormalities of human vitreous structure in diabetes. *Graefes Arch Clin Exp Ophthalmol* 1993;231:257–260.
 37. Sebag J. Diabetic vitreopathy. *Ophthalmology* 1996;103:205–206.
 38. Kroll P, Rodrigues EB, Hoerle S. Pathogenesis and classification of proliferative diabetic vitreoretinopathy. *Ophthalmologica* 2007;221:78–94.
 39. Kuppermann BD, Thomas EL, de Smet MD, Grillone LR; Vitrase for Vitreous Hemorrhage Study Groups. Pooled efficacy results from two multinational randomized controlled clinical trials of a single intravitreal injection of highly purified ovine hyaluronidase (Vitrase) for the management of vitreous hemorrhage. *Am J Ophthalmol* 2005;140:573–584.
 40. Azzolini C, D'Angelo A, Maestranzi G, et al. Intravitreal plasmin enzyme in diabetic macular edema. *Am J Ophthalmol* 2004;138:560–566.
 41. Sebag J. Is pharmacologic vitreolysis brewing? *Retina* 2002;22:1–3.
 42. Quiram PA, Leverenz VR, Baker RM, Dang L, Giblin FJ, Trese MT. Microplasmin-induced posterior vitreous detachment affects vitreous oxygen levels. *Retina* 2007;27:1090–1096.
 43. Stefansson E. Letter to the editor. *Retina* 2008;28:1175–1176.