The Emerging Role of Pharmacologic Vitreolysis

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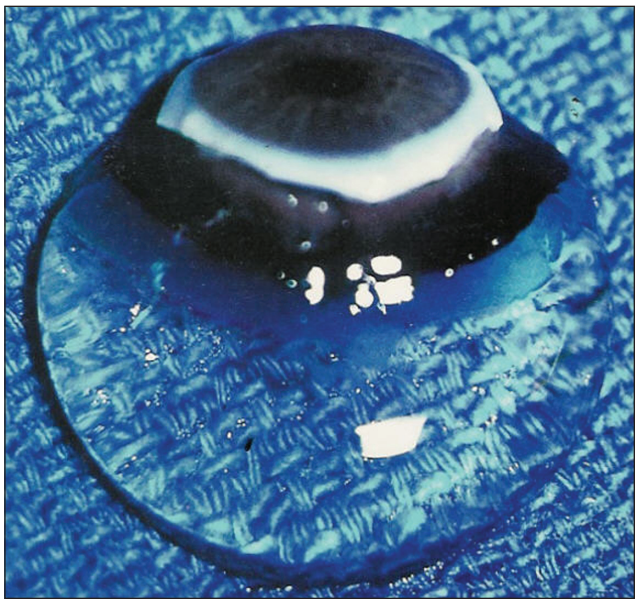
Vitreous has long posed a daunting challenge to both scientific investigation and clinical evaluation. However, owing to recent advances in research and clinical domains, the enigmatic[1] vitreous is now better understood in terms of its roles in health and disease.[2,3] As a result, diseases such as rhegmatogenous retinal detachment, advanced diabetic retinopathy, macular pucker, macular holes and perhaps even wet age-related macular degeneration are no longer solely diseases of the retina, but are considered vitreo-retinopathies, the latter three vitreo-maculopathies. Indeed, proliferative diabetic retinopathy has recently been classified by clinical criteria and renamed "proliferative diabetic vitreo-retinopathy" to emphasize the role of vitreous.[4]

Of particular interest in diabetic retinopathy and AMD is the prospect that more knowledge about the role of vitreous in these chronic diseases may enable intervention early in the natural history of these long-term conditions so as to mitigate the visual devastation that often accompanies advanced disease. While current interventions to eliminate the contribution of vitreous are surgical, the future will also likely see non-surgical therapy in the form of pharmacologic vitreolysis.

ANOMALOUS PVD

Transparent in youth (**Figure 1**), vitreous undergoes molecular re-arrangements during aging that result in structural changes in the gel vitreous leading to liquefaction, known as "synchisis." Concurrent dehiscence at the vitreo-retinal interface results in posterior vitreous detachment, the most common event in the posterior segment during life. PVD is thus the consequence of synchisis and vitreo-retinal dehiscence with collapse (syneresis) of the posterior vitreous away from the retina.[2,3] Since PVD occurs in the overwhelming majority of individuals, it is plausible that much like the phenomenon of apoptosis in various cells and tissues throughout the body, PVD is pre-programmed.[1,5] It is well known that in diabetic retinopathy[6,7] and age-related macular degeneration[8,9] total PVD protects against more advanced stages of disease. Thus, over time, nature may have evolved mechanisms to induce PVD and prevent untoward age-related sequelae. In a minority of cases, however, vitreous gel liquefaction occurs without sufficient dehiscence at the vitreo-retinal interface, precluding clean separation of the posterior vitreous cortex away from the retina. This is known as "anomalous PVD."[10]



SPECIMEN COURTESY OF THE NEW ENGLAND EYE BANK)

Figure 1. The human vitreous is still attached to the anterior segment after dissection of the sclera choroid and retina. Exquisite transparency and solid gel consistency are evident.

The consequences of anomalous PVD vary depending upon where the gel is most liquefied and where the posterior vitreous cortex is most firmly adherent to the retina. This unifying concept of vitreo-retinopathies explains the varied manifestations of anomalous PVD (**Figure 2**). If vitreous is most firmly adherent to the peripheral retina, such as in areas of retinal lattice, then retinal tears and detachment can be the consequence of anomalous PVD (right side of Figure 2). If vitreous adhesion to the macula is unusually strong, a variety of vitreo-maculopathies can result from anomalous PVD.

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As shown in Figure 2, vitreo-maculopathies differ based on whether or not there is a split within the posterior vitreous cortex tangential to the plane of the retina. Such cleavage within the posterior vitreous cortex is known as "vitreoschisis."[11] Extracellular matrix biology and genetic determinants of vitreo-retinal interface composition likely influence whether the posterior vitreous cortex splits tangentially during anomalous PVD, leaving the outer layer of the vitreous still attached to the macula (partial-thickness anomalous PVD), or whether the posterior vitreous cortex that remains adherent to the macula following anomalous PVD is full thickness.

Partial-thickness vitreous adherence to the macula following anomalous PVD results from splitting in the posterior vitreous cortex, known as vitreoschisis[11] (left side of figure 2). Histopathology studies[12] have shown that vitreoschisis is present in 80% of eyes with proliferative diabetic vitreo-retinopathy. Clinical investigations[13] using combined optical coherence tomography/scanning laser ophthalmoscopy detected vitreoschisis in half of eyes with macular holes and macular pucker. Persistent vitreous adhesion to the optic disc may also play a role in the pathogenesis of macular holes and cysts, since nearly 90% of eyes with macular holes have vitreo-papillary adhesion.[14] The general clinical setting is also important insofar as anomalous PVD may have very different effects in an individual with diabetes, or in an individual with a genetic predisposition to certain forms of age-related macular degeneration. In both conditions advanced glycation end-products in vitreous[15] and at the vitreo-retinal interface may play a role in anomalous PVD, secondary to the effects of hyperglycemia in the case of diabetes, and related to aging in AMD.

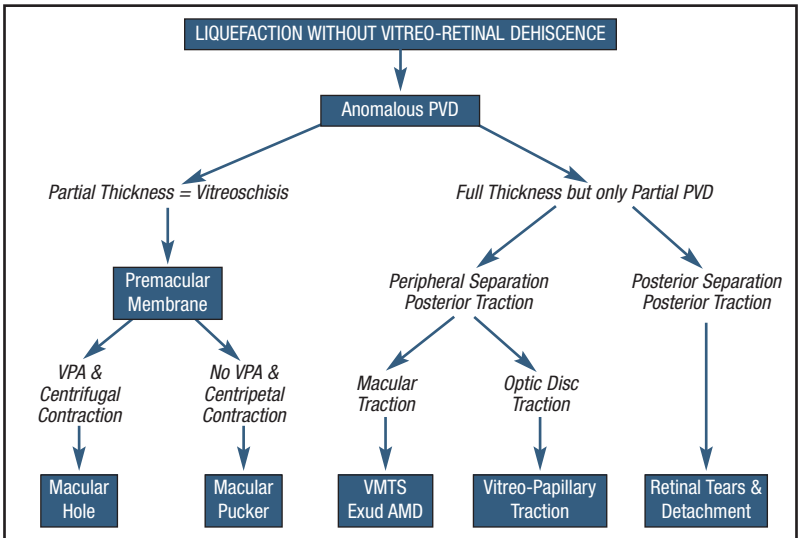
Peripheral separation of vitreous from retina with persistent adhesion of full-thickness vitreous cortex to the macula causes the vitreo-macular traction syndrome. This form of anomalous PVD may also be important in promoting exudative AMD, since recent studies[8,9] have shown that anomalous PVD with full-thickness adhesion at the vitreo-macular interface may promote choroidal neovascularization and wet AMD. Total PVD is more common in dry AMD and may indeed protect against wet AMD. The preventive therapy opportunities of these statistically and clinically significant observations are intriguing, especially if non-surgical treatments can be developed to prevent disease progression in one treatment session as opposed to multiple interventions over a protracted period of time.

PHARMACOLOGIC VITREOLYSIS

In 1998 the term "pharmacologic vitreolysis" was coined[16] to formalize a new field, one that was embarked upon with the notion that we had learned enough about the molecular biology of vitreous to effectively develop pharmacologic treatments. Initially, it was felt that this knowledge could be used to enhance vitreo-retinal interface surgery with pharmacologic adjuncts. However, it is now the hope that this novel form of therapy could one day supplant surgery by mitigating the molecular changes that underlie the pathophysiology of vitreous at earlier stages of disease. Further, pharmacologic vitreolysis may improve intraocular physiology and metabolism.[17]

An enhanced understanding of the role of vitreous in retinal disorders has led investigators to use pharmacologic vitreolysis in diseases such as diabetic retinopathy,[18] macular holes,[19] retinopathy of prematurity[20] and even congenital retinoschisis.[21] Since initial attempts all used enzymes as adjuncts to surgery, the term "enzymatic vitreolysis" was prevalent in the early literature.[22,23] However, the term "pharmacologic vitreolysis" was proposed[5] so that vitreolytic agents could be grouped according to their mechanism of action as either "enzymatic" or "non-enzymatic" (**Table 1**). Furthermore, it was proposed that these agents could be sub-categorized as either non-specific agents, such as tissue plasminogen activator,[24] plasmin,[25,26] microplasmin[27,28] and nattokinase;[29] or substrate-specific agents, such as chondroitinase,[30,31] dispase[32,33] and hyaluronidase.[31,34,35]

Although this approach does have intellectual appeal on theoretical grounds, the original classification system seems to have less relevance today, since other than two



The clinical manifestations of anomalous PVD vary depending upon where the gel is most liquefied and where the posterior vitreous cortex is most firmly adherent to the retina. In the macula, anomalous PVD varies depending upon whether the attached vitreous is full-thickness, or whether there is a split in the posterior vitreous cortex (vitreoschisis).

PVD = Posterior vitreous detachment; VMTS = Vitreo-macular traction syndrome; Exud AMD = Exudative age-related macular degeneration; VPA = Vitreo-papillary adhesion

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Figure 2. Schematic diagram of anomalous posterior vitreous detachment.

Table 1. Pharmacologic Vitreolysis Classification Based on Biochemical Activity

ENZYMATIC	NON-ENZYMATIC
Non-Specific:	• Urea/Vitreosolve[36]
• Tissue plasminogen activator[24]	• RGD peptides[37]
• Plasmin[25,26]	
• Microplasmin[27,28]	
• Nattokinase[29]	
Substrate Specific:	
• chondroitinase[30,31]	
• dispase[32,33]	
• hyaluronidase[31,34,35]	

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Table 2. Pharmacologic Vitreolysis Classification Based on Biologic Activity

LIQUEFACTANTS *(Agents that liquefy the gel vitreous)*	INTERFACTANTS *(Agents that alter the vit-ret interface)*
Non-Specific:	**Non-Specific:**
• tPA[24]	• tPA[24]
• plasmin[25,26]	• plasmin[25,26]
• microplasmin[27,28]	• microplasmin[27,28]
• nattokinase[29]	• nattokinase[29]
• vitreosolve*[36]	• vitreosolve*[36]
Substrate Specific:	**Substrate Specific:**
• chondroitinase[30,31]	• dispase[32,33]
• hyaluronidase[31,34,35]	• chondroitinase[30,31]
	• RGD-peptides*[37]

* non-enzymatic agents tPA = tissue plasminogen activator

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

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non-enzymatic approaches (Urea/Vitreosolve[36] and RGD peptides[37]) the overwhelming majority of drugs that have been tested to date are enzymes. It would seem, therefore, that an alternative classification might be more useful, especially if based upon biologic activity.

Rather than basing the classification of drugs for pharmacologic vitreolysis upon the properties of the agents themselves, grouping these drugs 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 vitreo-retinal interface ("interfactants"). **Table 2** lists the different pharmacologic vitreolysis agents currently in development classified on the basis of biologic activity. Of note is that several agents are believed to have both liquefactant and interfactant properties.

Another important consideration for future research and development: when implemented clinically, pharmacologic vitreolysis will be performed upon eyes with abnormal vitreous. This is not only true for all the ophthalmic conditions identified in Figure 2, but also systemic conditions such as diabetes, and probably ocular conditions such as myopia which features "myopic vitreopathy."[38] It is known, for example, that diabetes induces significant biochemical[39–42] and structural[43] effects upon vitreous, resulting in "diabetic vitreopathy"[44] and diabetic vitreo-retinopathy.[4]

Since diabetic vitreous is so different from normal vitreous, studies on the effects of pharmacologic vitreolysis upon normal vitreous may fail to develop agents that are effective in pathologic conditions. This consideration might partly explain why hyaluronidase (Vitrase) failed in phase III FDA clinical trials for treating vitreous hemorrhage in diabetic retinopathy. No pre-clinical studies were performed using Vitrase on diabetic vitreous. Another explanation for the failure of Vitrase relates to the fact that hyaluronidase is not an interfactant, only a liquefactant (Table 1). Thus, while hyaluronidase will liquefy gel vitreous, it will not induce vitreo-retinal dehiscence. In proliferative diabetic retinopathy, this will result in persistent traction upon neovascularization with subsequent recurrent vitreous hemorrhage and vision loss.

A notable exception to the foregoing is the study of Zhi-Liang et al.,[45] who investigated 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.[34,35] Plasmin alone did not result in PVD, but induced only a partial PVD in 10/10 (100%) subjects. This is disconcerting, since past studies[6,7] showed that a partially detached vitreous carries the worst prognosis for progressive diabetic retinopathy. Thus, plasmin might actually worsen the prognosis by inducing a partial (anomalous) PVD. While previous investigations using plasmin[18–21,47] and microplasmin[26,27,47] claimed to induce total PVD, those experiments were performed in non-diabetic vitreous. Pilot clinical studies[16,46] in diabetic eyes without controls found that plasmin was effective as an adjunct to surgery for diabetic vitreo-retinopathy, but there were no studies using plasmin in diabetic eyes without surgery. Thus, further work needs to be undertaken in diabetic subjects using these and other agents in a non-surgical setting to treat diabetic vitreo-retinopathy.

While the studies of Zhi-Liang et al. showed that neither hyaluronidase nor plasmin alone achieved effective pharmacologic vitreolysis, the combination of the two agents induced a total PVD in 8/10 (80%) eyes. It is important to emphasize that these studies were in an animal model of diabetic vitreopathy and the results need to be corroborated in humans. One plausible explanation, however, is that the liquefactant hyaluronidase induced gel liquefaction while the interfactant properties of plasmin induced sufficient dehiscence at the vitreo-retinal interface to induce total PVD in a much higher percentage of cases than has been observed to date in any clinical trials. Thus,

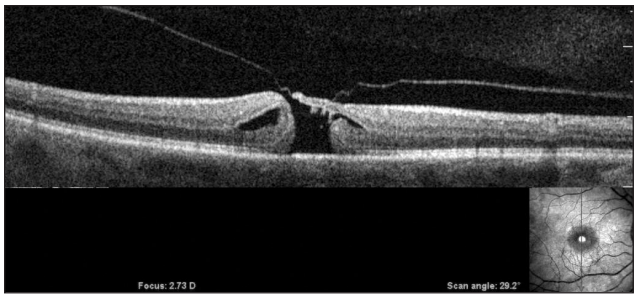

Figure 3. Anomalous PVD with macular hole. CombinedOCT/SLO imaging demonstrates anomalous PVD with persistent vitreo-foveal adhesion inducing a full-thickness macular hole and adjacent cystoid spaces.

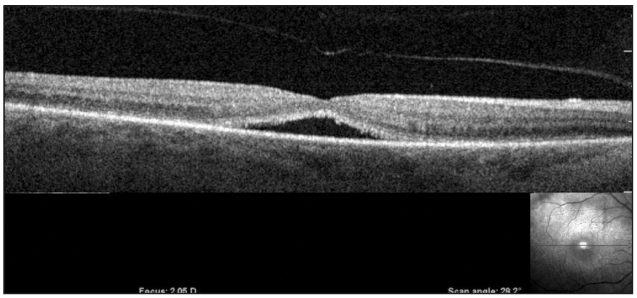

Figure 4. Resolution of macular hole after pharmacologic vitreolysis. Combined OCT/SLO imaging demonstrates resolution of macular hole one week after injection. A total PVD is evident. There is elevation of the foveal region.

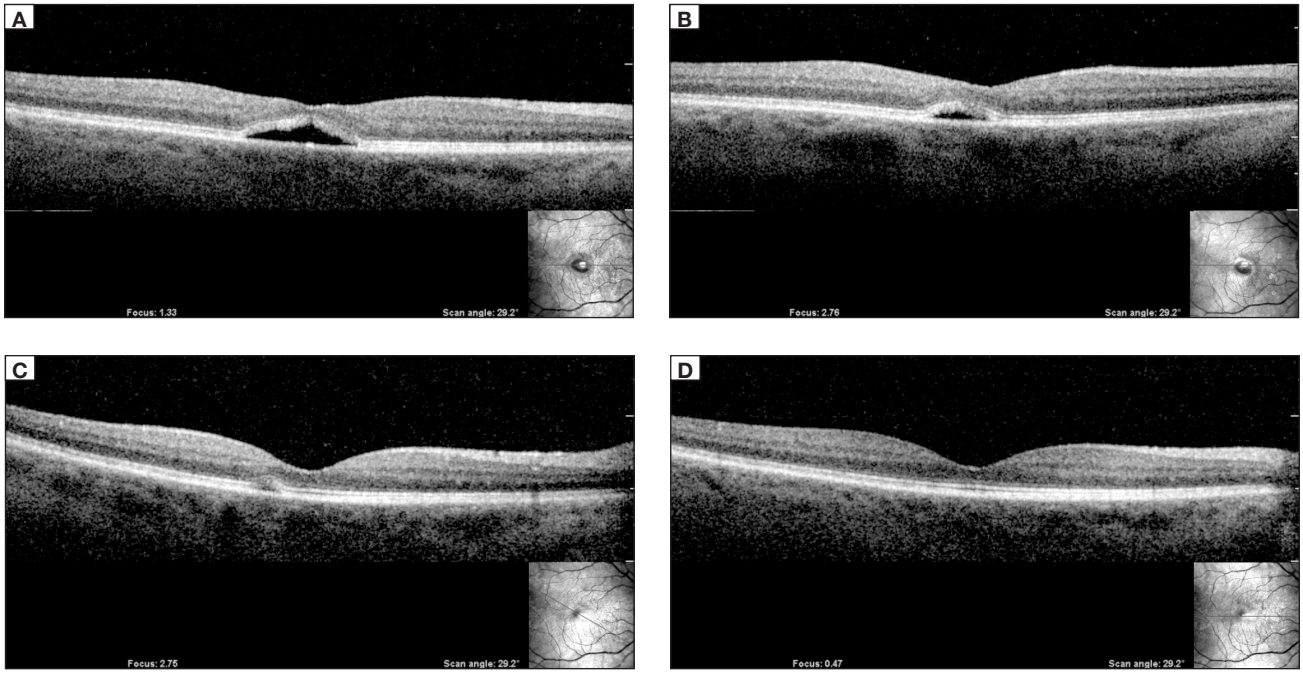

Figure 5. Final outcome of macular hole treated by pharmacologic vitreolysis. Combined SD-OCT/SLO demonstrates the restoration of normal macular anatomy 15 months after pharmacologic vitreolysis. A: Three months post-injection; B: Six months post-injection; C: 12 months post-injection; D: 15 months post-injection.

the concept[48] that combination pharmacologic vitreolysis can attain a therapeutic outcome that monotherapy could not achieve is intriguing.

Future clinical trial protocols should consider incorporating combination therapy whose value was previously suggested in theory[48] and has been proven in practice in non-diabetic animal models of vitreous[35] and in experimental models of diabetic vitreopathy.[45] Including outcome measures of ocular physiology and metabolism would also test the hypothesis that pharmacologic vitreolysis can induce salubrious effects on ocular physiology and metabolism.[49,50]

It is likely that the first drug to be approved for clinical pharmacologic vitreolysis will be microplasmin (ThromboGenics, NV). A multi-center phase 3 FDA trial to test the efficacy of microplasmin in relieving vitreo-macular traction has recently been completed and the results should be forthcoming soon. The earlier phase 2 trial[51] found that microplasmin induced resolution of macular holes without surgery in 6/20 (30%; p=0.03) cases. The following case report is one of the subjects in that study.

• ***Case Report:*** SC is a 62-year-old white woman with the chief complaint of distortions and vision loss in the right eye of three months' duration. Visual acuity was 20/200. Combined OCT/SLO demonstrated anomalous PVD with persistent adhesion of the posterior vitreous cortex to the fovea and underlying macular hole and cysts (**Figure 3**). She underwent injection in the office and two days later described the sudden onset of a "kaleidoscope effect." One week after injection the distortions were much reduced and vision improved. Visual acuity was 20/40. Combined OCT/SLO demonstrated complete PVD with closure of the macular hole and no evidence

of cysts (**Figure 4**). The elevation of the fovea, which is sometimes seen following surgical closure of macular holes, persisted for 15 months before resolving spontaneously (**Figure 5**).

While such an effect is dramatic in an individual case such as this, efficacy in the phase 2 study of pharmacologic vitreolysis with microplasmin was limited to 30% of cases. It is nonetheless plausible that with further research and development, pharmacologic vitreolysis will have a major impact on the future management of vitreo-retinal disease. If high-risk individuals can be identified early in the natural history of disease, they could be treated with pharmacologic vitreolysis prophylactically to induce an innocuous (and not anomalous) PVD. In this way, the number of people who lose vision due to retinal detachment, macular holes, advanced diabetic retinopathy, and perhaps even exudative age-related macular degeneration can be vastly reduced, perhaps even eliminated. **RP**

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