Published in final edited form as:

Retina. 2007 October ; 27(8): 1090–1096. doi:10.1097/IAE.0b013e3180654229.

Microplasmin-Induced Posterior Vitreous Detachment Affects Vitreous Oxygen Levels

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Abstract

Purpose—To determine if enzymatic induction of a posterior vitreous detachment (PVD) and/or vitreous liquefaction affects O₂ concentration in the vitreous cavity in animals with vascularized and avascular retinal circulations.

Methods—Either microplasmin or hyaluronidase was injected intravitreally into guinea pigs (avascular retinal circulation), brown Norway rats (vascularized retinal circulation without fovea), or cats (vascularized retinal circulation with fovea) with the contralateral eye used as a control. One to 2 weeks post injection, vitreal oxygen concentration was measured using a highly sensitive, platinum-based fluorophore O₂ sensor. In addition, control and microplasmin-injected rats, guinea pigs, and cats were exposed to 100% oxygen and vitreal O₂ levels were measured over time. Scanning electron microscopy (SEM) was used to evaluate the vitreoretinal interface for the presence of a PVD.

Results—In animals with a vascularized retinal circulation (brown Norway rats and cats), intravitreal injection of microplasmin with induction of a PVD significantly increased baseline O₂ concentration in the vitreous cavity compared to hyaluronidase injected eyes and controls in rats (35, 25, and 23 mm Hg, $P < 0.001$ and $P < 0.001$, respectively) and cats (26, 18, and 16 mm Hg, $P < 0.01$ and $P < 0.001$, respectively). Interestingly, intravitreal injection of hyaluronidase (vitreous liquefaction without induction of a PVD) did not significantly increase vitreal O₂ levels in any of the animal species ($P > 0.1$). Upon exposure to 100% oxygen by facemask, microplasmin injected animals showed a rapid increase in vitreal oxygen levels compared to hyaluronidase injected animals and controls, indicating that the presence of a PVD allows rapid O₂ exchange within the vitreous cavity. Similarly, once O₂ was discontinued, the O₂ concentration decreased in a similarly rapid rate. SEM showed smooth retinal surfaces in microplasmin-injected cat eyes, indicating the presence of a PVD which was not present in hyaluronidase injected or control eyes.

Conclusion—The results suggest that enzymatic-assisted PVD with microplasmin increases vitreal O₂ levels and increases the rate of O₂ exchange within the vitreous cavity.

One of the goals of vitreoretinal surgery is to separate the posterior vitreous interface from the inner limiting lamina and to create a posterior vitreous detachment (PVD). The presence of incomplete removal of the vitreous during repair of retinal detachment, proliferative diabetic retinopathy, proliferative vitreoretinopathy, macular hole, posterior hyaloidal contracture syndrome, and vitreomacular traction syndrome may result in surgical failure.¹ As previously described, mechanical posterior vitreous separation may not remove all of the vitreous from

the retinal surface during vitrectomy.² With the advent of a better understanding of the vitreous anatomy and biochemistry, enzymatic agents have been used to cleave the vitreoretinal juncture and manipulate the vitreous.^{3–6} Some enzymatic agents used to alter the vitreous such as chondroitinase and Dispase were found to damage the subretinal space and retina and cause electroretinographic (ERG) changes.^{7,8} More recently, enzymatic manipulation has been performed with agents with a more favorable profile such as hyaluronidase, plasmin, and microplasmin. Recombinant, human microplasmin is a 29 kD protein containing one protease active site of plasmin.⁹ Whereas hyaluronidase (Vitrase) has been shown to cause liquefaction of the vitreous without induction of a PVD,^{10,11} microplasmin and plasmin cause vitreous liquefaction and induction of a PVD.^{11–13}

Previous reports have shown that O₂ levels in the vitreous cavity increase following pars plana vitrectomy.¹⁴ In addition, stabilization of proliferative diabetic retinopathy and diabetic macular edema often occurs following vitrectomy, possibly due to increased oxygenation and removal of the vitreous scaffold.^{15–18} Although it has been shown that vitreous O₂ increases after mechanical removal of the vitreous, it is not known if chemical vitrectomy has the same effect. This study is designed to determine if induction of an enzymatic-assisted PVD and/or vitreous liquefaction affects O₂ concentration in the vitreous cavity in animals with vascularized (rat and cat) and avascular (guinea pig) retinas. The experiments described herein use three animal models to determine if inducing an enzymatic-assisted PVD and/or vitreous liquefaction affects O₂ concentration in the vitreous cavity.

Methods

All procedures were approved by the institutional animal care committee and adhered to the ARVO Statement for the Use of Animals in Ophthalmic and Vision Research. Guinea pigs were anesthetized with 0.4 mL ketamine and 0.3 mL xylazine, Brown Norway rats were anesthetized with 0.3 mL ketamine and 0.2 mL xylazine, and cats were anesthetized with 33 mg/kg ketamine, 1.0 mg/kg xylazine, and 0.04 mg/kg atropine for the experiments described below. Guinea pigs and rats were anesthetized and O₂ was measured while the animals breathed room air. To prevent excessive movement of the cats during O₂ measurements, they were intubated and maintained by ventilator on room air.

Intravitreal Injection

Guinea pig: 25 IU of bovine hyaluronidase (Sigma-Aldrich, St. Louis, MO H3631) or 0.6 units (0.05 mg) of microplasmin (ThromboGenics, Ltd., Dublin, Ireland) was injected using a 30-gauge needle into the midvitreous cavity. Brown Norway rat: 12.5 IU of hyaluronidase or 0.3 units (0.025 mg) of microplasmin was injected. Cats: 37.5 IU of hyaluronidase or 3.0 units (0.25 mg) of microplasmin was injected using a 30-gauge needle into the midvitreous cavity. The dose of microplasmin injected in cats and guinea pigs was based on doses determined to induce a PVD.^{12,19} The dose of microplasmin injected in rats was extrapolated from the volume of the guinea pig eye and the comparable number of units required to induce a PVD. The hyaluronidase (bovine) concentration was based upon the effective dose previously reported for the above animal species by Wang et al.¹⁰ and Hikichi et al.¹¹ Although human, recombinant microplasmin and bovine hyaluronidase were used in this study, the injections were well tolerated without evidence of anterior segment inflammation or vitritis. The number of eyes injected for the study was 11, 9, and 23 for guinea pigs, cats, and rats, respectively, as shown in Table 1. The noninjected eye of the animal served as the control. One to 2 weeks later, pO₂ measurements were conducted as described.

Oxygen Measurements in Midvitreal Cavity

Oxygen concentration was measured using a highly sensitive, platinum-based fluorophore O₂ sensor (Oxford Optronix, Ltd.). A 25-gauge needle was used to create an insertion track for the placement of the 250 μ m diameter pO₂ probe into the vitreous cavity (Figure 1). Baseline (before breathing 100% O₂) measurements of pO₂ were performed in the midvitreal cavity. Once measurements were performed, the guinea pigs and rats were exposed to 100% oxygen by facemask and cats were ventilated while O₂ levels were measured over time.

SEM Analysis

Following pO₂ experiments, scanning electron microscopy (SEM) was performed, as previously described,^{12,20,21} to evaluate the vitreoretinal interface for the presence of a PVD in microplasmin, hyaluronidase, and control eyes of each species (n = 3). Briefly, all eyes for scanning electron microscopy were fixed in 2% paraformaldehyde and 2.5% glutaraldehyde in 0.1 moles/L cacodylate buffer, pH 7.4, overnight. On the next day, corneas and lenses were removed and the whole eye cup with the retina and vitreous intact was fixed one additional day, washed with 7% sucrose, dehydrated in ethanol, dried to the critical point, and sputter coated in carbon. Photography was performed by electron microscopy (model DS-130 International Scientific Instruments Inc., Milpitas, CA). Two observers evaluated SEM photographs independently. Each observer evaluated the degree of vitreoretinal separation and if a continuous or discontinuous network of collagen fibrils covered the ILM or if the ILM was devoid of any collagen fibrils.

Results

To ensure formation of a PVD in microplasmin injected animals as previously described,¹² SEM was performed in microplasmin, hyaluronidase injected and control animals for all species. Microplasmin injected animals demonstrated a vitreoretinal interface free of cortical vitreous as compared to hyaluronidase injected and control animals. Representative SEM images of microplasmin and hyaluronidase injected and control eyes of the cat are shown in Figure 2.

Guinea Pigs (Avascular Retinal Circulation)

At room air, baseline midvitreal O₂ levels were similar for control, microplasmin, and hyaluronidase injected eyes (2.5, 2.2, and 1.2 mm Hg, respectively) (Table 1). Once baseline O₂ levels were established, the animals were exposed to 100% O₂ by facemask and midvitreal oxygen levels were monitored (Figure 3). Eyes injected with microplasmin showed a rapid increase in O₂ levels compared to hyaluronidase injected animals and controls, indicating that the presence of a PVD allows rapid O₂ exchange within the vitreous cavity. The upward slope of the pO₂ curve for microplasmin was 22 times faster than control and that for hyaluronidase was 3.6 times faster than control, respectively (Table 2). Similarly, once O₂ was discontinued, the O₂ concentration decreased in a similarly rapid rate in microplasmin injected animals, indicating that the presence of a PVD allowed increased exchange of O₂ in the vitreous cavity (Table 2).

Brown Norway Rats (Vascularized Retinal Circulation Without Fovea)

Midvitreal oxygen concentrations were measured in control, microplasmin, and hyaluronidase injected rat eyes. As shown in Table 1, baseline midvitreal O₂ concentrations in microplasmin injected eyes were significantly increased compared to hyaluronidase injected and control eyes (35, 25, and 23, $P < 0.001$ and $P < 0.001$, respectively). Upon exposure to 100% oxygen, eyes treated with microplasmin showed a rapid increase in midvitreal oxygen compared to controls,

indicating that the presence of a PVD facilitates O₂ exchange in the vitreous cavity (Figure 4 and Table 2).

Cats (Vascularized Retinal Circulation With Fovea)

Midvitreal oxygen concentrations were measured in control, microplasmin, and hyaluronidase-injected cat eyes. As shown in Table 1, baseline midvitreal O₂ concentrations in microplasmin injected eyes were significantly increased compared to hyaluronidase injected and control eyes (26, 18, 16 mm Hg, $P < 0.01$ and $P < 0.001$, respectively). PO₂ levels in hyaluronidase-injected and control eyes were not significantly different ($P > 0.1$). Upon exposure to 100% oxygen, eyes treated with microplasmin showed a rapid increase in midvitreal oxygen compared to controls (Figure 5), indicating that vitreous liquefaction and PVD induction by microplasmin facilitates O₂ exchange in the vitreous cavity to a greater degree than vitreous liquefaction alone with hyaluronidase.

Discussion

Our results reveal that animals with vascularized retinas (cats and rats) treated with microplasmin, achieving posterior vitreous separation and vitreous cavity liquefaction, show a significant increase in vitreal O₂ concentration as compared to control and hyaluronidase-injected eyes (Table 1). When microplasmin treated animals were exposed to 100% oxygen, there was an accelerated increase in oxygen levels in the midvitreal cavity compared to control or hyaluronidase treated eyes (Figures 3–5, Table 2). These results indicate that inducing a posterior vitreous separation and liquefaction, as seen in microplasmin eyes, may increase exposure of oxygen to the retina. Following injection of microplasmin, vitreal oxygen levels increased in animals with vascularized retinas (rats and cats) and not in animals with avascular retinas (guinea pigs), indicating that the increase in vitreal oxygen is likely arising from the retinal circulation (Table 1). In this study, we examined the foveated, vascularized retina of cat eyes to model the oxygen dynamics of the human retina.

Induction of a PVD and vitreous liquefaction did not increase baseline vitreal oxygen levels in animals with avascular retinal circulations (Guinea pigs) compared to animals with vascularized retinas, indicating that the increase in vitreal oxygen may arise from the retinal circulation. As previously described, species with avascular retinal circulations (choroidal circulation only) such as guinea pig possess barely detectable O₂ tension in the retina, but high O₂ tension in the choroidal structures.²² Once guinea pigs were exposed to 100% oxygen by facemask, the choroidal circulation was saturated by oxygen allowing increased exchange of oxygen through the retina to be detected in the vitreous. Our results show that oxygen exchange is facilitated in the presence of a PVD and vitreous liquefaction as illustrated in Figures 3–5.

Following injection with microplasmin, the retinal architecture and blood–retinal barrier was intact as shown in previously described studies^{19,20} and in our SEM data for all animal species. In control eyes, SEM shows the presence of cortical vitreous on the retinal surface (Figure 2A). In hyaluronidase-injected eyes, the cortical vitreous appears more densely compressed at the retinal surface following vitreous liquefaction without PVD formation (Figure 2B). In microplasmin injected eyes, SEM reveals a clean retinal surface with induction of a PVD without residual cortical vitreous (Figure 2C). In previous studies with microplasmin, there was no evidence of electrophysiologic or ultrastructural changes to the retina in multiple animal species.^{12,19} In our study, human, recombinant microplasmin, and bovine hyaluronidase were well tolerated in animal models with no evidence of anterior segment inflammation or vitritis. SEM showed normal retinal architecture (Figure 2). Thus, microplasmin appears to be a safe alternative to plasmin, which is currently used as a surgical adjunct in vitreoretinal surgery.^{23,24}

Previous studies have shown that injection of microplasmin increases the vitreous diffusion coefficient in porcine eyes using dynamic light scattering.²⁵ We were unable to determine if the elevated vitreal oxygen was due to an increase in oxygen diffusion or an increase in bulk flow (mixing) of oxygen in liquefied vitreous. The results from dynamic light scattering indicate increased liquefaction of the vitreous following microplasmin injection, allowing increased mixing and oxygen exchange. Although enzymatic compromise of the blood–retinal barrier is theoretical, as stated previously, no anterior or posterior segment inflammation occurred following injection and microscopic analysis revealed normal retinal architecture.

Our findings raise several interesting questions. In diabetic eyes, posterior vitreous separation has long been believed to reduce progression of proliferative diabetic retinopathy (PDR).¹⁷ Retinal ischemia drives elevated levels of vascular endothelial growth factor (VEGF) which induces retinal neovascularization and macular edema.²⁶ Pan-VEGF blockade by bevacizumab (Avastin) results in regression of neovascularization and reduction of leakage by angiography.²⁷ We propose that PVD induction with microplasmin could increase vitreal oxygen levels to reduce neovascularization and eliminate the vitreous scaffold upon which neovascularization forms. Several studies have noted a reduction in diabetic macular edema following posterior vitreous separation and ILM peeling, possibly indicating that increased oxygen tension following vitrectomy may reduce diabetic macular edema.^{15,16,18} These results would support the idea that a posterior vitreous separation with clean cleavage between the internal limiting lamina of the retina and posterior hyaloid of the vitreous by microplasmin could be a positive circumstance. Our studies show that a microplasmin induced PVD can increase vitreous cavity oxygen concentration. This is a well known concept from eyes that have had vitreous surgery. When the vitreous gel, including the posterior hyaloid, is removed from the retinal surface, the vitreous cavity has been found to have an increase in oxygen concentration.¹⁴

Our results reveal that rats and cats treated with microplasmin, achieving posterior vitreous separation and vitreous cavity liquefaction, show a significant increase in vitreal O₂ concentration as compared to control and hyaluronidase injected eyes (Table 1). This finding suggests that it may be useful to perform chemical vitrectomy to increase vitreal oxygen concentrations in patients with ischemic retinal diseases. Additional studies may examine animal models of ischemic retinal diseases to measure changes in retinal oxygen levels²⁸ or quantitate reversal of neovascularization following injection of microplasmin.²⁹

Acknowledgments

The authors thank Steven Bassnett, PhD, of Washington University for use of the oxygen measuring device and Janet Schofding for assistance with handling of experimental animals.

Supported by NIH grants EY02027 and EY014803.

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Fig. 1.
Oxyprobe is inserted into the vitreous cavity. A flashing light aids in visualization of the probe.

Fig. 2. Scanning electron microscopy of cat vitreoretinal interface. **A**, Control showing surface with dense network of cortical vitreous. **B**, Hyaluronidase. Condensed cortical vitreous is present at the vitreoretinal interface following vitreous liquefaction without posterior vitreous detachment. **C**, Microplasmin. Smooth retinal surface free of cortical vitreous following induction of a posterior vitreous detachment.

Guinea pig (avascular retina)-midvitreal O₂

Fig. 3. Guinea pig. Comparison of midvitreal oxygen concentration of control, microplasmin, and hyaluronidase treated eyes over time after exposure to 100% oxygen. Arrows indicate start and stop of 100% oxygen treatment.

Rat (vascularized retina)-midvitreal O₂

Fig. 4. Brown Norway rat. Comparison of midvitreal oxygen levels in control, microplasmin, and hyaluronidase treated eyes over time following exposure to 100% oxygen. Arrows indicate start and stop of 100% oxygen treatment.

Fig. 5.
Cat. Comparison of midvitreal oxygen concentration in control, microplasmin, and hyaluronidase treated eyes after exposure to 100% oxygen. Arrows indicate start and stop of 100% oxygen treatment.

Table 1
pO₂ Levels (mm Hg) in the Mid Vitreous Cavity of Guinea Pig, Brown Norway Rat, and Cat Eyes

Guinea Pig				Brown Norway Rat				Cat	
Con	MP	HY		Con	MP	HY		Con	MP
2.5 ± 1.2 (4)	2.2 ± 2.0 (8)	1.2 ± 1.0 (3)		23 ± 2 (5)	35 ± 5 (15)	25 ± 4 (8)		16 ± 2 (6)	26 ± 2 (5)
—	<i>P</i> > 0.1	<i>P</i> > 0.1		—	<i>P</i> < 0.001	<i>P</i> > 0.1		—	<i>P</i> < 0.001
	P > 0.1				P < 0.001				P < 0.01

pO₂ levels in the mid vitreous humor were measured 1–2 weeks after intravitreal injection of microplasmin (MP) or hyaluronidase (HY). Results are expressed as means ± SD (number of analyses). *P* values in bold represent differences between MP and HY measurements.

Table 2
Mid Vitreous pO₂ Level Data for Guinea Pigs and Rats Following Exposure to 100% Oxygen

	Guinea Pig			Brown Norway Rat		
	Con	MP	HY	Con	MP	HY
Delay up (min)	2.8 ± 0.8 (4)	0.3 ± 0.2 (8) <i>P</i> < 0.001	1.0 ± 0.6 (5) <i>P</i> < 0.01	0.4 ± 0.2 (4)	0.1 ± 0.1 (12) <i>P</i> < 0.001	0.2 ± 0.1 (8) <i>P</i> < 0.05
Slope up (mm Hg/min)	4.5 ± 2.0 (4)	97 ± 69 (8) <i>P</i> = 0.01	16 ± 9 (5) <i>P</i> < 0.05	37 ± 21 (4)	205 ± 128 (12) <i>P</i> < 0.05	101 ± 70 (8) <i>P</i> > 0.1
Slope down (mm Hg/min)	-3.4 ± 1.2 (4)	-96 ± 70 (8) <i>P</i> < 0.05	-48 ± 46 (5) <i>P</i> > 0.1	-28 ± 14 (4)	-68 ± 37 (12) <i>P</i> > 0.1	-46 ± 21 (8) <i>P</i> > 0.1

Mid vitreous pO₂ levels in guinea pigs and rats were monitored following breathing of 100% O₂ (see Figures 3 and 4). Analyses were conducted 1–2 weeks after intravitreal injection of microplasmin (MP) or hyaluronidase (HY). Delay up is the time between the change to 100% O₂ and the first observable increase in pO₂ level. *P* values in bold indicate differences between MP and HY.