

In Vivo Assessment of Pharmacologic Vitreolysis in Rabbits With the Digital Fluoroscopy System

Jeong Hun Bae,^{1,2} Han Seok Park,¹ Joon Mo Kim,¹ Byung Ro Lee,³ Sung Chul Lee,² Tamer Tandogan,⁴ Gerd U. Auffarth,^{4,5} Michael J. Koss,^{5,6} and Chul Young Choi^{1,4}

¹Department of Ophthalmology, Kangbuk Samsung Hospital, Sungkyunkwan University School of Medicine, Seoul, Republic of Korea

²Department of Ophthalmology, Yonsei University College of Medicine, Seoul, Republic of Korea

³Department of Ophthalmology, College of Medicine, Hanyang University, Seoul, Republic of Korea

⁴David J. Apple International Laboratory for Ocular Pathology and International Vision Correction Research Centre, Heidelberg, Germany

⁵Department of Ophthalmology, University of Heidelberg, Heidelberg, Germany

⁶Department of Ophthalmology, University of Southern California, Los Angeles, California, United States

Correspondence: Chul Young Choi, Department of Ophthalmology, Kangbuk Samsung Hospital, Sungkyunkwan University School of Medicine, 29 Saemunan-ro, Jongno-gu, Seoul, 110-746, Republic of Korea; sashimi0@naver.com.

Submitted: November 24, 2014

Accepted: June 13, 2015

Citation: Bae JH, Park HS, Kim JM, et al. In vivo assessment of pharmacologic vitreolysis in rabbits with the digital fluoroscopy system. *Invest Ophthalmol Vis Sci.* 2015;56:4817-4822. DOI:10.1167/iovs.14-16137

PURPOSE. The purpose of this study was to demonstrate the efficacy of the digital fluoroscopy system (DFS) for the in vivo assessment of pharmacologically induced posterior vitreous detachment (PVD) and vitreous liquefaction in a rabbit model.

METHODS. Twenty eyes from 10 New Zealand white rabbits were divided into 5 groups. In each group, one rabbit received an intravitreal injection of 2.0 U plasmin in the right eye and 0.5 U plasmin in the left eye. Intravitreal injection of 0.1 mL balanced salt solution (BSS) was given in the right eye, and no injection was given in the left eye of another rabbit used as a control. Intraocular fluid dynamics were assessed by the DFS, using a contrast agent in each group at different time intervals (6 hours, 12 hours, 1 day, 3 days, and 7 days). After rabbits were killed, both eyes were enucleated. Scanning electron microscopy was used to confirm the morphological alterations of the vitreoretinal interface as observed in the DFS.

RESULTS. Complete PVD was observed after 12 hours with 2.0 U plasmin injection, whereas complete PVD was observed only after 3 days in eyes injected with 0.5 U plasmin. Eyes that received BSS injection or did not receive an injection failed to show complete PVD even after 7 days. Complete vitreous liquefaction was observed after 7 days with 2.0 U plasmin injection, but no eyes with 0.5 U plasmin or BSS injection showed complete liquefaction. We could clearly confirm the presence of PVD and the degree of vitreous liquefaction by using DFS.

CONCLUSIONS. Digital fluoroscopy system appears to be a useful tool for the evaluation of pharmacological vitreolysis in rabbits with clear in vivo visualization of PVD and vitreous liquefaction.

Keywords: digital fluoroscopy system, plasmin, posterior vitreous detachment, vitreolysis, vitreous liquefaction

The vitreous has an integral role in various retinal diseases, including macular holes, macular pucker, retinal detachment, and diabetic retinopathy.¹⁻³ Removal of the posterior vitreous cortex from the retinal surface and relief of vitreoretinal traction is the most important purpose of vitrectomy for the treatment of these diseases. Despite developments in techniques and equipment, mechanical induction of posterior vitreous detachment (PVD) is often incomplete, and vitreous fibrils remain on the retinal surface.⁴ Residual vitreous may act as a scaffold for cellular proliferation and cause tractional problems. Furthermore, the high costs and potential risks of vitrectomy, including retinal detachment, hemorrhage, and endophthalmitis, have spurred efforts to find a nonsurgical treatment option.

Since pharmacologic vitreolysis was first described by Sebag et al.² in 1998, there has been much interest in the use of intravitreal agents for vitreolysis.⁵ Several agents, such as hyaluronidase, dispase, plasmin, and microplasmin, have been suggested to cleave the vitreoretinal junction and to alter

vitreous structure.⁶⁻⁸ Therefore, appropriate and objective methods to evaluate the status of posterior vitreous and the vitreoretinal interface are required for accurate assessment of treatment efficacy. Various technologies such as slit-lamp biomicroscopy, B-scan ultrasonography, and optical coherence tomography (OCT) are used to evaluate PVD or vitreous liquefaction.⁹ Although time-efficient, noninvasive, real-time imaging is possible with these technologies, obtaining clear images of PVD and confirming the degree of PVD or vitreous liquefaction is not always possible and is dependent on examiner skill.

The digital fluoroscopy system (DFS; Axiom Artis dFC; Siemens, Princeton, NJ, USA) is an imaging technique enabling real-time imaging of a patient's inner anatomical structures by using fluoroscopy. The x-ray images obtained by DFS can be produced as a digital video sequence and displayed on a monitor. Dynamic images can give examiners additional control over image quality and interpretation. Furthermore, real-time

subtraction of digital fluoroscopic images has led to the use of this technology in a wide range of diagnostic and interventional applications including coronary angiography.^{10,11} The digital subtraction technique is used to selectively visualize a certain structure over various surrounding structures. Images are produced using a contrast agent by subtracting the precontrast image or mask from postcontrast images once the contrast agent has been introduced into a structure. All images are processed in real time by a computer. In ophthalmology, the DFS could be a useful method for evaluating the status of vitreous humor with intraocular fluid dynamics. The purpose of this study was to demonstrate the efficacy of the DFS for in vivo assessment of pharmacologically induced PVD and vitreous liquefaction in rabbit eyes.

METHODS

Animals and Preparation of Plasmin Solution

Our experiments were performed in accordance with the Association for Research in Vision and Ophthalmology statement for the use of animals in ophthalmic and vision research. New Zealand white male rabbits (2.5–3.0 kg) were used in the study. The rabbits were housed in a controlled environment with a 12-light:12-dark cycle, and food and water were given ad libitum. Rabbits were anesthetized by intramuscular injection of ketamine hydrochloride, 35 mg/kg, and xylazine hydrochloride, 5 mg/kg, during all procedures. This study was approved by the animal ethics and care committee of the Kangbuk Samsung Hospital and conducted in accordance with the Declaration of Helsinki.

Plasmin (Sigma-Aldrich Corp., St. Louis, MO, USA) was acquired in a powder form and dissolved in sterile balanced salt solution (BSS) to final concentrations of 0.5 U and 2.0 U per 0.1 mL, and stored at -20°C until used.

Induction of PVD

Twenty eyes of 10 rabbits were divided into 5 groups. In each group, one rabbit received an injection of 2.0 U/0.1 mL plasmin in the right eye and 0.5 U/0.1 mL plasmin in the left eye. The other rabbit received an injection of 0.1 mL BSS in the right eye and a sham injection in the left eye. Pars plana injection was administered at 6 hours, 12 hours, 1 day, 3 days, and 7 days before DFS examination for groups 1 to 5, respectively. Before injection, the rabbit eyes underwent slit-lamp biomicroscopy and indirect ophthalmoscopy, which were repeated immediately after injection and at 6 hours, 12 hours, 1 day, 3 days, and 7 days post injection. In addition, for baseline assessment of PVD, B-scan ultrasonography was performed in all rabbit eyes before injection.

After anesthesia with ketamine hydrochloride and xylazine hydrochloride, intravitreal injection was performed as follows. Pupils were dilated with 0.5% tropicamide and 0.5% phenylephrine eyedrops, and topical proparacaine 0.5% was applied for corneal anesthesia. After the application of 5% povidone-iodine solution, anterior chamber paracentesis was performed for removal of 0.1 mL aqueous humor. The plasmin preparations and/or BSS were injected through a 30-gauge needle inserted into the midvitreous cavity, 1.5 mm posterior from the limbus in the superotemporal quadrant. Immediately after injection, 0.5% moxifloxacin ophthalmic solution (Vigamox, Alcon Laboratories, Elkridge, MD, USA) was applied to the ocular surface.

Evaluation of the Vitreous Humor by Using the DFS

A cardiac C-arm angiography system (Siemens) was used for digital fluoroscopic evaluation. The system has a flat-panel

detector using alpha-Si coupled with a CsI (TI) scintillator. The flat-panel detector captures 956×954 -pixel images at 30 frames per second with a pixel pitch of 184 μm and provides 4 fields of view: 10, 16, 20, and 25 cm. Images were evaluated in fluoroscopy mode as well as in cine mode (image acquisition).

Each anesthetized rabbit was placed dorsoventrally on the horizontal, flat panel of the DFS. Topical 0.5% proparacaine and 5% povidone-iodine solution were applied on the ocular surface. Iodixanol (320 mg/mL, Visipaque; GE Healthcare, Wauwatosa, WI, USA) was used to visualize the vitreous humor, using the DFS. The contrast agent was mixed with BSS in a 1:1 ratio, and 0.1 mL diluted contrast agent was injected through a 30-gauge needle into the midvitreous cavity. For visualization of vitreous motion, rabbit eyeballs were moved with radiolucent wooden sticks. Images of the flow and movement of contrast agent in the rabbit eyes were recorded at 30 frames per second. Using the recorded DFS images, the status of PVD and vitreous liquefaction was assessed by three masked graders (GUA, MJK, and SCL) and determined based on the agreements of two or more graders. Complete PVD was defined as free movement of the posterior vitreous membrane detached from the optic disc and retina. Partial PVD was divided into the two subgroups stage 1 partial PVD and stage 2 partial PVD. Stage 1 partial PVD was defined as PVD with attachment to the optic disc and retina posterior to the equator, and stage 2 partial PVD was defined as PVD with attachment to the optic disc and retina anterior to the equator. No PVD was defined as lack of visible posterior vitreous membrane and movement of the contrast agent.

Histologic Evaluation

Rabbits were killed using intravenous injections of KCl immediately after DFS examination, and both of the eyes were enucleated and fixed in 4% glutaraldehyde in water for 24 hours at 4°C . The posterior segment was carefully separated from the anterior segment of the globe, oriented, and opened into 4 parts. The retina was dehydrated and dried to remove all volatile substances from the tissues. Then, a conformal gold coating was applied to the tissues using a sputter-coating machine, and photographs were taken using scanning electron microscopy (SEM; model S-2380N; Hitachi, Tokyo, Japan). The ultrastructure of the vitreoretinal interface was evaluated.

RESULTS

Clinical Examination

Before injection, none of the rabbit eyes had PVD on baseline assessment with indirect ophthalmoscopy and B-scan ultrasonography. All rabbits tolerated the injection well with no signs of pain or hemorrhage. In all plasmin-injected eyes, mild anterior chamber flare and vitreous opacity were observed after injection. However, the inflammation resolved spontaneously within 3 days. Indirect ophthalmoscopy revealed no sign of retinal detachment or optic nerve pallor.

Assessment of PVD and Vitreous Liquefaction Using the DFS

Table 1 shows the status of PVD in each group. Posterior vitreous detachment was assessed with dynamic evaluation for posterior vitreous movement as outlined by the radiopaque contrast agent (Fig. 1; see also Supplementary Videos S1, S2). Partial PVD was first observed at 6 hours in plasmin 2.0 U-injected eyes and at 12 hours in plasmin 0.5 U-injected eyes. Complete PVD was observed 12 hours after injection in

TABLE 1. Diagnostic Findings of Posterior Vitreous Detachment by the Digital Fluoroscopic System

Treatment	Time Point After Intravitreal Injection				
	6 h, Group 1	12 h, Group 2	1 day, Group 3	3 days, Group 4	7 days, Group 5
Plasmin, 2.0 U	Partial (S2)	Complete	Complete	Complete	Complete
Plasmin, 0.5 U	No	Partial (S1)	Partial (S1)	Complete	Complete
BSS, 0.1 mL	No	No	No	Partial (S1)	Partial (S1)
Sham injection*	No	No	No	No	No

BSS, balanced salt solution; S1, stage 1; S2, stage 2.

* An imitation injection procedure using a syringe without needle.

plasmin 2.0 U-injected eyes and 3 days after injection in plasmin 0.5 U-injected eyes. Neither BSS- nor sham-injected eyes showed evidence of complete PVD within 7 days.

The degree of vitreous liquefaction in each group is shown in Table 2. In eyes with complete liquefaction, intravitreally injected contrast agent was gradually diluted with diffusion into the posterior cavity of the eyeball. However, little movement or dilution of the contrast agent was noticed in the eyes, with no liquefaction (Fig. 2; see also Supplementary Videos S3, S4). Partial vitreous liquefaction appeared in the DFS as condensed radiopaque space together with diluted medium flow into the vitreous-liquefied cavity. Complete vitreous liquefaction was only observed in the eye at 7 days after an injection of 2.0 U plasmin. The eyes injected with 0.5 U plasmin and BSS showed partial vitreous liquefaction at 1 and 7 days after injection, respectively. The degree of PVD and vitreous liquefaction was dose-dependent, and the higher doses of plasmin induced more complete and earlier PVD and vitreous liquefaction.

Results of PVD and vitreous liquefaction assessment by DFS were in good agreement among graders. The observed agreement was 0.85 (17 of 20 eyes) for PVD and 0.90 (18 of 20 eyes) for vitreous liquefaction. Fleiss' κ coefficient for intergrader reliability was -0.053 (95% confidence interval [CI]: -0.071 to -0.017) for PVD and -0.034 (95% CI: -0.034 to -0.017) for vitreous liquefaction (Stata 14 software; Stata Corp., College Station, TX, USA).

Histology

Scanning electron microscopy in eyes that did not to have PVD according to the DFS revealed thick collagen fibrils covering the inner retinal surface, suggesting the absence of PVD. In eyes with complete PVD according to the DFS, SEM photographs showed a smooth internal limiting membrane surface covered by only a few residual collagen fibrils. Eyes with partial PVD on DFS showed more residual fibrils than those with complete PVD (Fig. 3). There was a positive correlation between the dosage of plasmin and the degree of PVD. Eyes that received 0.5 U plasmin had many more collagen fibrils on the inner retinal surface than those that received 2.0 U plasmin.

DISCUSSION

The results of our study demonstrate that the DFS is effective and provides a high level of accuracy in determining the status of PVD and degree of vitreous liquefaction after intravitreal injection of plasmin in rabbit eyes. Findings with the DFS correspond well with histologic confirmation on SEM. The DFS also shows real-time and high-resolution imaging for 3-dimensional motion of the posterior vitreous membrane and for intravitreal fluid movement by the contrast agent. To the best of our knowledge, this is the first report of the ophthalmic

use of the DFS for the evaluation of PVD and vitreous liquefaction.

Recently, there has been considerable interest in the clinical evaluation of the status of PVD or vitreous liquefaction, in part to assess the efficacy of pharmacologic vitreolysis for the prevention or treatment of certain vitreoretinal disorders. In previous studies, PVD has been evaluated by a variety of methods including B-scan ultrasonography, slit-lamp biomicroscopy, OCT, and electron microscopy.^{9,12} Kičová et al.⁹ suggested that B-scan ultrasonography combined with slit-lamp biomicroscopy was the most reliable method to clinically detect a PVD, with a positive predictive value of 0.83. In eyes with complete PVD, a continuous and undulating echogenic band can be seen in front of the retina on ultrasonography, whereas eyes with partial PVD reveal an echogenic band with focal attachment to the retina and are less mobile than those with complete PVD. However, ultrasonography has limitations in providing a clear image of the posterior vitreous membrane, especially just behind the lens or close to the retinal surface, and in assessing whether the vitreous is liquefied or not. Furthermore, the reliability of ultrasonography depends highly on the skills of the examiner and the clarity of the medium. Slit-lamp biomicroscopy is a useful tool that allows direct inspection of the vitreous, but it is limited by its lack of reproducibility and its dependence on the skills of the examiner and on medium opacity.¹²

The presence of PVD has been evaluated with OCT, which provides 2-dimensional and high-resolution images of the posterior vitreoretinal structure. With a PVD, OCT usually shows a linear hyper-reflective signal above the retinal surface and can demonstrate a shallow PVD that is undetectable with a conventional ultrasound device. Despite being a useful tool for detecting PVD in the central retina, OCT has only a limited view of the perimacular area and vitreous above.⁹ With a scanning depth of 2 mm, OCT could not detect the posterior hyaloid far from the retina or outside the posterior pole in cases with complete PVD. Limitations of OCT also include the difficulty of obtaining clear images in patients with dense medium opacity or whose cooperation is poor.

Fluoroscopy provides high-quality, real-time moving images for various diagnostic and interventional procedures such as coronary/cerebral angiography, discography, barium enemas, and orthopedic fracture reduction surgery. Fluid flow in the body can be evaluated by other imaging modalities, such as computed tomography or magnetic resonance imaging with contrast enhancement. However, those modalities acquire images at specific moments in time, making it difficult to observe the dynamic flow of intraocular fluid and to perform real-time imaging. With the DFS, we could assess the status of PVD and vitreous liquefaction just behind the lens as well as in the far peripheral retina in real time with intact ocular structures. Therefore, we suggest that the DFS is a useful method for studying intraocular fluid dynamics and for investigating the pathophysiology of PVD and vitreous liquefaction. Because x-rays, a form of ionizing radiation, are

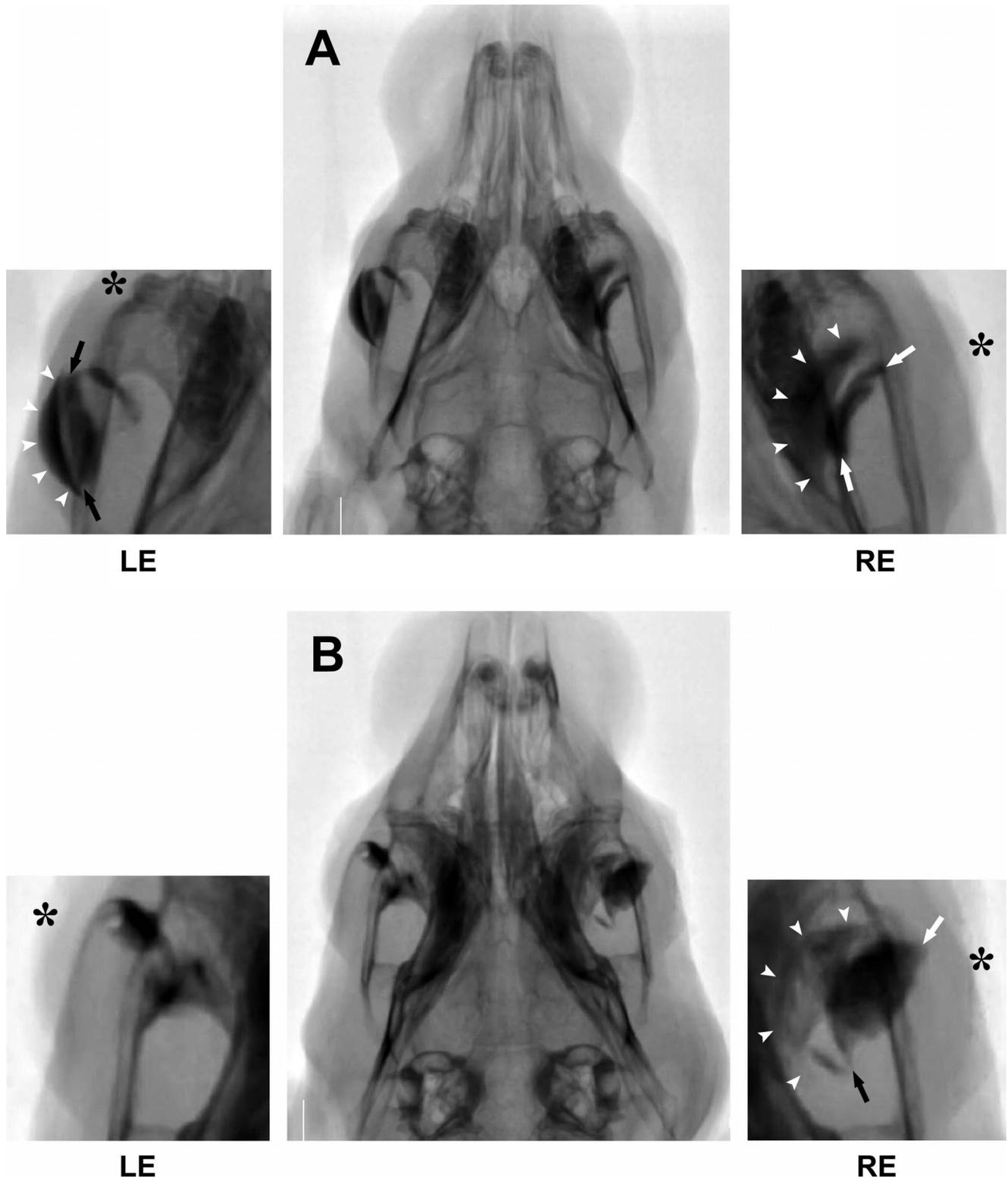


FIGURE 1. Digital fluoroscopic images of posterior vitreous detachment (PVD) in rabbit eyes. **(A)** One day after intravitreal injection of 2.0 U plasmin in the right eye (RE) and 0.5 U in the left eye (LE). In the RE, complete detachment of the posterior vitreous membrane from the retina is noted (*white arrows*; complete PVD is shown in the *enlarged photo*), whereas the other eye shows a partially detached posterior vitreous membrane with attachment to the posterior pole and the midperipheral retina (*black arrows*; stage 1 partial PVD [*enlarged photo*]). The retina is outlined by the contrast agent (*white arrowheads*). **(B)** Six hours after intravitreal injection of 2.0 U plasmin in the RE and 0.5 U in the LE. In the RE, nearly complete PVD (*white arrow*) is seen, with attachment to the posterior pole (*black arrow*) and a localized accumulation of contrast agent on the retina (*white arrowheads*; stage 2 partial PVD [*enlarged photo*]), whereas the posterior vitreous membrane is not visible without movement of the contrast agent in the LE (no PVD [*enlarged photo*]). *Corneal side.

TABLE 2. Diagnostic Findings of Vitreous Liquefaction by the Digital Fluoroscopic System

Treatment	Time Point After Intravitreal Injection				
	6 h, Group 1	12 h, Group 2	1 day, Group 3	3 days, Group 4	7 days, Group 5
Plasmin, 2.0 U	No	Partial	Partial	Partial	Complete
Plasmin, 0.5 U	No	No	Partial	Partial	Partial
BSS, 0.1 mL	No	No	No	No	Partial
Sham injection*	No	No	No	No	No

* An imitation injection procedure using a syringe without needle.

used, fluoroscopy poses the potential risk of radiation. However, modern fluoroscopy with digital imaging technology and flat-panel detector systems can reduce the radiation dose and allow safer diagnostic profiles, especially for ophthalmic purposes, with shorter durations and a narrow region of radiation exposure.^{13,14}

For the visualization of the vitreous cavity, iodixanol, an iodinated and nonionic contrast agent, was used in fluoroscopic imaging. Iodixanol is iso-osmolar with an osmolality of 290 mOsm/kg water, and its electrolytes are balanced with sodium and calcium in a ratio equivalent to that of blood. In the vitreous cavity, iodixanol showed a distinct distribution and movement according to the status of the PVD, which provided information on the stereoscopic image and anatomical range of the PVD. Furthermore, the intravitreal density gradient of iodixanol enabled a quantitative differentiation of the degree of vitreous liquefaction. Iodixanol was delivered into the vitreous cavity by intravitreal injection, which might have affected the PVD. In this study, however, the intravitreal injection of iodixanol did not affect the study outcomes because there was no more than 15 minutes between injection and enucleation, which is too short a time period to induce PVD. Some studies have shown that iodixanol causes less renal damage than ionic contrast agents and is associated with fewer neurological side effects than other nonionic contrast agents.^{15,16} Although there have been no reports of the ocular toxicity of iodinated contrast agents, nonionic and iso-osmolar contrast agents like iodixanol are generally expected to have better safety profiles than non-iso-osmolar agents and are approved for a wide range of diagnostic procedures using fluoroscopy.¹⁷

For pharmacologic vitreolysis in rabbit eyes, plasmin was used in this study at different doses. Plasmin is a nonspecific serine protease that works on various glycoproteins such as laminin and fibronectin, both of which are present at the vitreoretinal interface. Plasmin also facilitates PVD by activating endogenous metalloproteinase without degrading the type

IV collagen of the internal limiting membrane.¹⁸ Recently, the U.S. Food and Drug Administration approved the use of microplasmin, a truncated form of plasmin, for the treatment of vitreomacular traction and macular holes. In the present study, plasmin provided a predictable outcomes of PVD and vitreous liquefaction depending on its dosage and duration, and the DFS showed clear images of plasmin-induced vitreolysis in the rabbit eyes. Therefore, plasmin can be used as a control drug for comparison with other vitreolytic agents in the DFS study of pharmacologic vitreolysis.

In this study, the κ coefficient indicated a poor reliability even though the observed agreement between graders was high. The low κ coefficient was probably due to a high prevalence of the given response in a small sample size, and an interpretation based only on the κ might give a misleading result.¹⁹ Thus, the observed agreement would be more appropriate for analysis rather than the contradictory κ value.

This study had several limitations. First, because studies designed to evaluate PVD and vitreous liquefaction with the DFS have not been performed to date, there are no previously validated objective and efficient parameters for ocular examination by DFS. Thus, efforts to find the optimal settings and equipment for the use of DFS in evaluating pharmacologic vitreolysis are required. Second, baseline assessment of vitreous status before injection could not be performed with the same DFS method due to the long washout period of iodixanol from the vitreous cavity, the potential toxicity of additional ionizing radiation in rabbit eyes and the possible delayed effect of intravitreal injection of iodixanol on vitreous status. Third, vitreous movement observed with the DFS was passively induced by moving the eyeballs of the anesthetized rabbits with wooden sticks. This vitreous movement might be somewhat different from that induced by voluntary eye movements. Fourth, the application of DFS in human eyes has not yet been performed and requires more data for its safety and efficacy. The DFS, however, offers high-quality

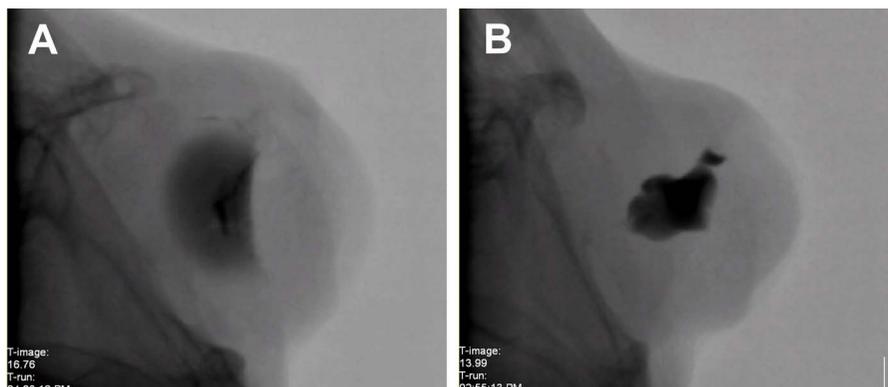


FIGURE 2. Digital fluoroscopic images of vitreous liquefaction in rabbit eyes. (A) Seven days after intravitreal injection of 2.0 U plasmin. The radiopaque contrast agent is distributed and diluted evenly in the vitreous cavity, which indicates that the vitreous is completely liquefied. (B) Seven days after sham injection. As the vitreous was not liquefied, the contrast agent is concentrated in the midvitreal cavity without dispersion.

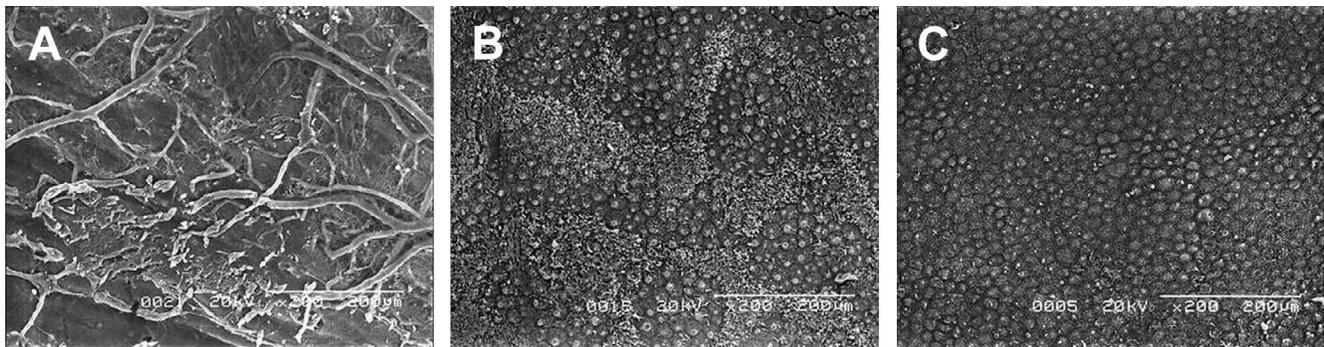


FIGURE 3. Scanning electron photomicrographs of the inner retina surface. (A) In a sham-injected eye, a dense network of collagen fibrils covered the whole retinal surface. No posterior vitreous detachment (PVD) is seen. (B) One day after intravitreal injection of 0.5 U plasmin, a smooth retinal surface was partially observed with aggregated collagen fibrils. Partial PVD. (C) One day after intravitreal injection of 2.0 U plasmin, there was a smooth retinal surface with only a few degraded collagen remnants. Complete PVD. Magnification: $\times 200$; scale bars: = 200 μm .

images for evaluating the status of the posterior vitreous and vitreous liquefaction in an animal model and can be expected to be useful in studies of ocular fluid dynamics and angiography.

In conclusion, the DFS clearly showed the differential status of PVD and vitreous liquefaction induced by different doses and durations of intravitreal plasmin in rabbit eyes. Despite the potential risks of the contrast agent and radiation, the DFS provides a clear visualization of posterior vitreous movement in real time as well as the degree of vitreous liquefaction in gray-scale images. Thus, our results demonstrate the novel feasibility of the DFS as a new imaging modality for evaluating pharmacologic vitreolysis in animal models, with clear in vivo visualization of PVD and vitreous liquefaction. Further studies are warranted.

Acknowledgments

Supported by medical research funds from Kangbuk Samsung Hospital and Samsung Biomedical Research Institute Grant C-B1-317-1.

Disclosure: **J.H. Bae**, None; **H.S. Park**, None; **J.M. Kim**, None; **B.R. Lee**, None; **S.C. Lee**, None; **T. Tandogan**, None; **G.U. Auffarth**, None; **M.J. Koss**, None; **C.Y. Choi**, None

References

- Johnson MW. Posterior vitreous detachment: evolution and complications of its early stages. *Am J Ophthalmol.* 2010;149:371-382.
- Sebag J, Gupta P, Rosen RR, 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.
- Hikichi T, Fujio N, Akiba J, Azuma Y, Takahashi M, Yoshida A. Association between the short-term natural history of diabetic macular edema and the vitreomacular relationship in type II diabetes mellitus. *Ophthalmology.* 1997;104:473-478.
- Gandorfer A, Ulbig M, Kampik A. Plasmin-assisted vitrectomy eliminates cortical vitreous remnants. *Eye.* 2002;16:95-97.
- Sebag J. Pharmacologic vitreolysis. *Retina.* 1998;18:1-3.
- Tezel TH, Del Priore LV, Kaplan HJ. Posterior vitreous detachment with dispase. *Retina.* 1998;18:7-15.
- Narayanan R, Kuppermann BD. Hyaluronidase for pharmacologic vitreolysis. *Dev Ophthalmol.* 2009;44:20-25.
- Stalmans P, Benz MS, Gandorfer A, et al. Enzymatic vitreolysis with ocriplasmin for vitreomacular traction and macular holes. *N Engl J Med.* 2012;367:606-615.
- Kičová N, Bertelmann T, Irle S, Sekundo W, Mennel S. Evaluation of a posterior vitreous detachment: a comparison of biomicroscopy, B-scan ultrasonography and optical coherence tomography to surgical findings with chromodissection. *Acta Ophthalmol.* 2012;90:e264-268.
- Detrano R, Markovic D, Simpfendorfer C, et al. Digital subtraction fluoroscopy: a new method of detecting coronary calcifications with improved sensitivity for the prediction of coronary disease. *Circulation.* 1985;71:725-732.
- Vogel RA, Mancini GB, Bates ER. Cardiac applications of digital subtraction angiography. *Int J Card Imaging.* 1985;1:233-240.
- Hoehn F, Mirshahi A, Hattenbach LO. Optical coherence tomography for diagnosis of posterior vitreous detachment at the macular region. *Eur J Ophthalmol.* 2009;19:442-447.
- Spahn M. Flat detectors and their clinical applications. *Eur Radiol.* 2005;15:1934-1947.
- Dragusin O, Breisch R, Bokou C, Beissel J. Does a flat panel detector reduce the patient radiation dose in interventional cardiology? *Radiat Prot Dosimetry.* 2010;139:266-270.
- Jo SH, Youn TJ, Koo BK, et al. Renal toxicity evaluation and comparison between visipaque (iodixanol) and hexabrix (ioxaglate) in patients with renal insufficiency undergoing coronary angiography: the RECOVER study: a randomized controlled trial. *J Am Coll Cardiol.* 2006;48:924-930.
- Sundgren P, Bååth L, Maly P. CNS-effects from subarachnoid injections of iohexol and the non-ionic dimers iodixanol and iotrolan in the rabbit. *Acta Radiol.* 1995;36:307-311.
- Spencer CM, Goa KL. Iodixanol. A review of its pharmacodynamic and pharmacokinetic properties and diagnostic use as an x-ray contrast medium. *Drugs.* 1996;52:899-927.
- Kohno T, Sorgente N, Ishibashi T, Goodnight R, Ryan SJ. Immunofluorescent studies of fibronectin and laminin in the human eye. *Invest Ophthalmol Vis Sci.* 1987;28:506-514.
- Feinstein AR, Cicchetti DV. High agreement but low kappa: I. The problems of two paradoxes. *J Clin Epidemiol.* 1990;43:543-549.