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Efficacy of autologous plasmin for idiopathic macular hole surgery

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PURPOSE. *To determine whether a single intravitreal injection of autologous plasmin or a combination of plasmin and intraocular gas without peeling the internal limiting membrane (ILM) will close idiopathic macular holes.*
METHODS. *Eight eyes of seven patients with an idiopathic macular hole were studied. The degree of posterior vitreous detachment (PVD), vitreal liquefaction, closure of the macular hole, visual acuity, and complications following intravitreal plasmin or plasmin with gas were investigated. The removed ILM was examined by electron microscopy.*
RESULTS. *A PVD was created in seven out of eight eyes exposed to plasmin or plasmin with gas, however, the macular hole was not closed by either. Closure occurred in two eyes using conventional vitrectomy after the plasmin with gas injection, but peeling the ILM was required in the remaining six eyes. Vitreal fibers and glial cells were not observed on the vitreal surface of the extracted ILM.*
CONCLUSIONS. *A PVD was created safely and reliably although closure of the macular hole did not occur with either plasmin or with plasmin and gas injection. However, vitreous surgery became easier, and it required a shorter time to close the macular hole with intravitreal plasmin. (Eur J Ophthalmol 2005; 15: 787-94)*

KEY WORDS. *Autologous plasmin, Idiopathic macular hole, Posterior vitreous detachment, Vitrectomy*

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INTRODUCTION

There is good evidence that macular holes (MHs) result from tangential traction of the vitreous on the macular region (1, 2). In some cases, there is evidence that a contraction of a fibrous membrane consisting of glial cells and the internal limiting membrane (ILM) surrounding the hole can lead to MHs (3, 4). Thus, the successful closure of MHs should be achieved by creating a posterior vitreous detachment (PVD) (5, 6) or by removing the ILM (7-12) in these cases.

However, even if there is a closure of the MH following these surgical procedures, surgical complications may occur, such as formation of retinal tears (13-15), visual field loss (16, 17), maculopathy due to light damage (18), choroidal neovascularization (19), and failure to improve vision (20). In addition, the face-down position that the patient must maintain following the injection of gas for tamponading the retina is a significant strain on the patient. Some of these complications can be reduced if a PVD is already created and if the operation time is shortened.

The concept of pharmacologic vitrectomy with plasmin

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was introduced by Margherio et al and Trese et al (21, 22), and these procedures have made substantial contributions toward improving the success rate of closing traumatic MHs. The authors used autologous plasmin derived from the patients' blood and, after isolation and purification, the plasmin was injected into the vitreous cavity. We have tried to replicate the method used by Trese et al (22) except that we have added used suppressants of protein denaturation in the preparation of plasmin to isolate purified plasminogen. The purpose of this study was to determine whether plasmin, activated from plasminogen just before surgery, alone or plasmin and gas injection will lead to the closure of MHs. Our ultimate goal was to make MH surgery an ambulatory treatment.

PATIENTS AND METHODS

Patient selection

Applicants for this surgery were submitted to the Juntendo University's Institutional Review Board, and approval was obtained for eight eyes of seven patients. Patients were informed on the purpose and procedures of the surgery, and also that if closure was not achieved after plasmin injection, conventional treatment would be implemented. They were also advised of the benefits of the new procedure to them and to the medical staff if closure was made by plasmin injection alone or plasmin plus gas injection. After receiving this information, an informed consent

Table I - SUMMARY OF PATIENTS

	Pre-operative					Operative	
Patient No. Age	**EYE**	**Pre-op VA**	**Size of hole(μm)**	**OCT (stage)**	**B-mode (PVD)**	**1st procedure**	**Result of 1st procedure**
1(F) 56 y.o	OS	20 200	300	Stage 3	(-)	Plasmin alone	PVD(+) but not closed
2(F) 66 y.o	OS	20 200	300	Stage 3	(-)	Plasmin + SF6	PVD(+) but not closed
3(F) 73 y.o	OD	20 200	300	Stage 3	(-)	Plasmin + SF6	PVD(+) but not closed
4(F) 54 y.o	OD	20 200	200	Stage 3	(-)	Plasmin + SF6 ↓ PEA+IOL+VTX+SF6	PVD(-)RD(+) but not closed
5(F) 63 y.o	OS	20 100	200	Stage 3	(-)	PEA+IOL+ +Plasmin+VTX+ +ILM+SF6	PVD(+) but not closed
6(F) 64 y.o	OD	20 200	300	Stage 2-3	(-)	PEA+IOL+ +Plasmin+VTX+ +ILM+SF6	PVD(+) and closed
7(M) 60 y.o	OD	20 100	300	Stage 3	(-)	PEA+IOL+ +Plasmin+VTX+SF6	PVD(+) and closed
	OS	20 100	500	Stage 3-4	(-)	PEA+IOL+ +Plasmin+VTX+ +ILM+SF6	PVD(+) and closed

was received from all of the patients. Surgery was performed on only one eye even if a MH was present in both eyes because this treatment was new. However, in the seventh patient, the surgery on the first eye was completed in a short time, and the patient requested surgery be performed on the fellow eye with plasmin as in the first eye.The ages of the patients ranged from 54 to 73 years with an average of 62 years. There were two eyes of one male patient and six eyes of six female patients. The preoperative vision ranged from 20/200 to 20/50, and the diameter of the hole was 200 µm for two eyes, 300 µm for five eyes, and 500 µm for one eye.

Optical coherence tomography (OCT) showed that all eyes had either Stage 2 or 3 idiopathic macular holes. Eyes with other membranes or with other eye diseases were excluded. All eyes were phakic, and those cases with cataracts affecting vision were excluded.mn We confirmed that a PVD was not present in all of the eyes by B-mode ultrasonography (Tab. I) The holes were examined in detail before surgery by slit-lamp microscopy with a 90 diopter Goldmann lens. Lattice degeneration in the lower peripheral region was confirmed in only Case 2.

Preparation of autologous plasminogen

Thirty milliliters of blood was collected from each patient, and centrifuged to separate the plasma. In order to suppress protein denaturation, 10 IU of aprotinin, 5 mM

(to be continued)

Operative			Post-operative			
2nd procedure	**Result of 2nd procedure**	**3rd procedure**	**Final Result**	**Post op VA**	**Complications**	**F/U (Mo)**
SF6	not closed	VTX+ +ILM+SF6	Closed	20 40	(-)	12
VTX+SF6	not closed	VTX+ +ILM+SF6	Closed	20 100	Retinal tear	12
PEA+IOL+VTX+ +ILM+SF6	closed		Closed	20 100	(-)	12
VTX+ +ILM+SF6	closed		Closed	20 200	Macular degeneration	12
VTX+ +ILM (wider) + SF6	closed		Closed	20 100	(-)	12
			Closed	20 60	(-)	10
			Closed	20 30	(-)	6
			Closed	20 60	(-)	6

F= Female; M= Male; OS= Left eye; OD= Right eye; VA= Visual acuity; OCT= Optical cohrence tomography; SF6= SF6 gas injection; PEA= Phacoemulsification and aspiration; IOL= Intraocular lens implantation; PVD= Posterior vitreous detachment; VTX= Vitrectomy; ILM= Internal limiting membrane removal; F/U= Follow-up period

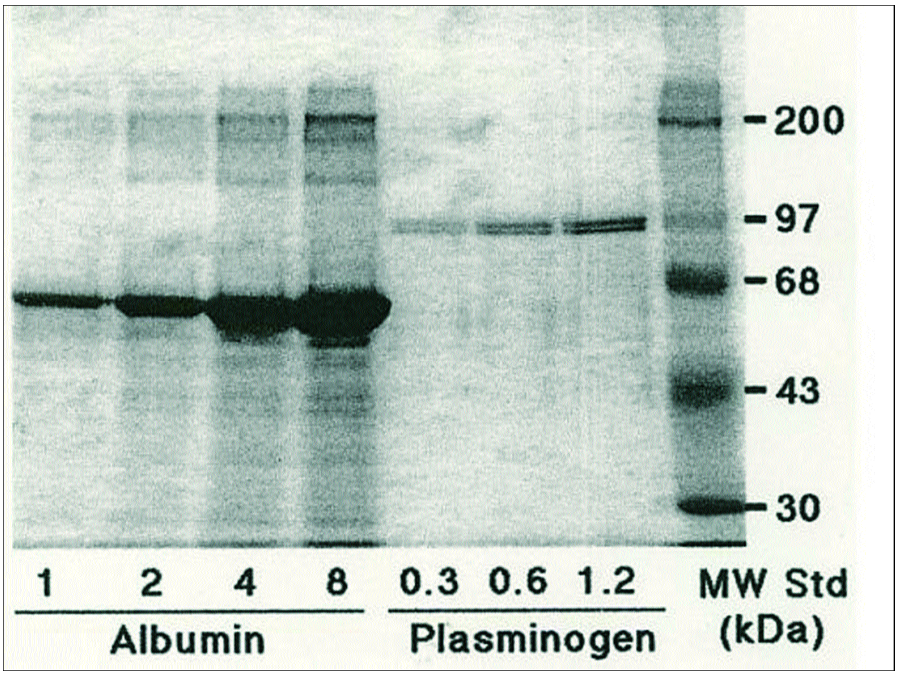

Fig. 1 - *SDS-PAGE of purified plasmin. There are clear lines at 97 kDa.*

benzamizine, and 1 mM EDTA were added to the plasma, and affinity chromatography was performed using lysine Sepharose. Because epsilon-amino caproic acid was used in the extraction, gel filtration chromatography using Sephadex G250 was performed for dechlorination. A dialysis membrane was used for condensation, and purified plasminogen was filtered through a 0.22 µm Millipore filter for sterilization.

The preparation of plasminogen required about 8 hours. To confirm the absence of contamination including anaerobic bacteria, the isolated plasminogen was cultured on blood agar medium, chocolate agar medium, and thioglycolate medium for 48 hours.

The plasminogen was frozen and stored, and the activity of plasminogen was measured by its absorbance at 405 nm using D-val-leu-lys-p-nitroanilide (S-2251). The purity of the plasminogen was confirmed by electrophoresis (SDS-PAGE; Fig. 1). The main difference between this study and that of Trese et al (22) was that aprotinin and benzamizine were added to prevent protein denaturation, and that urokinase was used to activate plasminogen to plasmin just prior to its use during surgery (23).

Surgical techniques

We confirmed that plasminogen was completely aseptic and that adequate conversion of plasminogen to stable plasmin by urokinase was achieved. The plasmin, 0.2 IU plasmin/0.1 mL, was injected into the vitreous cavity in the operating room with 27 G needle at 3.5 mm posterior to limbus. For all cases, phacoemulsification and aspiration (PEA) and intraocular lens implantation (IOL) were performed during the vitreous surgery. We performed plasmin injection and vitreous surgery simultaneously, we injected plasmin, then waited for more than 10 minutes before starting vitreous surgery.

Electron microscopic examination

The ILM removed during vitrectomy was examined to determine whether any remnants of the vitreous or fibrous tissues remained attached. The removed ILM was fixed in a mixture of glutaraldehyde and paraformaldehyde followed by postfixation in osmium tetroxide. The tissues were then dehydrated in alcohol, embedded in epoxy resin, and ultrathin sections were cut. The sections were examined with an electron microscope (JOEL JEM-1010, Tokyo, Japan).

RESULTS

Initially, we considered it important to achieve closure of the MH without ILM removal because the safety of ILM

removal had not yet been confirmed. The clinical features of the eight eyes are shown in Table I. In all eyes, no signs of inflammation were found and a complete PVD was created following intravitreal plasmin by B-mode ultrasonography or by the findings during vitreous surgery except in one case (Patient 4). In those cases with PVD, PVD was complete and no attachments remained at optic nerve. However, the MH was not closed by plasmin alone or plasmin with intraocular gas tamponading.

Patient 1 was treated with only intravitreal plasmin, and although a PVD was confirmed, the MH was not closed postoperatively. With time, there appeared to be a gradual progression to closure but because it did not close within 1 week, gas injection and a face-down position was implemented. However, the MH remained open. According to Hikichi et al (24), PVD can be performed by plasmin and SF6 gas injections, so we assumed that the injected gas had not come in contact with the macular region. Because additional gas injection still did not lead to closure, vitrectomy with gas injection was performed but the MH remained opened. Because the patient desired a faster cure, surgery was performed and the ILM was removed. This sealed the hole and vision improved.

Patient 2 received a simultaneous injection of plasmin and SF6 gas. As in Case 1, the injected gas may not have come in contact with the macular region adequately because insufficient gas was injected. A retinal tear in lattice degeneration was detected about 15 minutes after plasmin injection in the peripheral retina, which is a complication of this procedure. The tear indicated that the plasmin-induced vitreous detachment extended all the way to the periphery. We believe that this is one type of complication that may develop in cases where lattice degeneration is found in the periphery.

Patient 3 received intravitreal plasmin and SF6 gas simultaneously, and again a complete PVD was created but the MH did not close (Fig. 2). Patient 4 received intravitreal plasmin and SF6 gas simultaneously, and there was an increase in the central scotoma about 1 hour after the injection. Ultrasound B-mode showed that PVD had not occurred, and a retinal detachment was confirmed by OCT in the macular region. On the same day, vitreous surgery was performed. An intraoperative examination showed that the liquefaction was mild and PVD had not developed.

We concluded from the first four patients that intravitreal plasmin alone or plasmin with SF6 gas injection did not lead to closure of the MH, and that ILM removal was required. Therefore, the initial treatment for Cases 5 and 6 consisted of plasmin injection and vitreous removal followed by ILM peeling. Then there was liquid-air exchange and SF6 gas injection. An early closure of the MHs was detected in both cases.

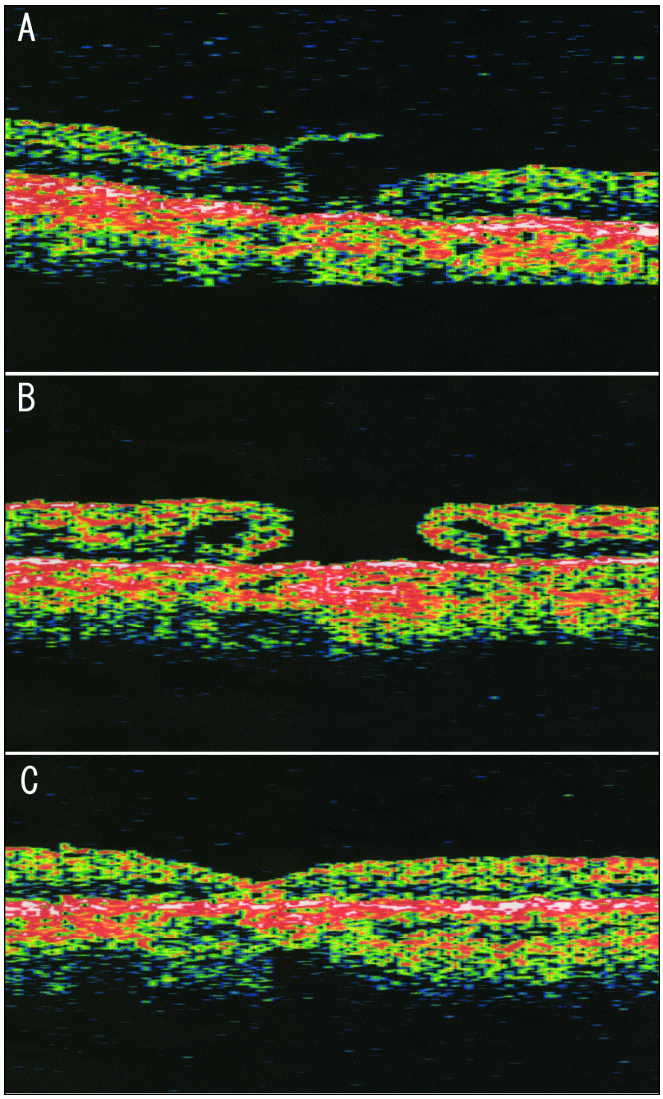

Fig. 2 - *Optical coherence tomography findings of Patient 3.* **(A)** *Before surgery.* **(B)** *After plasmin and gas injection.* **(C)** *After vitreous surgery.*

In Case 7, PEA + IOL, plasmin, vitreous removal, liquid-air exchange, and SF6 injection were performed without ILM removal, and the hole was closed in both eyes.

Intraocular inflammation due to the plasmin injection did not occur in all eight eyes, and in seven out of eight eyes, a PVD was created with plasmin or plasmin with SF6 gas injection. The procedure of vitreous surgery was simplified by the creation of a PVD and liquefaction of the vitreous in all cases.

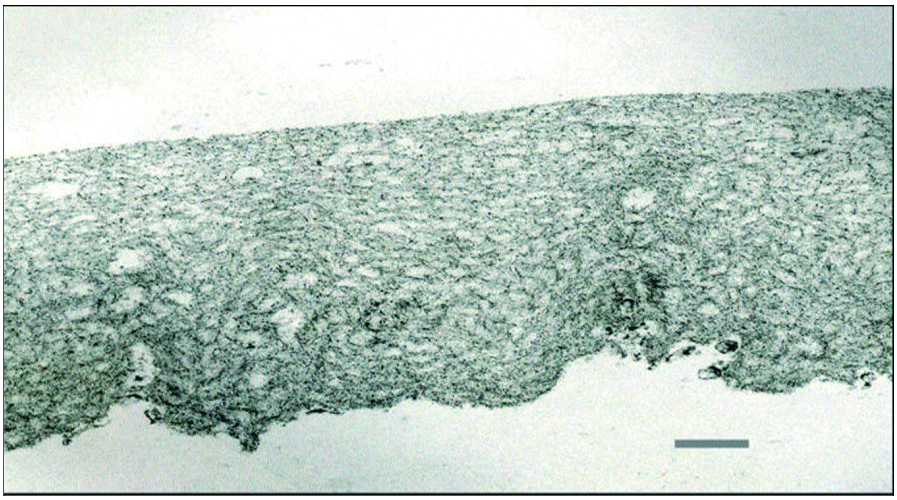

Fig. 3 - *Transmission electron microscopic findings of internal limiting membrane (ILM) removed. No vitreous fibers remained on the surface for ILM in all eyes removed.*

Electron microscope examination showed no signs of vitreal fibers or fibrous tissues on the removed ILM. In addition, cellular membranes of Müller cells were not observed.

DISCUSSION

The closure rate of idiopathic MHs was first reported by Kelly and Wendel (5) and Wendel et al (6) to be 58% and 73%, respectively. Because MHs are due to the traction caused by the vitreous, the creation of a PVD alone was thought to be sufficient to close the MH. It was soon realized that holes in the early stage of ocular diseases may close, but for larger holes or those surrounded by a membrane, ILM peeling or membrane removal was required to close the MH (7-12).

When removing the ILM, visibility is improved and operation time is reduced if indocyanine green is used to make the ILM visible (9, 11). Moreover, if closure does not occur by removal of the vitreous alone, gas injection and a face-down position may be required in order to block the passage between the fluid cuff and vitreous cavity (25).

Because complications can develop during the creation of a PVD, the frequency of these complications can be reduced if this procedure can be performed without manipulation of the vitreoretinal interface and can be done in a shorter time. This would then be one advantage of using an enzyme to liquefy the vitreous during vitreous surgery.

Laminin and fibronectin are the components that form the vitreoretinal interface (26). There have been proposals to chemically dissociate this interface (27, 28). There are several reports on clinical use with plasmin derived from autologous blood (21, 22, 29, 30).

We also attempted the method described in their report, but because the activity of the resulting plasmin was unstable we added a protein denaturation suppressant during the preparation. We were then able to achieve a highly active plasmin that was stable. Our plasmin showed maximum activity 5 to 10 min after intravitreous injection (23), but quickly deactivated thereafter. It was most effective when injected 5 to 10 min before the start of the vitreous surgery.

Although PVD may be mechanically induced during vitreous surgery, any remaining vitreous cortex may lead to non-closure or reopening of the hole. Trese et al (22) stated that plasmin injection causes not only a PVD but also removal of membranes surrounding the hole. We examined the ILM obtained during vitreous surgery with plasmin by electron microscopy and confirmed that there was no membrane or cells on the ILM.

Thus, in the seven cases, with the exception of Case 4, a PVD with liquefaction of the vitreous had developed due to the plasmin injection. This allowed the vitreous to be removed in a shorter time.

In Patient 4, the liquefaction of the vitreous was mild; PVD did not occur but a retinal detachment had developed. Although plasmin activity measured prior to injection was sufficient, it was ineffective in the vitreoretinal interface and the retinal detachment may have developed because of the contraction of the vitreous in the periphery. We do not have a good explanation as to why the retinal detachment developed. We assume that the retinal detachment was rhegmatogenous. But it is not clear

whether the plasmin played a role in its development by creating posterior vitreous separation or the injection itself played a role. We will have to examine the injection procedure in more detail so that a more reliable procedure can be used in which plasmin can reach the interface.

In conclusion, intravitreal injection of plasmin caused a liquefaction of vitreous and formation of a PVD. The MH did not close, but the surgical procedures became simpler and were done in a shorter time. In this way, plasmin injection during vitreous surgery might be useful in the initial steps to treat MH. However, if there is a point of strong pathologic vitreoretinal adhesion, retinal breaks may occur.

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