分泌顆粒体剥離の作成と網膜血管新生に対する抑制効果についての研究

（課題番号：08672003）

平成8年度～平成9年度科学研究費補助金
基盤研究（C）研究成果報告書

平成11年2月

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はしがき

平成８年から、文部省科学研究費補助金（基盤研究Ｃ２）の助成のもとに行われた「後部硝子体剥離の作成と網膜血管新生に対する抑制効果についての研究」は２年間の研究期間を終了し、ここに研究成果報告書をまとめることになった。未だ計画のすべてが達成されたわけではなく、今後も更なる検討が必要ではあるが、いくつかの新知見をが得られたと考えている。本研究成果は既に学術雑誌への掲載が決まっているので、投稿論文をこの報告書に代える。報告書の作成にあたり、ご指導、ご助言を頂いた関係者の皆様に心からお礼申し上げる。

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研究経費

平成８年度	900千円
平成９年度	1,300千円
計	2,200千円

研究発表

（1）学会誌等

Taiichi Hikichi, Norihiko Yanagiya, Masanori Kado, Jun Akiba, Akitoshi Yoshida: Posterior vitreous detachment induced by injection of plasmin and sulfur-hexa-fluoride in the rabbit vitreous. Retina, in press.

（2）口頭発表

Taiichi Hikichi, Norihiko Yanagiya, Masanori Kado, Jun Akiba, Akitoshi Yoshida: Posterior vitreous detachment induced by injection of plasmin and sulfur-hexa-fluoride in the rabbit vitreous. ARVO Annual Meeting　　May 1998　Florida, USA

Introduction

Two important goals of vitreous surgery are separation of the vitreous from the retina and release of vitreous traction. Especially in some retinal diseases such as macular holes[1] or diabetic macular edema,[2] removal of the vitreous cortex from the retina is the primary surgical maneuver. However, surgical removal of the vitreous cortex is sometimes difficult and carries the risk of complications such as retinal breaks, retinal detachment, or retinal nerve fiber damage.[3,4]

In the natural history of some retinal disorders, the role of vitreoretinal adhesion is well established. Complete posterior vitreous detachment (PVD) may prevent retinal neovascularization in eyes with diabetic retinopathy and retinal vein occlusion.[5,6] The prognosis of proliferative diabetic retinopathy is much better in eyes with complete PVD than in eyes with no or partial PVD.[7] Vitreous traction on the fovea is a major cause of idiopathic macular holes, and vitreous separation from the fovea may greatly reduce the risk of macular hole formation.[8]

Verstraeten and associates[9] demonstrated that plasmin could be a useful biochemical adjunct of mechanical vitrectomy and facilitate PVD formation. Separating the vitreous cortex from the retina without vitrectomy would be more valuable clinically. In the present study, to investigate the possibility of PVD formation without vitrectomy, we evaluated if vitreous injection of plasmin and sulfur-hexa-fluoride (SF_6) could induce PVD without vitrectomy.

Materials and Methods

Preparation of Plasmin Solution

Plasmin (CalBiochem, La Jolla, CA) was stored at -20°C until administration, at which time the powder was reconstituted in sterile balanced salt solution (BSS) at room temperature to a final concentration of 1 unit of 0.1 mL.[9]

Injection of Plasmin and SF6

Fifteen male New Zealand white rabbits (2-2.5 kg) were housed in the animal care facilities of Asahikawa Medical University, Asahikawa, Japan. The animals were fed standard laboratory chow and treated in accordance with the Principles of Laboratory Animal Care of the National Institutes of Health. Rabbits were anesthetized with a combination of intramuscularly injected ketamine hydrochloride (20 mg/kg) and chlorpromazine hydrochloride (10 mg/kg) and topical proparacaine. Five rabbits each were randomly assigned to one of three groups. One eye of each rabbit in group I received a pars plana injection in the midvitreous cavity of 1 unit of human plasmin (0.1 mL reconstituted in sterile BSS) and 0.5 mL of SF_6. One eye of each rabbit in group II received a vitreous injection of 1 unit of human plasmin (0.1 mL) only. One eye of each rabbit in group III received a vitreous injection of 0.5 mL of SF_6 only.

The fellow eye of each rabbit was injected with BSS (0.1 mL).

Clinical Examination

Before the study and at periodic intervals after intravitreal injection, indirect ophthalmoscopic and slit-lamp biomicroscopic examinations with and without a preset lens (+90 diopters, Nikon, Tokyo, Japan) were performed. We also monitored the intraocular pressure (IOP) levels of the injected eyes using a calibrated pneumotonometer (Model 30 Classic Pneumotonometer, Mentor O&O, Inc., Norwell, MA) after application of one drop of 0.4% oxybuprocaine hydrochloride at base line and periodic intervals after intravitreal injection, and confirmed that the IOP did not increase. The mean IOP of three measurements was calculated at each time point. When the IOP rose after the intravitreal injection of SF_6, paracentesis was performed and the IOP was decreased to the pre-injection level.

Electrophysiologic Examination

Seven days after intervention, electroretinography (ERG) was performed in all rabbits. The rabbits were anesthetized with 10 mg/kg intravenous pentobarbital sodium, the pupils were dilated, and topical anesthetic was applied to the cornea. Rabbits were dark-

adapted for 1 hour before the measurements were recorded. The study eye was held open with a Barraquer-type wire speculum, and the fellow eye was carefully patched to avoid all stimulation. For recording, we used a photic stimulator (SLS 4100), a biophysical amplifier (AVM-10), and an averager (DAT-1100; Nihon-Kohden, Tokyo). Time constants were set at 2 sec for ERG a and b waves, and at 0.003 sec for oscillatory potentials. Dark-adapted ERGs were recorded with a light stimulus set at 5,000 lux of corneal illuminance (stimulus duration, 0.003 sec), and recordings were made from the suction-cup type corneal electrode (Kyoto Contact Lens Co. Kyoto, Japan) by averaging 10 responses to the light stimulus at 0.1 Hz. An inactive needle electrode was placed in the eyelid subcutaneously.

Scanning Electron Microscopic Examination

After ERG examination, all rabbits were euthanized. The 12-o'clock position was marked with a suture placed at the limbus. The enucleated eyes were immediately cut open at the pars plana and placed in 0.1 M phosphate buffer (pH 7.4) containing 2.5% glutaraldehyde and 0.25 M sucrose. After 24 hours fixation, the lens was carefully separated from the posterior segment of the globe. The posterior segment of the globes then was oriented the anteroposterior axis. After rinsing the buffer, specimens for scanning electron microscopy were fixed in 1% osmium tetroxide for 60 minutes and dehydrated through a graded ethanol series, sputter-coated in palladium platinum, and photographed using a Hitachi S-4100 microscope (Hitachi, Tokyo).

Results

Indirect ophthalmoscopy and slit-lamp observation indicated that the vitreous was mildly hazy with few infiltrating cells 1 day post-injection with plasmin. These vitreous findings decreased 3 days after injection with plasmin. In eyes that received plasmin and SF_6, the vitreous findings were more apparent. SF_6 resorbed in all eyes 7 days post-injection Reactions in the vitreous in the

eyes that received BSS alone were minimal.

In eyes that received plasmin and SF6 injection (group I), scanning electron micrographs showed a smooth retinal surface, consistent with a bare internal limiting lamina (Figure 1). These findings were found in both the superior and inferior retina. The vitreous remained attached to the retina at the vitreous base. The retinal surface in eyes injected with plasmin alone (group II), SF6 alone (group III), or BSS appeared to be covered with sparse collagen fibers (Figure 2 and 3).

During electrophysiologic examination 7 days after intervention, the a- and b-wave amplitudes did not differ among eyes that received an injection of plasmin and SF6, plasmin alone, SF6 alone, and BSS.

Discussion

Verstraeten and associates[9] demonstrated that injection of plasmin in the rabbit vitreous in combination with a core vitrectomy produced PVD without mechanical peeling of the posterior vitreous cortex from the retinal surface. Vitreous injection of plasmin caused a transient decrease in the ERG b-wave amplitude with excellent recovery. Those authors speculated that the decrease in the ERG amplitude resulted from the high osmolarity of the plasmin solution. Their histologic observations revealed no evidence of retinal damage. In the present study, we injected plasmin combined with SF6, one of the most popular gas vitreous substitutes to induce PVD. Fineberg and associates[10] reported that no electroretinographic, histologic, or ultrastructural abnormalities in the retina were found in the owl monkey, when the vitreous was injected with SF6.

Plasmin is known to have proteolytic activity against laminin and fibronectin,[11-13] which are components of the internal limiting lamina and thought to bridge and bind vitreous collagen fibers between the posterior vitreous cortex and the internal limiting lamina. Thus, plasmin should exert its activity on the vitreoretinal interface. However, based on the study of Verstraeten

and associates,[9] an injection of plasmin alone did not produce PVD. Another iintervention such as a core vitrectomy or SF_6 injection seems necessary to cause PVD. With core vitrectomy, mechanical oscillation, which is produced by cutting and aspirating the vitreous, might induce separation of vitreous fibers from the internal limiting lamina. Lincoff and colleagues[14] reported that intravitreal injection of the gas induced a loss of hyaluronan and aggregation of vitreous collagen fibers. Biochemical changes such as these in the vitreous might play a role in inducing separation of vitreous fibers from the internal limiting lamina by weakening the adhesion between the internal limiting lamina and vitreous when treated with plasmin.

Our study demonstrated that a vitreous injection of plasmin combined with SF_6 could induce PVD without vitrectomy in rabbit eyes, and also demonstrated that a vitreous injection of plasmin alone or SF_6 alone could not induce PVD. A phase II clinical trial of plasmin, which is isolated from the patient's own serum for use during vitrectomy, is currently being organized in United States.[15] Although the present study has limitations because we used an animal model, if intravitreous gas injection is substituted for vitrectomy, the technique would be simpler, cheaper, and less invasive. A clinical trial is necessary to confirm the efficacy of this technique.

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Fig. 1. Scanning electron micrograph of eyes with both plasmin and SF_6 injection (group I). The retinal surface is smooth, which is consistent with a bare internal limiting membrane.

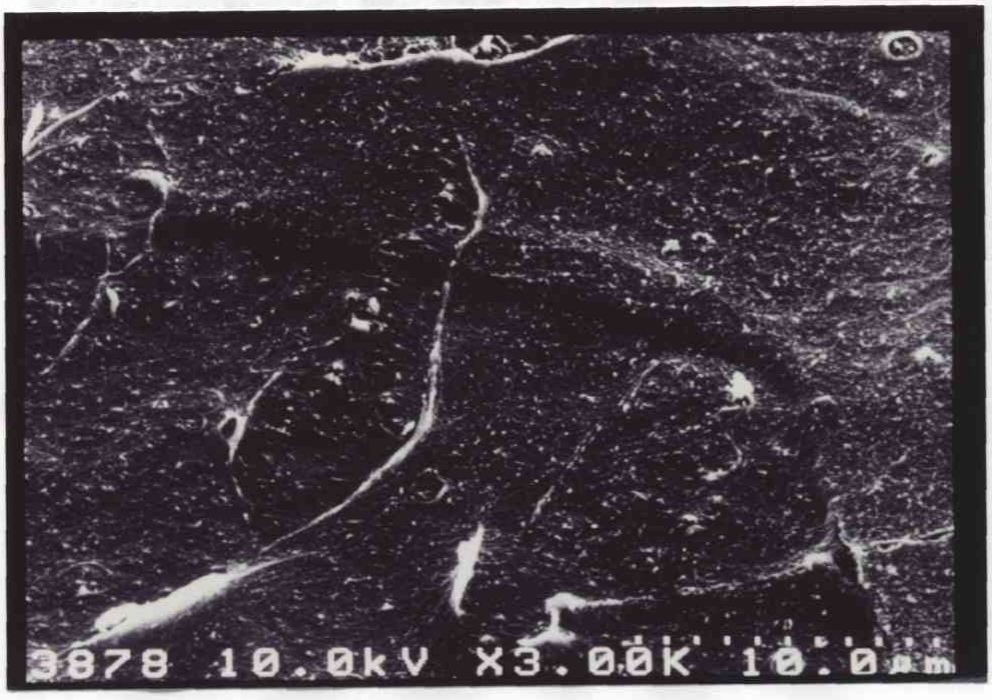

Fig. 2. Scanning electron micrograph of eyes with plasmin injection alone (group II). The retinal surface appears to be covered with sparse collagen fibers.

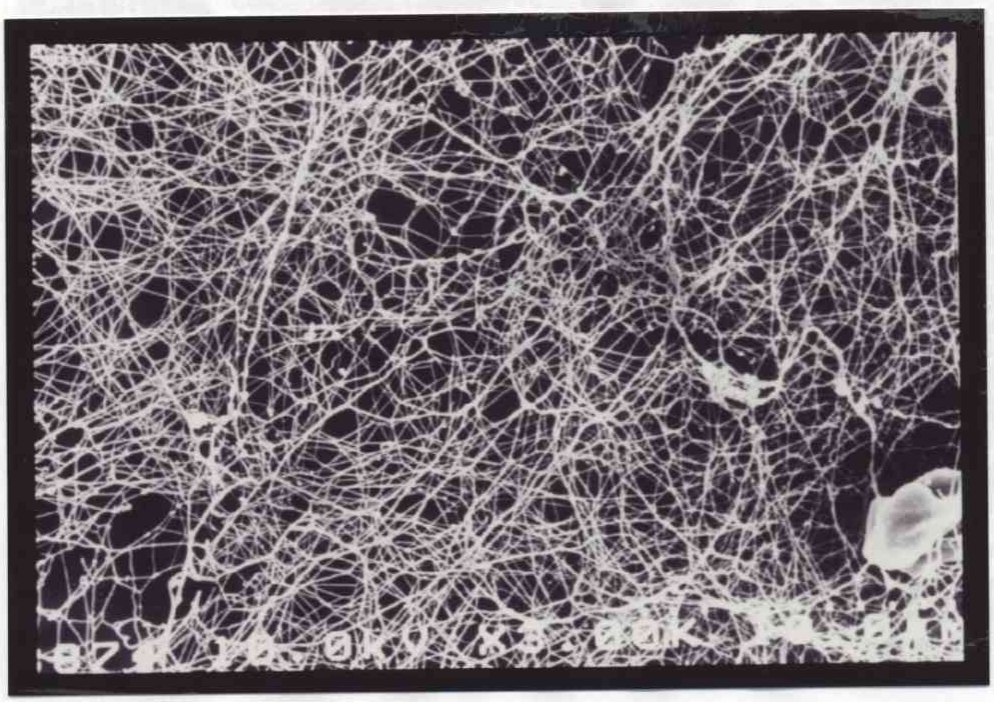

Fig. 3. Scanning electron micrograph of eyes with plasmin injection alone (group III). The retinal surface appears to be covered with sparse collagen fibers.