

DISPASE FACILITATES POSTERIOR VITREOUS DETACHMENT DURING VITRECTOMY IN YOUNG PIGS

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Purpose: To evaluate the role of intravitreal dispase in conjunction with pars plana vitrectomy to facilitate the creation of a posterior vitreous detachment (PVD) in young pig eyes.

Methods: Twenty-four eyes of 24 animals were randomized to receive an intravitreal injection of dispase (50 μ g/0.05 mL) or phosphate buffered saline (PBS) immediately after core vitrectomy and before attempted creation of a posterior cortical vitreous detachment. Following a 15-minute waiting period, surgical creation of a posterior vitreous separation was attempted by aspiration of the posterior vitreous immediately adjacent to the optic disk. Eyes were evaluated postoperatively by clinical examination (1, 4, and 8 weeks) and electroretinography (4 and 8 weeks), after which they were enucleated for light, scanning, and transmission electron microscopy.

Results: Based on intraoperative findings and postoperative scanning electron microscopy, eyes receiving intravitreal dispase exhibited a higher incidence of PVD compared to eyes receiving PBS ($P = 0.029$). Electroretinographic responses recorded at postoperative weeks 4 and 8 were similar in both dispase and PBS eyes compared to the unoperated fellow eyes. Clinical examinations, including indirect ophthalmoscopy, were indistinguishable between the PBS eyes and 11 of 12 eyes in the dispase group. Light and transmission electron microscopy demonstrated no differences in the retina between the dispase eyes and the PBS operated controls.

Conclusion: Dispase is a useful adjunct in facilitating surgical creation of a PVD in young pig eyes.

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Whereas the precise nature of the vitreoretinal adhesion is not yet fully understood, the atraumatic cleavage of this adhesion is nevertheless important in the management of numerous vitreoretinal diseases. The vitreoretinal adhesion is determined, at

least in part, by an extracellular matrix “glue” attaching the collagen fibrils of the cortical vitreous to the internal limiting lamina (ILL) adjacent to the Müller cell pedicles on the inner surface of the retina.¹ The ILL is composed of proteoglycans, fibronectin, laminin, and collagen types I and IV.² It has been suggested that this extracellular matrix, especially fibronectin and laminin, is important for stabilization of the vitreoretinal attachment.^{3,4}

The evaluation of various pharmacologic adjuncts, either alone or in conjunction with pars plana vitrectomy, to help with vitreoretinal separation has been

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the subject of numerous previous studies.⁵⁻⁷ In 1993, work in rabbits suggested that the intravitreal injection of plasmin followed by vitrectomy was effective in producing a retinal surface devoid of cortical vitreous.⁸ Chondroitinase was subsequently shown to be a helpful adjunct to vitrectomy in monkeys, allowing a complete separation of the vitreous cortex from the ILL.⁹ More recently, there have been contradictory reports regarding the efficacy of pharmacologic vitreolysis in rabbits using intravitreal hyaluronidase.^{10,11}

Dispase is a metalloenzyme known to be a potent fibronectinase and type IV collagenase obtained from *Bacillus polymyxa*. Dispase has been used to cleave the basal membrane of different tissues such as skin and corneal epithelium.¹² This enzyme has been shown to have a specific action on fibronectin and collagen type IV, components of inner limiting lamina that help mediate the adhesion of vitreoretinal interface.¹³

Tezel et al¹⁴ in 1998 showed partial or complete posterior vitreous separation in postmortem adult domestic pig and human eyes incubated with different concentrations of dispase. However, the presence and extent of posterior vitreous detachment (PVD) was only evaluated by gross examination and observation of the adherence of whole vitreous after tilting the open eye cup for a limited time to avoid the confounding factor of the postmortem release of proteinases. Kang et al¹⁵ in 1998 also demonstrated that intravitreal dispase is effective in producing posterior vitreous separation in rabbits, but safety was not assessed.

We chose young pigs for our experimental model because of the anatomic similarity of the pig eye to the human eye and the strong attachment of the pig vitreous to the retina.¹⁶⁻¹⁸ The purpose of this study is to evaluate the potential efficacy and safety of intravitreal dispase-assisted vitrectomy in facilitating separation of the posterior cortical vitreous from the retinal surface in the young pig.

Methods

Preparation of Dispase

Dispase (Gibco, Grand Island, NY) was prepared under sterile conditions one day before surgery by diluting it with sterile phosphate buffered saline (PBS) to 50 μ g/0.05 mL (pH = 7.4). The dispase was then filtered and stored at -5 degrees Celsius.

Animals

Twenty-four *Sus scrofa* swine aged 3 to 7 weeks were treated in accordance with the animal resolution of the Association for Research in Vision and Oph-

thalmology. All pigs were randomly assigned to either the dispase or the PBS groups. The dispase group consisted of 12 animals that received 50 μ g (0.05 mL) of dispase into the midvitreal cavity of one eye immediately after core vitrectomy, followed by a 15-minute waiting period, then active aspiration of the cortical vitreous adjacent to the optic nerve head. The PBS group consisted of 12 animals that received 0.05 mL of calcium and magnesium free PBS into the midvitreal cavity of one eye immediately after core vitrectomy, followed by a 15-minute waiting period, then active aspiration of the cortical vitreous adjacent to the optic nerve head.

Procedure

We induced general anesthesia in the pigs with a 2-3 cc intramuscular injection of a 1:1 combination of xylazine (TranquiVed, Vedco, St. Joseph, MO, 100 mg/mL) and ketamine hydrochloride (KetaVed, Phoenix Scientific, St. Joseph, MO, 100 mg/mL). If additional anesthesia was needed during the procedure, 1 cc of intramuscular ketamine was used. For pupil dilatation we used cyclopentolate hydrochloride 1% (AK-Pentolate 1%, Akorn, Abita Springs, LA) and phenylephrine hydrochloride 10% (AK-Dilate, Akorn). The conjunctival sac was irrigated with 5% povidone iodine solution.

All animals were initially examined with indirect ophthalmoscopy to exclude any preexisting vitreoretinal abnormalities. The nictitating membrane was removed to facilitate instrument access to the sclerotomies. We created standard three-port pars plana vitrectomy sclerotomies into which we placed 19-gauge cannulas (Grieshaber, Fort Worth, TX) to protect the vitreous base. A core vitrectomy with a 30-degree fundus contact lens was performed utilizing a Daisy (Storz, St. Louis, MO) vitrectomy unit to remove the axial vitreous gel.

The sclerotomies were plugged, the infusion was turned off, and each study eye was randomized to receive either 0.05 cc of PBS solution or 0.05 cc of dispase (50 μ g) injected via a 30-gauge needle into the midvitreal cavity through the pars plana. The surgeons were masked as to the material injected. After each injection, an indirect ophthalmoscopic examination was performed to look for possible bleeding or trauma related to needle contact. After 15 minutes, active aspiration with the vitrector tip using a maximum aspiration of 100 mmHg was applied at the edge of the optic disk to induce a mechanical detachment of the posterior vitreous cortex.

Following attempted vitreous cortex peeling, additional vitrectomy was performed to remove any of the

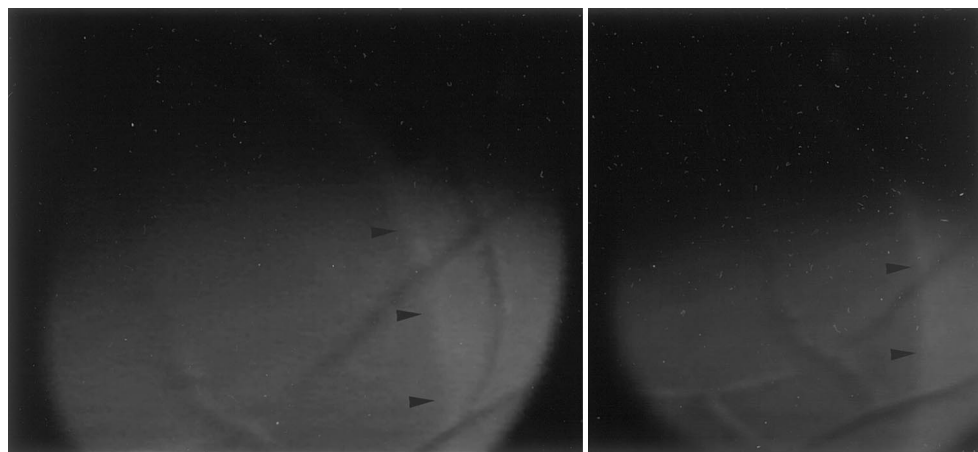


Fig. 1. Photographs of a dispase-treated pig eye undergoing pars plana vitrectomy during active aspiration of posterior vitreous cortex. A circumlinear line (arrowheads) can be seen on the retinal surface corresponding to the boundary between attached and detached posterior vitreous cortex.

elevated cortical vitreous and to wash out any residual drug. The sclerotomies and conjunctiva were sutured with 7/0 Vicryl and gentamycin (20 mg) was injected into the subconjunctival space.

Classification of PVD

Based on intraoperative observations during active aspiration of the cortical vitreous, PVD was classified as follows: Total PVD—Identification of a continuous, crescent-shaped line on the retinal surface that moved anteriorly from the edge of the disk (Figure 1) in all four quadrants as the cortical vitreous was aspirated and/or when an unequivocal Weiss ring was created; Partial PVD—Identification of a continuous, crescent-shaped line in less than four quadrants and when stretching of the vitreous was observed with aspiration over the retinal surface where no such line was observed; No PVD—No identification of a line on the retina surface in any quadrant and when stretching of the vitreous with aspiration was observed over the entire retinal surface. In eyes where the clinical findings during attempted posterior vitreous separation were difficult to interpret, scanning electron microscopy (SEM) was performed to determine the degree of any vitreous detachment and these eyes were subsequently classified into one of the three defined groups cited above.

Postoperative Evaluation

In the dispase group the animals ($n = 12$) were followed for up to 8 weeks postoperatively (12 at 1 week, 9 at 4 weeks, and 4 at 8 weeks). Animals were euthanized at 1, 4, and 8 weeks for histologic evaluation of toxicity. In the PBS group, 4 out of 12 animals were evaluated during the same periods as the dispase eyes (4 at 1 week, 2 at 4 weeks, and 1 at 8 weeks) and

the other 8 animals were evaluated only for the extent of PVD and killed immediately after surgery. Evaluation consisted of slit lamp microscopy and indirect ophthalmoscopy (at 1, 4, and 8 weeks), electroretinography (at 4 and 8 weeks), and histopathologic examination of the retina by light microscopy. In selected eyes SEM and/or transmission electron microscopy (TEM) were performed to examine retina and vitreo-retinal interface.

Electrophysiology

Before each electroretinograph (ERG) the animals were dark adapted for 1 hour. Under anesthesia, the ERG recordings were made using an UTAS-2000 (LKC Technologies, Gaithersburg, MD). Full-field electroretinograms were recorded from both eyes of each animal using a computer-based system with the Jet contact lens electrodes. Two ERG responses were elicited: dim blue scotopic (average of 40 responses) and single flash photopic (average of 20 responses).¹⁹

Clinical Examination

After ERG testing, each animal was examined clinically with slit lamp biomicroscopy and indirect ophthalmoscopy. A Genesis model camera manufactured by Kowa (Nagoya, Japan) was used for taking anterior segment and fundus photographs of each eye.

Based on the classification used in the Endophthalmitis Vitrectomy Study,²⁰ we graded media clarity in the pig eyes as follows: Grade 0, clear view; Grade 1, impaired view, with second-order vessels visible; Grade 2, inability to see second-order vessels, but with some retina vessels visible; Grade 3, inability to see retinal vessels, but with red reflex; Grade 4, no red reflex.

Histologic Examination

The animals were killed with intracardiac sodium pentobarbital. After enucleation, the eyes underwent immediate sharp razor penetration in the superior pole subjacent to the pars plana to ensure rapid penetration of fixative. Care was taken to avoid damage to the adjacent retina and lens. The eyes remained immersed in 2% paraformaldehyde plus 2.5% glutaraldehyde (0.1 mol/L phosphate buffer and pH 7.4) for a minimum of 24 hours at 4 degrees Celsius.

For light microscopy, the globe was embedded in paraffin and 5- μ m-thick horizontal sections were made through the optic nerve head and stained with hematoxylin and eosin. For SEM, a unique posterior calotte (including part of each quadrant of the eye) was removed, dehydrated in ethanol, dried in carbon dioxide liquid to the critical point, sputter-coated in gold, and photographed using the JSM 6,400 scanning microscope (Jeol, Peabody, MA). For TEM (1,200 Ex, Jeol), the tissue was dehydrated in an ethanol series, exchanged with propylene oxide, infiltrated, and embedded in Spurr embedding medium. The sections (70 nm) were cut with a DDK diamond knife on a Reichert Ultracut S microtome and mounted on slotted grids (Electron Microscopy Sciences, Fort Washington, PA) with supporting Formvar (SPI, West Chester, PA) film. Sections were stained for 15 minutes with KMnO₄ and for 5 minutes with Sato's lead stain.

Statistical Analyses

Data on the incidence of PVD were analyzed using the exact two-sided Wilcoxon–Mann–Whitney test using Statxact-4 for Windows (CYTEL Software Corp., Cambridge, MA). A confidence level of $P < 0.05$ was considered statistically significant.

Results

No intraoperative complications, including hemorrhage or retinal tears, were encountered during attempted peeling of the posterior vitreous cortex. Based on clinical observation during surgery, posterior vitreous separation was classified as follows: in the dispase group ($n = 12$), seven eyes were noted to have a total PVD, none had a partial PVD, none had no PVD, and five were unable to be clinically graded. In the PBS group ($n = 12$), none were graded as total PVD, five were found to have a partial PVD, three had no PVD, and four were unable to be clinically graded. The nine eyes (five in the dispase group and four from the PBS group) in which intraoperative clinical classification of the extent of posterior vitreous separation was not clear were processed for SEM to determine

Table 1. Incidence of PVD, Surgical and Scanning Electron Microscope Classification

PVD	Dispase Group ($n = 12$)	PBS Group ($n = 12$)
Total	7	2
Partial	5	7
None	0	3

PVD, posterior vitreous detachment; PBS, phosphate buffered saline.

the state of posterior vitreous cortex in different areas of the retinal surface.

After SEM analysis, the overall incidence of total PVD in the dispase group was higher (7/12) compared with the PBS group (2/12). The incidence of partial PVD was 5 out of 12 in the dispase group compared to 7 of 12 in eyes receiving PBS. There were no dispase eyes and three PBS eyes in which it was not possible to detach the posterior vitreous cortex at all. There was a statistically significant increase in the incidence of PVD in dispase eyes compared to the PBS controls ($P = 0.029$) (Table 1).

In the dispase group ($n = 12$), indirect ophthalmoscopy performed at postoperative week 1 demonstrated seven eyes with the media totally clear (G0) and with no visible retinal pathology. Two eyes had a mild vitreous haze (G1), one eye had a moderate vitreous haze (G2), and in two eyes there was only a red reflex (G3). Of those five animals with some grade of media haziness 1 week after surgery, two animals were killed at 1 week (G1 and G2) and each demonstrated normal retinal histology. Two other animals spontaneously cleared by week 4 and also demonstrated no histologic evidence of retinal damage. One animal still had a few areas of residual hemorrhagic vitreous clots as well as some segmental vessel sheathing in one quadrant when examined at week 4.

In the PBS group, four animals were evaluated at postoperative week 1. Two of these eyes had a completely clear vitreous (G0) and the other two had mild vitreous haze only (G1). One animal (G1) was killed for histology at postoperative week 1 and showed a normal retinal histology. By 4 weeks, three of the four animals had a completely clear vitreous and no evidence of visible retinal pathology.

A cataract was visible on slit lamp examination in 2 out of 12 eyes (diagnosed at postoperative week 1) in the dispase group and in 1 out of 4 eyes (diagnosed at postoperative week 8) in the PBS group. All of the eyes with lens opacities demonstrated a localized posterior subcapsular cataract that did not impair the fundus view. In the dispase group, we found two eyes with a mild, diffuse, subepithelial corneal haze,

Scotopic Response



Photopic Response

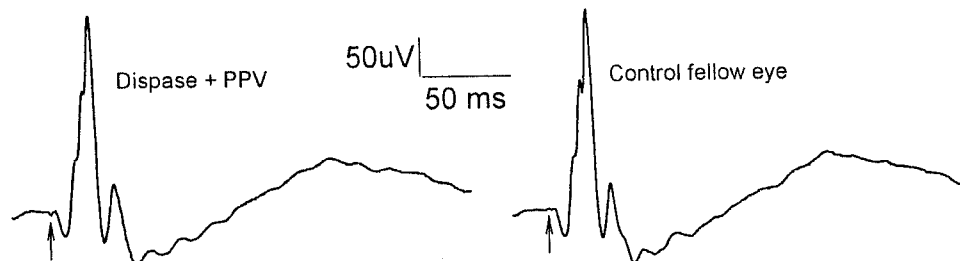


Fig. 2. Comparison of dim-blue scotopic responses (top) and single flash photopic responses (bottom) recorded at postoperative week 8 between dispase eyes and unoperated fellow eye controls. No significant differences were seen in the waveforms between the operated and unoperated fellow eyes at either 4 (data not shown) or 8 weeks of follow-up. PPV, pars plana vitrectomy.

whereas no corneal changes were seen in eyes of the PBS animals. A similar mild corneal haze is commonly found after vitrectomy in pigs without any injected drug (personal observation). Removal of the nictitating membrane (an external ocular membrane responsible for lubrication, occlusion, and humidification of the ocular surface) to improve surgical access is the likely cause of this keratopathy.

There were no significant differences in ERG patterns between the dispase and the PBS eyes or between the operated and unoperated fellow eyes within each group. The scotopic and photopic b-wave amplitudes of both the dispase and PBS eyes were comparable to those of the unoperated fellow eyes (Figure 2).

Light microscopy demonstrated normal retinal histology in both the dispase and the PBS eyes and this was indistinguishable from the unoperated control eyes. In one dispase eye (the same eye noted to have a persistent vitreous haze) there was agglutination of inflammatory cells over the optic nerve head that corresponded to a hemorrhagic/fibrin clot seen on clinical examination. No alterations of histology around the vessel walls were found and there were no indications of intraretinal hemorrhage, disorganization, or necrosis of the retina.

Scanning electron microscopy (Figure 3) of the inner retinal surface from the dispase and PBS eyes demonstrated the imprint of Müller cell footplates under the ILL in the areas where an intraoperative posterior vitreous separation was observed. The inner retinal surface had a fairly smooth undulating surface without collagen fibrils, suggesting a bare ILL. In

areas where no posterior vitreous separation was seen intraoperatively, there was a persistence of collagen fibrils consistent with attached cortical vitreous.

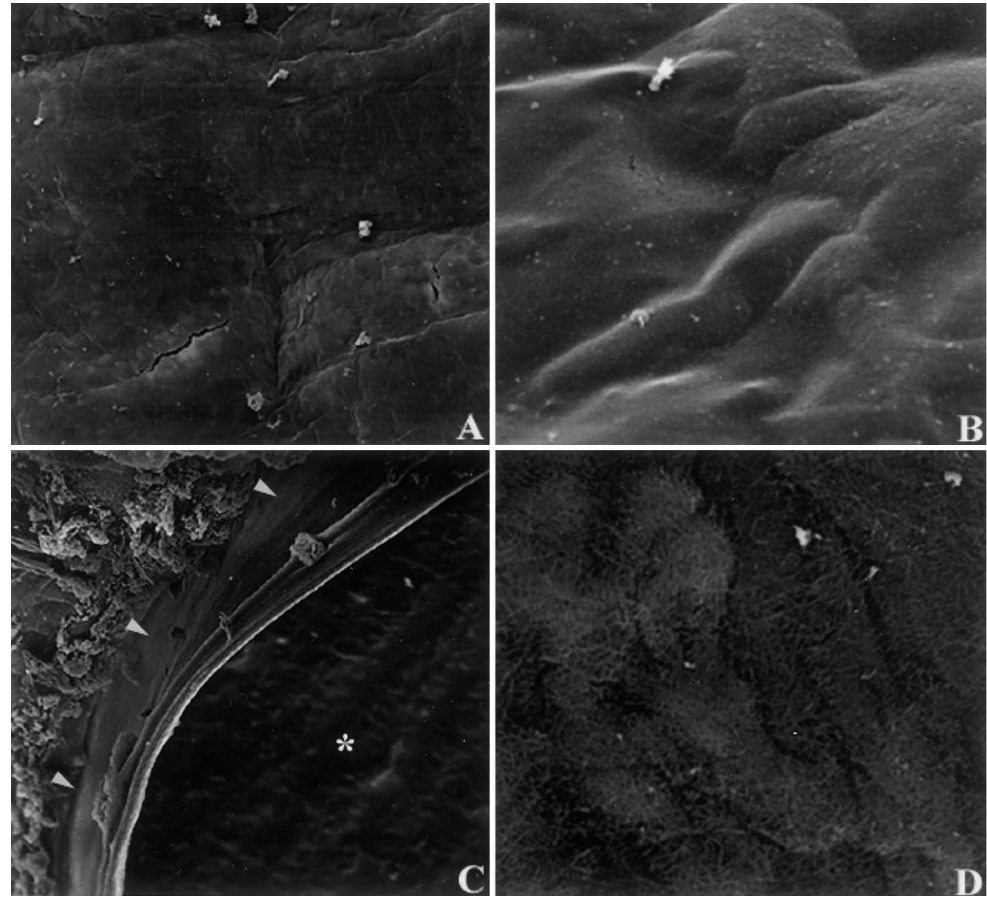
Transmission electron microscopy (Figure 4) demonstrated rare collagen fibers adjacent to the ILL in areas where an intraoperative PVD was accomplished. In contrast, in retinal areas of eyes where the vitreous could not be separated intraoperatively, condensed collagen fibers were found to insert into the ILL. The ILL was intact with a normal appearance in both the dispase and the PBS eyes. In operated and unoperated fellow eyes, the overall retinal morphology was well preserved and similar. Scattered vacuoles in the nerve fiber layer, ganglion cell layer, and the inner and outer segments, believed to be artifacts caused by the fixative process, were seen in both surgical groups as well as in unoperated eyes.

Discussion

Posterior cortical vitreous is very adherent to the inner retina in young porcine eyes. Intravitreal dispase (50 μ g in 0.05 cc), in conjunction with pars plana vitrectomy, facilitated the surgical creation of a PVD relative to PBS controls in these young pig eyes. A complete PVD was successfully created in 7 of 12 pig eyes with dispase compared to 2 of 12 eyes receiving PBS. No posterior vitreous separation at all was observed in no Dispace eyes and in 3 of 12 PBS eyes.

Dispace appeared nontoxic to the retina at the dose and time course utilized in this study in 11 of the 12 eyes. Both dispase and PBS eyes demonstrated some

Fig. 3. **A, B,** Scanning electron photomicrographs of the inner retina surface from which the posterior vitreous cortex was completely detached during dispase-assisted vitrectomy (original magnification **A**, $\times 350$). **B,** The retinal surface appears completely devoid of vitreous collagen fibrils and presents the smooth undulating surface of the internal limiting lamina overlying Müller cell footplates (original magnification $\times 3,500$). **C,** Scanning electron microscopy of a partial posterior vitreous detachment (PVD) in an eye of the control group. Note residual cortical vitreous attachment (arrowheads) on part of retinal surface adjacent to bare internal limiting lamina (asterisk) (original magnification $\times 350$). **D,** A region where cortical vitreous attachment to the retina persists in an eye operated with dispase where a complete PVD could not be created (original magnification $\times 3,500$).



transient vitreous inflammation postoperatively. The one eye that showed a persistent inflammatory reaction in the vitreous at the end of follow-up was the eye that exhibited considerable vitreous hemorrhage related to closing of the sclerotomy. That eye had only a red reflex at 1 week and by 4 weeks it still had numerous small hemorrhagic/fibrin clots in the vitreous as well as some localized retinal vascular sheathing. Despite these ophthalmoscopic findings, no ERG differences were present between this dispase eye and its untreated fellow eye, nor were there any other retinal morphologic alterations or signs of inflammatory infiltration, hemorrhage, or disorganization of the retinal structure.

A previous study has suggested that intravitreal dispase can be toxic to the retina. Frenzel et al²¹ produced retinal detachment with proliferative vitreoretinopathy in rabbits with intravitreal injection of 0.05 IU (approximately 70 μg) over a 10-week follow-up. We have tested different approaches with different doses of dispase (unreported data) and found that with higher doses or longer exposure times we observed evidence of dispase toxicity including scattered intraretinal hemorrhages and lens subluxation.

We found that an intravitreal dose of 50 μg given after core vitrectomy and then washed out of the vitreous cavity after 15 minutes appeared safe.

Our clinical impression as to whether we were able to create a posterior vitreous separation was confirmed by SEM. In areas where we believed that we had successfully separated the posterior vitreous cortex, we observed the retina surface to be fairly smooth, to be devoid of collagen fibrils, and to have assumed an undulating pattern corresponding to the pedicle of Müller cells covered by the ILL. In contrast, in areas where we believed that the cortical vitreous remained attached on the basis of intraoperative observations, a prominent collagen meshwork over the inner retinal surface was present on SEM.

By TEM, the normal anatomy of the ILL was confirmed to be preserved both in dispase and in PBS eyes. The overall retinal morphology was comparable in operated eyes (dispase or PBS) and the unoperated fellow eyes. The presence of scattered vacuoles was observed in some part of the retina in both operated and nonoperated fellow eyes. Similar findings have been previously described as a fixation artifact in

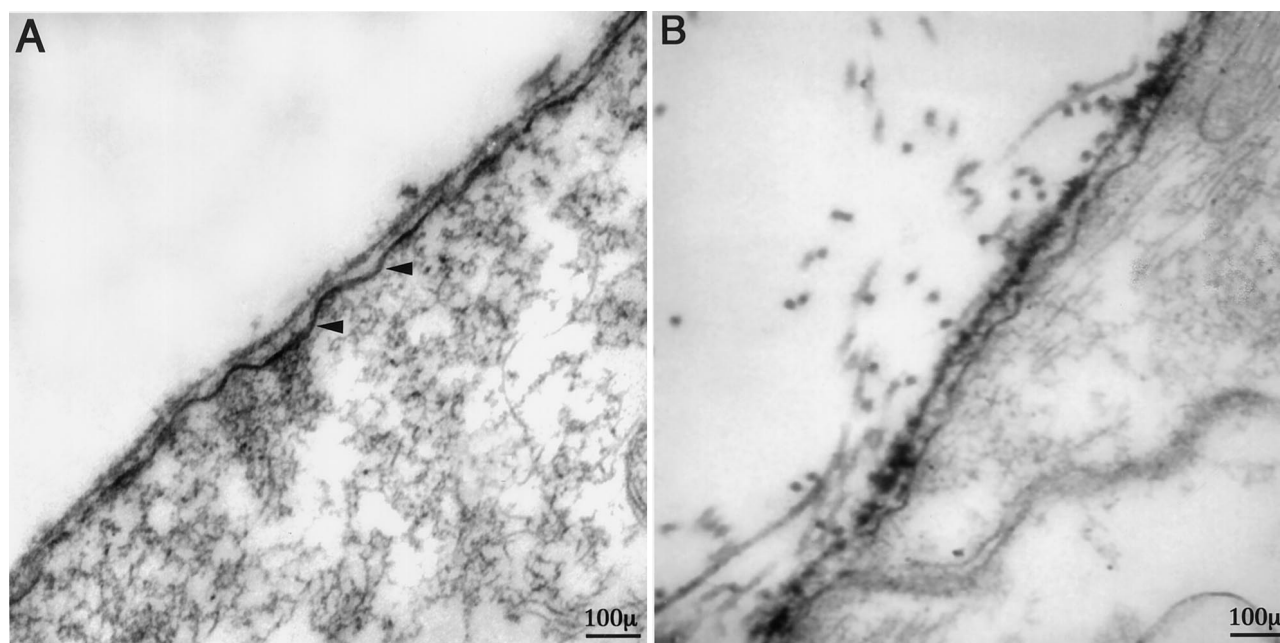


Fig. 4. **A**, Electromicrograph of a porcine inner retina after surgical creation of a posterior vitreous detachment in an eye receiving 50 μ g/0.05 mL of dispase during vitrectomy. Internal limiting lamina (ILL) (arrowheads) is noted to be present and attached over the inner retina surface. There are a few remnants of collagen fibrils attached to the retina (original magnification $\times 12,000$). **B**, Electromicrograph of a pig eye undergoing vitrectomy with phosphate buffered saline in which partial vitreous detachment was obtained. A segment of inner retina where the vitreous remained attached is included and demonstrates adjacent collagen fibrils remaining attached to the ILL (original magnification $\times 12,000$).

rabbit retina and we believe that our similar findings in the pig represent the same phenomenon.^{22,23}

The potential benefits of an enzymatic assisted procedure to allow easier and less traumatic separation of adherent cortical vitreous from the retina surface have led to a number of studies seeking to identify the best potential adjunctive agents. Moorhead et al postulated that bacterial collagenase in conjunction with vitrectomy in rabbits might be capable of digesting a laboratory induced vitreal/epiretinal scar tissue (cicatricial collagen), but toxicity was a noted problem.^{24,25} Collagenase seems to have lower specificity for vitreous and basal membrane collagen (which plays an important role in the vitreoretinal adhesion) as opposed to cicatricial collagen.⁵

Verstraeten et al⁸ in 1993 proved that plasmin could be useful as adjunct to mechanical vitrectomy in rabbit eyes and Margherio et al²⁶ showed that autologous plasmin-assisted vitrectomy facilitated the removal of cortical vitreous from the retina surface in four pediatric patients with traumatic macular hole. A multicentered, prospective clinical trial of plasmin to facilitate posterior vitreous separation is being planned (M. Trese, personal communication, 2001). Autologous plasmin is a biologic substrate made up from the patient's own blood and is currently not widely available. Unlike autologous plasmin, dispase is commercially available, inexpensive, and easy to prepare.

Hageman and Russell⁹ in 1994 demonstrated that chondroitinase—the enzyme that lyses chondroitin sulfate—in combination with vitrectomy produces disinsertion of the vitreous in monkeys. A Phase I clinical trial in humans was performed but no results of this have been published nor has a Phase II study been initiated.

A multicentered prospective clinical trial by Le Mer et al²⁷ in 1999 demonstrated that 25 μ g of intravitreal tissue plasminogen activator injection 15 minutes before vitrectomy in eyes with proliferative diabetic retinopathy did not improve the final visual acuity or decrease the incidence of retinal tears during membrane removal compared to the control group that underwent vitrectomy alone. In addition, Hrach et al²⁸ observed clinical, electrophysiologic, and histologic alterations in cat eyes receiving intravitreal doses greater than or equal to 50 μ g.

Another enzyme that has been broadly studied is hyaluronidase, which degrades hyaluronic acid, decreasing vitreous viscosity and leading to vitreous liquefaction.^{10,29} However, other studies have concluded that hyaluronidase is not able to produce a PVD in the rabbit.^{11,30}

Our results demonstrate that intravitreal dispase, in combination with pars plana vitrectomy, has the potential to safely and effectively facilitate the separa-

tion of the posterior cortical vitreous from the inner retinal surface in this experimental model.

Key words: dispase, inner limiting lamina, pharmacologic vitreolysis, posterior vitreous detachment, vitrectomy, vitreoretinal interface, vitreous.

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