A New Model of Proliferative Vitreoretinopathy

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PURPOSE. To design a new model of proliferative vitreoretinopathy (PVR) that would not rely on the addition of exogenous cells. The release of endogenous cells from surrounding attachments seems to be an early event in the pathogenesis of PVR. Because the proteolytic enzyme dispase dissociates tissues, the hypothesis was that an intraocular injection of dispase could trigger events that would cause PVR. The requirement for a surgical retinal break at the time of dispase injection was also examined.

METHODS. One eye of Dutch Belted rabbits was injected with 0.003 U to 1.0 U dispase in the subretinal space or vitreous cavity. Control rabbits received a saline injection. An intentional retinal tear was created in animals in some groups. Observations were made for at least 10 weeks after surgery.

RESULTS. Proliferative vitreoretinopathy developed in response to subretinal or intravitreal dispase, with or without creation of a controlled retinal break. Increased severity of PVR correlated with increasing doses of dispase. Evidence of PVR included preretinal membranes, distortion of myelin wings and retinal vessels, fixed retinal folds, and traction retinal detachment. Proliferative vitreoretinopathy did not develop in saline-treated control animals.

CONCLUSIONS. Dispase initiated the development of PVR without the addition of exogenous cells, growth factors, or cytokines typically found in PVR membranes. A cascade of events was probably triggered by dispase, causing native cells and factors to produce PVR. The dispase model of PVR was technically easy to perform, permitted a clear view of the retina, and had a high success rate in development of PVR.

Proliferative vitreoretinopathy (PVR), the principal cause of failed retinal reattachment surgery, is characterized by the migration and proliferation of cells that form cellular membranes in the vitreous and on the retina. Cell types identified in PVR membranes include retinal pigment epithelial (RPE) cells, glial cells, fibroblasts, and inflammatory cells. Extracellular matrix (ECM) molecules, including fibronectin; collagen types I, II, III, and IV; and laminin, have been detected in epiretinal membranes. Contraction of the cellular membranes causes tractional detachment of the retina with loss of vision as the detachment progresses.

The visual prognosis for patients with PVR is markedly diminished once significant PVR is established. Current surgical treatments for PVR include removal of epiretinal membrane by vitrectomy and reduction of transvitreous traction by scleral buckling. The clinical use of pharmacologic agents has had limited success because of toxic effects. The difficulty of maintaining satisfactory vision in PVR patients with the current therapies indicates the critical need for alternative forms of therapy.

Various in vivo models of PVR have been designed to study pathogenesis and therapy. Numerous models have relied on the addition of cells or factors reported to be found in PVR and often include other manipulations such as lensectomy, vitrectomy, or both. Rabbit models that involve the intravitreal injection of fibroblasts or RPE cells have been widely used. The time course of membrane formation and retinal detachment depends on the type and number of cells introduced. Other models include the intravitreal injection of platelets combined with additional components. A noncellular model of PVR involving the injection of platelet-derived growth factor and fibronectin also included vitreous extrusion, a potential source of traction on the retina.

Our goal was to design a new model of PVR in which pathogenesis was caused by endogenous cells and factors. The proteolytic enzyme dispase was used to create a microenvironment in the vitreous cavity within which contractile membranes and retinal detachments resembling PVR in humans formed. Dispase is a nonspecific, neutral metalloproteinase from Bacillus polymyxa that frees cells from surrounding matrix. It is commonly used for cell harvesting and tissue dissociation. We examined the dependence of dispase-induced PVR on the creation of intentional retinal tears that exposed RPE cells, disrupted the vitreoretinal interface, and provided RPE and/or retinal cells direct physical access to the vitreous cavity. We report in this study that PVR developed in rabbit eyes following the introduction of dispase into either of two sites in the eye, with or without the formation of an intentional tear in the retina.

METHODS

One eye only of Dutch Belted rabbits (1-2 kg; Birchwood Farms, Red Wing, MN) was used. Rabbits were anesthetized with 35 mg/kg ketamine (Phoenix, St. Joseph, MO) and 7.0 mg/kg xylazine (Miles, Shawnee Mission, KS). Topical anesthesia, 0.5% proparacaine (Alcon, Humacao, PR), was applied as well. Pupils were dilated with 1% tropicamide and 2.5% phen-
A 27/30 gauge needle was inserted through the sclera approximately 3 mm posterior to the limbus. A beveled 30–33-gauge cannula was inserted into the subretinal space at the inferior edge of the optic disc. In experimental animals, 0.01 ml to 0.02 ml dispase solution (0.3–10 U/ml) was injected into the subretinal space to form a localized retinal detachment 1 to 3 disc diameters (dd) in size. In control animals, the same volume of saline was injected in the same way. Optionally, 1 hour later, a controlled retinal tear (2–3 dd) was made in the area of the dispase bleb.

**Subretinal Dispase with or without Retinal Tear**

A sclerotomy was performed approximately 3 mm posterior to the limbus. A beveled 30–33-gauge cannula was inserted into the subretinal space at the inferior edge of the optic disc. In experimental animals, 0.01 ml to 0.02 ml dispase solution (0.3–10 U/ml) was injected into the subretinal space to form a localized retinal detachment 1 to 3 disc diameters (dd) in size. In control animals, the same volume of saline was injected in the same way. Optionally, 1 hour later, a controlled retinal tear (2–3 dd) was made in the area of the dispase bleb.

**Intravitreal Dispase and Endodiathermy**

A sclerotomy was performed as described. Using a tapered-tip endodiathermy probe (Storz, St. Louis, MO) set at 12%, 9 to 12 retinal breaks, approximately 1 dd apart, were created in the inferior retina. Dispase solution (0.06–1.0 U/0.1 ml) was injected over the optic disc or wings using a 27/30 gauge needle.

**Intravitreal Dispase**

A 27/30 gauge needle was inserted through the sclera approximately 3 mm posterior to the limbus. Dispase solution (0.01–0.5 U per 0.01–0.1 ml) or saline in control animals was injected over the optic disc and/or wings.

**Follow-up**

Clinical examinations were performed and/or fundus photographs were taken on approximately postoperative days 1 and 7 and then every other week, typically through postoperative week 10. Observations were made regarding anterior segment inflammation; clarity of the cornea and lens; pupillary dilation; vascular tortuosity and dilation; hemorrhage; distortion of medullary wings; presence of intravitreal haze and membranes; and formation of retinal breaks, traction, and detachments. Proliferative vitreoretinopathy grading was based on a modification of Fastenberg et al.3 and on qualitative observations.

**Histopathology**

At the conclusion of an experiment, selected animals were perfused with 4% paraformaldehyde. Enucleated eyes were embedded in paraffin and sectioned for light microscopy. Histologic examination was performed after hematoxylin and eosin staining.

**RESULTS**

**Subretinal Dispase**

The ability of dispase to disrupt cell interactions suggested its use as an agent for inducing PVR. Because RPE cells have been found in epiretinal membranes, the disruption of RPE cell interactions was possibly important in the production of PVR. Consequently, dispase was injected into the subretinal space, and a controlled retinal break was made in some rabbits 1 hour after the injection of dispase.

Fourteen rabbits received a subretinal injection of 0.003 U to 0.2 U dispase, producing a localized retinal detachment, termed a bleb. Subsequently, in 11 rabbits a retinal tear was made at the apex of the bleb, whereas in 3 rabbits no further manipulations were performed. Rabbits that received a subretinal injection of saline plus an intentional tear served as controls (n = 3). In all rabbits, the localized retinal detachment was discernible for several days (Fig. 1A). However, by postoperative days 7 through 14, the retina was reattached. Also, in 13 of 14 experimental rabbits and in 2 of 3 control rabbits, hemorrhage, most often preretal, was evident on postoperative day 1. By postoperative day 7, hemorrhage diminished and was no longer evident by postoperative days 14 through 21. Vitreous haze was observed in only two rabbits, each of which received the highest dose of dispase (0.1–0.2 U). In all rabbits, the cornea, anterior chamber, and lens remained clear, and no conjunctivitis occurred. The pupil dilated widely (5–9 mm).

Proliferative vitreoretinopathy developed in 13 of 14 rabbits that received subretinal dispase (Fig. 2A). Of the 11 rabbits that also had a retinal tear, 10 developed PVR, while three of three rabbits without an intentional retinal tear developed PVR. At a dose of 0.012 U dispase or more, PVR developed in 100% of rabbits that received subretinal injection. (Fig. 2A). Overall, with increased doses of dispase, the severity of PVR increased. The fundus photographs in Figures 1A, 1B, and 1C illustrate the development and extent of PVR in a rabbit that received a subretinal injection of 0.012 U dispase and a retinal tear. Proliferative vitreoretinopathy did not develop in control rabbits, despite the intentional retinal tears (Fig. 1D).

We concluded that the introduction of dispase into the subretinal space produced PVR and that the formation of a controlled retinal break, other than the insertion site of the cannula, was unnecessary. Because hemorrhage was observed in two of three control rabbits, none of which developed PVR, we concluded that hemorrhage was not an indicator of the prospective development of PVR. However, PVR did not develop in rabbits in which no hemorrhage occurred.

**Intravitreal Dispase Plus Endodiathermy**

Because one of our goals was to design a simple procedure with minimal manipulations, we examined whether a subretinal injection of dispase was required or whether the injection of dispase at a more accessible location in the eye was sufficient to induce PVR. The intravitreal space near the optic disc and medullary wings was chosen as the injection site in subsequent experiments that also included the creation of intentional retinal breaks.

Retinal breaks were created in 16 rabbits by endodiathermy immediately followed by an injection of 0.06 U to 1.0 U dispase over the optic disc and/or medullary wings. The appearance of the endodiathermy lesions on postoperative days 1 and 2 (circular areas of whitened retina) was similar in all rabbits, regardless of the amount of dispase injected (compare Figs. 3A and 3C). Hemorrhage, most often preretal, was evident in all but 1 rabbit from postoperative day 1 until approximately postoperative day 8. In 14 of 16 rabbits, vitreous haze was a common feature until postoperative days 7 through 14. The anterior chamber remained clear, conjunctiva...
FIGURE 1. Fundus photographs of rabbits that received a subretinal injection of 0.012 U dispase plus a retinal tear (A, B, C) or of saline (D) plus a retinal tear. (A) Postoperative day 9. Rolled edges of retina were seen around the tear (star). Some vitreous haze and hemorrhage (arrow) were present. (B) Postoperative day 21. In the same rabbit, the retina was reattached at the tear (star), and a thick preretinal membrane was visible (arrow). (C) Postoperative day 105. The medullary wings were rolled and elevated (arrowbeads) in the same rabbit. Clumps of fibrous tissue were evident (stars). (D) Postoperative day 21. Site of subretinal injection is barely visible (star). No signs of proliferative vitreoretinopathy, such as vascular tortuosity, preretinal membranes, and retinal traction, were observed. d, optic disc.

Vitrus did not occur, and the pupil dilated widely in all rabbits. A posterior cortical cataract developed in two rabbits that received the highest dose of dispase (1.0 U), yet a partial view of the fundus was still possible, permitting assessment for PVR.

Proliferative vitreoretinopathy developed in 13 of 16 rabbits that received intravitreal dispase after endodiathermy (Fig. 2B). At a dose of 0.5 U to 1.0 U dispase, 90% of rabbits developed PVR. As with subretinal dispase, the severity of PVR that developed in response to endodiathermy and intravitreal dispase increased with greater doses of dispase. Although hemorrhage was a common factor in eyes in which PVR developed, PVR did not develop in all eyes with hemorrhage. The fundus photograph in Figure 3B depicts the extent of PVR on postoperative day 76 in a rabbit that received 0.5 U intravitreal dispase after endodiathermy. Proliferative vitreoretinopathy did not develop in a rabbit that received 0.06 U intravitreal dispase after endodiathermy (Fig. 3D).

Therefore, the introduction of dispase into the vitreous cavity, accompanied by the creation of retinal breaks, resulted in the development of PVR in a dose-related manner. Next, the requirement for retinal breaks with an intravitreal injection of dispase was assessed.

Intravitreal Dispace Only

Cell types other than RPE cells are found in PVR membranes, and the role of each cell type in PVR is not well defined. Therefore, to de-emphasize attempts to loosen RPE cells from surrounding attachments, experiments were performed with intravitreal dispase only, without intentional retinal breaks.

Forty-three rabbits received an injection of 0.01 U to 0.5 U dispase in the vitreous cavity over the optic disc and/or medullary wings. If obvious surgical complication occurred, such as the needle touching the retina, the animal was eliminated from the study (n = 4). Control rabbits received saline in a similar manner (n = 7). From postoperative day 1 until approximately postoperative days 14 through 21, preretinal and vitreous hemorrhages were observed in all dispase-treated rabbits but in none of the control rabbits. Vitreous haze was evident for up to 21 days in rabbits that received a dispase dose of 0.05 U or higher. The cornea and anterior chamber remained clear in all rabbits, and conjunctivitis did not occur. Pupil dilation of 7 mm to 9 mm was recorded in 93% of dispase-injected or saline-injected rabbits, with dilation of 3 mm to 6 mm recorded in the remainder of the rabbits. Cataract developed in 11
rabbits injected with dispase at a concentration equal to or higher than 0.05 U. Cataract formation was possibly caused by trauma to the lens that was not conspicuous during the injection process. Alternatively, dispase itself caused the cataract formation. Six rabbits with cataract were eliminated from further analysis, because a clear view of the fundus was not obtained. In all, 87% of rabbits injected in the vitreous cavity with dispase or saline without complication were usable.

Proliferative vitreoretinopathy developed in 27 of 33 rabbits that received intravitreal dispase (Fig. 2C). A higher occurrence and more severe PVR resulted from the intravitreal injection of higher doses of dispase. Proliferative vitreoretinopathy developed in 29% of rabbits injected with 0.01 U to 0.02 U dispase, in 94% injected with 0.05 U to 0.07 U, and 100% injected with 0.1 U to 0.5 U. Mean scores of severity were 0.6, 3.6, and 4.3, respectively. With increased dispase concentrations, the incidence of cataract formation, 0%, 25%, and 50%, respectively, also increased.

The photographs in Figures 4A and 4B illustrate the progressive development of PVR in a rabbit that received 0.05 U dispase intravitreally. Even though hemorrhage was observed in all dispase-treated rabbits, the ensuing progression to PVR varied depending on the dispase dose. Proliferative vitreoretinopathy did not develop in a rabbit that received an intravitreal dose of 0.01 U dispase (Fig. 4C), nor in rabbits that received intravitreal saline (Fig. 4D).

Histologic examination of eyes from dispase-injected rabbits revealed the presence of epiretinal cellular membranes. The photographs in Figures 5A, 5B, 5C, and 5D provide the histopathologic evidence for formation of an epiretinal membrane in an eye of a rabbit in which stage 4.5 PVR developed in response to the intravitreal injection of 0.07 U dispase. A photograph from another region of the same eye (Fig. 5E) reveals a more histopathologically normal looking retina, similar to the retina from an uninjected rabbit eye (Fig. 5F).

We concluded that an intravitreal injection of dispase alone was sufficient to trigger the reproducible development of PVR in the rabbit, and that a dose of 0.05 U to 0.07 U dispase most effectively induced PVR. Apparently, cellular activity required for the development of PVR was not impeded by the absence of an intentional retinal tear.

**DISCUSSION**

**Proliferative Vitreoretinopathy in Response to Dispase**

In this report, we describe a new in vivo model in which PVR was induced successfully when dispase was injected into the subretinal space or the vitreous cavity. Several criteria, including dose and ease of the procedure, were considered in determining optimal conditions for the model. Because with the intravitreal injection method PVR was consistently induced with 0.05 U dispase or more, epiretinal cellular membranes similar to those seen in clinical PVR were detected, the procedure was the least complex, and rabbits were anesthetized for less time, the vitreous cavity will be the site of dispase injection in our future work. Further, we concluded that an intravitreal injection of 0.05 U to 0.07 U dispase was the most effective dose for induction of PVR, because of the acceptable level of vitreous haze, pupil dilation, and cataract formation. Also, the degree of severity of PVR induced with 0.05 U to 0.07 U dispase will be useful for future research in reducing the incidence or severity of PVR.

**Exposure of RPE Cells**

We originally postulated that disruption of RPE cell attachments and/or provision of access to the vitreous cavity might be important components of the procedure for inducing PVR with dispase. However, we found that formation of retinal tears was unnecessary, because injection of dispase alone was sufficient to induce PVR. Merely forming retinal breaks did not cause PVR in rabbits in the present study or in other studies. Variable results have been reported regarding the incidence of traction retinal detachments in rabbits when procedures included other manipulations plus the formation of retinal
Figure 3. Fundus photographs of rabbits that received endodiathermy treatment plus an intravitreal injection of 0.5 U dispase (A, B) or of 0.06 U dispase (C, D). (A) Postoperative day 2. The retina was whitened near the diathermy marks (arrows) inferior to the optic disc. The diathermy marks are out of focus because of vitreous haze. (B) Postoperative day 76. A traction retinal detachment developed in the same rabbit resulting in fixed retinal folds (arrowheads). Contractile preretinal membrane (arrow), medullary wing (star). (C) Postoperative day 1. With a lower dose of dispase, the retina was also whitened near the diathermy marks (arrows). (D) Postoperative day 62. In the same rabbit, no intravitreal membranes or retinal detachments were apparent. Retinal pigment epithelial cell atrophy was visible at the sites of diathermy (arrows).

Thus, we concluded that in rabbit models direct exposure of RPE cells and mechanical disturbance of the vitreoretinal interface were unnecessary precursors for the processes that cause PVR.

Endogenous Cells in the Dispase Model of PVR

The basis of many models of PVR has been the introduction of exogenous cells typically found in PVR membranes. However, reservations about cellular models exist. The applicability of the dermal fibroblast model to the clinical situation is questionable. In experiments using RPE cells, cells must be grown in tissue culture to accumulate a sufficient number of cells, during which time many in vivo characteristics may be lost. The time course for membrane formation in response to the injection of cells can be rapid, within 1 to 2 weeks, in contrast to formation in humans, which progresses more slowly. In some cases, cells were allogeneic or xenogeneic, creating an immunologic factor not present in the clinical situation. Although PVR developed without disruption of RPE cells, it is possible that RPE cells may be part of the contractile membranes in the dispase model, because RPE cells have the ability to migrate and contract collagen gels. Further, because hemorrhage occurs in the dispase model, blood-borne components such as leukocytes and platelets may contribute to PVR.

Possible Changes in the Microenvironment of the Vitreous

The composition of the vitreous is important in the pathogenesis of PVR, and the introduction of dispase into the vitreous...
cavity apparently triggers a cascade of events that change the microenvironment and cause formation of contractile membranes. Dispase could trigger several pathways.

First, retinal hemorrhage was evident in dispase-treated rabbits, signifying breakdown of the blood-retinal barrier and release of blood-borne cytokines, growth factors, and inflammatory cells. The importance of the breakdown of the blood-retinal barrier in the pathogenesis of PVR has been suggested by several investigators. Elevated levels of cytokines and growth factors have been demonstrated in eyes of PVR patients, and cells associated with PVR have been shown to respond to cytokines and growth factors by migration, proliferation, or contraction or a combination of those effects. The extent of the breakdown of the blood-retinal barrier, rather than the mere occurrence, may be a critical factor.

The activity of dispase may be similar to that of innate matrix metalloproteinases that degrade ECM, thus permitting the movement of cells during processes of development, tissue remodeling, migration, and metastasis. Dispase degrades matrix molecules, and cells may be released from surrounding attachments by the activity of dispase. Also, fragments of ECM are known to be chemotactic and to direct migration of cells. Thus, ECM fragments generated by dispase activity may influence the behavior of nearby cells. Moreover, growth factors sequestered in the ECM may be released on the degradation of ECM by dispase.

Furthermore, several other mechanisms may be activated. A wound-healing process may be initiated including the formation of fibrin, the release of platelet-derived growth factor, and the activation of the plasminogen-plasmin system. Hyaluronic acid, a component of the vitreous, may direct cell migration into the vitreous through interaction with the ligand CD44. CD44 is known to be expressed on fibroblasts, Müller cells, and cultured RPE cells, preferentially on dividing cells. Finally, the activated cells themselves may secrete factors into the vitreous that can act in an autocrine or paracrine manner.

The complexity and variability of the pathogenesis of PVR in humans indicate that different mechanisms probably contribute to the development of PVR. Multiple pathways and interactions are probably responsible for the development of PVR in the dispase model as well.

Advantages of the Dispase Model of PVR

The injection of dispase into the vitreous cavity effectively and reproducibly induced the development of PVR. Native cells and factors were recruited to participate in the pathogenic process that led to the formation of contractile membranes. Thus, the model is not based on assumptions concerning the
Figure 5. Hematoxylin and eosin-stained sections of an eye from a rabbit that received an intravitreal injection of 0.07 U dispase (A, B, C, D, E) and of an eye from a healthy uninjected rabbit (F). (A) Posterior equatorial retina away from the region of the medullary wing. The retinal architecture has become folded (arrows) because of the presence of a contractile fibroglial membrane (stars) on the internal surface of the internal limiting membrane. (B) Higher magnification of the region illustrated in (A). The internal limiting membrane appears to be intact in this plane of the section. The epiretinal membrane is composed of cells with spindle-shaped nuclei (arrowheads) and homogeneously distributed chromatin. The cell borders are relatively indistinct but appear to be enmeshed in extracellular matrix consistent with collagen. (C) The internal contour of the retina in this region has an undulating appearance associated with a fibroglial membrane on the internal limiting membrane. The retina itself remains attached. (D) The epiretinal membrane (star) is particularly cellular in this region. The underlying retinal architecture remains relatively undisturbed. (E) This region of retina appears relatively normal with an intact internal limiting membrane and attached retina. (F) The retinal architecture is normal with no evidence of epiretinal membrane formation or retinal detachment. Magnification, (A, C) x200; (B, D, E, F) x400.
role or contribution of specific cells or factors in the development of PVR. Dispase is an inexpensive and readily available enzyme, and the intravitreal injection procedure is easy to perform and produces little trauma. The anterior chamber remained clear, and the pupil usually dilated widely. Because the lens remained free of opacity in most cases, a clear view of the fundus was obtained. Neither mechanical vitrectomy nor gas compression vitrectomy was performed, thus reducing risks such as gas-induced cataract. The time course of development of PVR and the histologic appearance of the cellular membranes that form correspond to observations made in the clinical setting.

Future work on the dispase model will include the characterization of cell types within the PVR membranes, a histologic examination of the progression of PVR, and the determination of the effectiveness of dispase in other animals. Studies on the prevention and treatment of PVR will be enhanced by the availability of this new model of PVR.

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References


Polyunsaturated Fatty Acids Are Lower in Blood Lipids of Usher's Type I but Not Usher's Type II

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PURPOSE. Previous studies have shown that persons with retinitis pigmentosa and Usher’s syndrome have lower blood levels of long-chain polyunsaturated fatty acids (PUFAs). In this study, the fatty acid composition of phospholipids from plasma and red blood cells (RBCs) was compared in persons with Usher’s syndrome type I; Usher’s syndrome type II, or no retinal disease (control subjects).

METHODS. Blood was drawn from fasting volunteers and separated into plasma and RBCs by centrifugation. Lipids were extracted and phospholipids were obtained by thin-layer chromatography. Fatty acid methyl esters were prepared and analyzed by gas-liquid chromatography.

RESULTS. There were no differences in plasma or RBC phospholipid fatty acid composition between control subjects (n = 54) and persons with Usher’s syndrome type II (n = 20). However, all 20- and 22-carbon PUFA levels from RBCs of persons with Usher’s syndrome type I were lower than those from control subjects and persons with Usher’s Syndrome type II. Likewise, plasma levels of 20:3n-6, 20:5n-3, and 22:6n-3 were lower in Usher’s syndrome type I compared with the control group. In contrast, plasma levels of 18:1n-9 and RBC levels of 16:0 and 18:1n-9 were higher in the group with Usher’s syndrome type I.

CONCLUSIONS. Plasma and RBCs from Usher’s syndrome type I, but not type II, have lower levels of long-chain PUFAs than plasma and RBCs from control subjects. (Invest Ophtalmol Vis Sci. 1998;39:2164–2166)

Numerous studies have shown that some humans and animals with inherited retinal degenerations have lower plasma and red blood cell (RBC) levels of long-chain polyunsaturated fatty acids than control subjects.