Pathogenesis of the Vitreous Cloud Emanating From Subretinal Hemorrhage

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Objective: To determine the cellular mechanism that allows subretinal hemorrhage to cloud the vitreous.

Methods: We simulated subretinal hemorrhage in a rabbit model by injecting autologous blood beneath the retina. At the first appearance of a cloud in the vitreous a vitrectomy was performed and using a surgical microscope, the retina was searched for breaks. After enucleation and fixation, the retina was searched for microscopic breaks using light and electron microscopy. The vitreous was then examined to determine the character of the cell population in the cloud. In a related study, we sampled and examined the vitreous for its cellular content in patients undergoing vitrectomy to clear cloudy vitreous emanating from subretinal hemorrhage.

Results: We found no breaks in the living retina of the animal models or the patients. Microscopic examination of serial sections of the rabbit retina revealed necrosis except for the internal limiting membrane. Fragments of the erythrocytes were seen within the damaged retina and on both sides of the internal limiting membrane. Electron microscopy suggested that the erythrocytic fragments had migrated across the internal limiting membrane. The vitreous cloud in both rabbits and patients contained only fragments of erythrocytes.

Conclusions: Thick subretinal hemorrhage causes necrosis of the overlying retina. Fragments of the erythrocytes infiltrate the retina and cross an intact internal limiting membrane to cloud the vitreous.

Clinical Relevance: Rapid necrosis of the retina occurs over thick subretinal hemorrhage and indicates the need for early displacement of the hemorrhage from the macula if function is to be preserved and breakthrough prevented.

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METHODS

Twenty New Zealand albino rabbits, weighing 3.5 to 4 kg, were approved for use in the study by the institutional review board of Cornell Medical College, New York, NY. All experiments conformed to the Association for Research in Vision and Ophthalmology statement for the use of animals in research. The animals were anesthetized with an intramuscular injection of ketamine hydrochloride (35 mg/kg) and xylazine hydrochloride (5 mg/kg). Anesthesia of the conjunctiva was obtained with topical 0.5% proparacaine hydrochloride. The pupils were dilated with topical 1% cyclopentolate hydrochloride.

Blood was withdrawn from the ear vein of the anesthetized animal and immediately injected beneath the posterior retina adjacent to the myelinated fibers at the inferior edge of the optic disc (Figure 1). To facilitate injection, the eye was propotosed and the injecting needle advanced to the subretinal space while the retina was being observed through the pupil with the surgical microscope. Volumes of 0.05
ml of blood were injected into 2 eyes, 0.1 mL into 11 eyes, 0.15 mL into 4 eyes, and 0.2 mL into 3 eyes. Only 1 eye of each animal was injected.

The eyes were observed ophthalmoscopically at 3-hour intervals between 8 AM and 8 PM. At the first sign of vitreous clouding, 5 animals were anesthetized as before, the eyes vitrectomized, and the retinal surface over the hemorrhage was searched for a defect using the surgical microscope. Samples of the vitreous cloud were taken, fixed in a 4% paraformaldehyde solution, and stained with hematoxylin-eosin for microscopic study.

The 5 animals whose eyes were vitrectomized and the 14 animals whose eyes were observed for 3 to 15 days were killed with intravenous pentobarbital sodium and the eyes enucleated and fixed in a solution of either 10% formalin or 2.5% glutaraldehyde. The posterior halves of the formalin-fixed eyes were embedded in paraffin and sectioned at 7-µm intervals in the area of the subretinal hemorrhage. The sections were stained with hematoxylin-eosin, periodic acid–Schiff, or sham with immunoperoxidase for examination by light microscopy. The area of hemorrhage in the eyes, fixed in glutaraldehyde and osmium tetroxide, was dehydrated in a series of graded alcohol, and embedded in epoxy resin–epoxy adhesive (Epon-Araldite; Electron Microscopy Sciences, Washington, Pa). One-micrometer-thick sections were cut, stained with toluidine blue, and examined using light microscopy to locate the area of maximum retinal necrosis. The retinal block was trimmed accordingly and ultrathin sections (60 to 70 nm) cut, mounted on copper grids, and examined using an electron microscope (JEOL 100 CX II; JEOL USA, Inc, Peabody, Mass) operating at 80 kV. Images were recorded on Kodak Electron Image film (Eastman Kodak, Rochester, NY).

**RESULTS**

**OPHTALMOSCOPY AND SURGICAL MICROSCOPY**

Volumes of 0.05 mL of injected blood remained beneath the retina and did not cloud the vitreous. Volumes of blood greater than 0.05 mL, that spread and caused an extensive but shallow retinal elevation, also remained beneath the retina. Volumes of blood of 0.1 mL or more, that remained aggregated, broke through the retina between 48 and 72 hours, except for 1 eye in which it was first detected at 96 hours. Breakthrough was manifested by a cloud in the vitreous over the subretinal hemorrhage (Figure 2).

An examination of the retina over the hemorrhage using the surgical microscope during vitrectomy revealed a smooth unbroken retinal surface in all eyes.

**LIGHT MICROSCOPY**

Sections of retina from eyes injected with 0.05 mL of blood showed loss of the outer segments, but the remainder of the retina was intact. When volumes of blood greater than 0.05 mL spread beneath the retina, the effect was more extensive, but still limited to loss of the outer segments. The retina from eyes injected with more than 0.5 mL of blood that remained aggregated, at breakthrough and at 1 to 15 days after breakthrough, showed increasing levels of necrosis except for the internal limiting membrane (ILM). The necrotic retina was infiltrated with fragments of erythrocytes and fragments were present on both sides of the ILM (Figure 3). Samples of the vitreous cloud revealed only fragments of erythrocytes (Figure 4).

The fragments were identified as erythrocytic in origin because they had morphologic and tintorial properties of erythrocyte fragments seen in routine histopathologic sections of resolving hemorrhages in other body tissues. The fragments remained unstained in the periodic acid–Schiff-stained sections, a distinctive property of erythrocyte cytoplasm. In the blank or sham-stained immunoperoxidase-stained sections, the erythrocytic fragments displayed characteristic endogenous peroxidase activity.

**ELECTRON MICROSCOPY**

Transmission electron microscopy of the ILM revealed fragments of erythrocytes on both sides of the ILM. The fragments were isodense and displayed intense osmiophilia, a characteristic of erythrocytes. The size and shape of the fragments on the external and internal sides of the ILM were similar (Figure 5). Penetration of the ILM was implied in sections that showed partial portions of what appears to be a single fragment on both sides of the ILM. Apparent aggregation occurs at the level of the ILM (Figure 6). The fragment of the erythrocyte is without an external membrane (Figure 7).
HUMAN STUDIES

A rabbit model of vitreous clouding from subretinal hemorrhage revealed that the vitreous cloud is composed of fragments of erythrocytes that have migrated across the ILM. Whether the same phenomenon occurs in patients was questionable because whole erythrocytes have been observed in samples obtained from cassettes of vitrectomy procedures done to clear the vitreous in eyes that have experienced clouding from a subretinal hemorrhage. To resolve the issue we examined a collection of pathologic specimens coded as vitreous hemorrhage, then performed a biopsy, and examined the vitreous cloud emanating from subretinal hemorrhage in patients undergoing vitrectomy. For contrast we also performed a biopsy of the vitreous in eyes with preretinal hemorrhage.

Methods

Eighty-two microscopic specimens coded only as vitreous hemorrhage were obtained from the Department of Pathology of the New York Eye & Ear Infirmary Hospital, New York, and studied for cellular content. The specimens were the contents of vitrectomy cassettes that had been centrifuged and processed as cell blocks, sections of which were stained with hematoxylin-eosin.

Biopsy specimens of vitreous hemorrhage from 8 patients were obtained prospectively by pars plana vitrec-
tomy. Four patients were known to have had subretinal bleeding from age-related macular degeneration and 4 to have had preretinal bleeding from proliferative diabetic retinopathy. The duration of the vitreous hemorrhages varied from 1 to 12 months. The vitrectomies were done by 4 surgeons of the New York Presbyterian Hospital, New York, to restore vision. The specimens consisted of 0.2 mL of vitreous obtained from the densest part of the vitreous cloud at the beginning of the vitrectomy. The surgeons made an effort to avoid contaminating the specimens with fresh bleeding from the entrance sites. The specimens were aspirated from the outflow tube of the vitrectomy system and immediately fixed in a 4% paraformaldehyde solution. Multiple smears from each specimen were made on coated glass slides and stained with hematoxylin-eosin for examination by light microscopy. In eyes in which the source of the vitreous cloud was known to be subretinal hemorrhage, the retinal surface overlying the site of the hemorrhage was examined for a break after the vitreous was clear, with the ×20 ocular magnification of the surgical microscope.

Results

Seventy-five of 82 specimens in the pathology collection of the New York Eye & Ear Infirmary contained a mix of erythrocytes in various stages of degeneration, fragments, and ghost cells. All of the specimens contained normal-looking erythrocytes regardless of the apparent age of the other cells present. Seven specimens were remarkable in that fragments of erythrocytes predominated. There were normal-looking erythrocytes present but no degenerated erythrocyte forms and, importantly, no ghost cells. The medical records of patients in only 2 of the 7 specimens were made available, but both revealed the origin of the vitreous hemorrhage to have been a subretinal hemorrhage from age-related macular degeneration.

The biopsy specimens of the vitreous obtained prospectively from the 4 patients with subretinal bleeding uniformly contained fragments of the erythrocytes (Figure 8). The diameter of the fragments varied between 0.5 and 4 µm. The smaller fragments tended to be round. The larger fragments were lobulated and appeared to be aggregates of small fragments. Examination of the retina over the subretinal hemorrhage using the surgical microscope at the conclusion of the vitrectomy revealed a smooth unbroken ILM.

The cell population of the vitreous obtained from eyes with preretinal bleeding contained erythrocytes in various stages of degeneration and varied according to the duration of the hemorrhage. The specimen of 1 month’s duration contained normal-appearing erythrocytes and a few acanthocytes (Figure 9). Specimens of 5 months’ duration contained a larger number of acanthocytes (Figure 10). At 12 months ghost cells and fragments predominated (Figure 11).
The abrupt clouding of the vitreous associated with thick subretinal hemorrhage suggests that a retinal break has occurred. In the experimental animal model no break could be detected. The ILM remained intact regardless of the amount of hemorrhage or degree of retinal necrosis. Nor could a break be found in the 4 human eyes studied using the surgical microscope. The vitreous cloud in both the rabbit model and in the patients was composed of fragments of erythrocytes that had migrated across the intact ILM. Scanning electron microscopy of the ILM in a primate model did not reveal pores or holes that could provide access. A similar phenomenon has been observed in blood staining of the cornea. McDonnell et al. have described fragments of erythrocytes from anterior chamber hemorrhage that have migrated across an apparently intact Descemet membrane and infiltrated the extracellular stroma of the cornea.

Whole erythrocytes found in the specimens obtained from cassettes of vitrectomy procedures have a normal contour regardless of the duration of the vitreous cloud indicating that their presence in the vitreous prior to fixation had been brief and are, therefore, contaminants of the surgical procedure. Old preretinal hemorrhages may contain many fragments, but the presence of ghost cells confirms their preretinal origin.

A retrospective review of 11 patients with subretinal hemorrhage at New York Presbyterian Hospital revealed that clouding of the vitreous was reported between 7 and 18 days after the occurrence of the subretinal bleed. In the rabbit, breakthrough occurred between 48 and 96 hours. In both patients and rabbits clouding of the vitreous occurred only in the presence of a thick subretinal hemorrhage. In the rabbit, microscopy revealed extensive necrosis of the retina except for the ILM, apparently an effect of the separation of the retina from the choroid and the blocking of metabolic exchange. We suggest that the same process occurs in the human retina but more slowly because the human retina is thicker and derives some metabolic support from the retinal vascular system. The rabbit and human response to thin subretinal hemorrhage is also similar. Thin subretinal hemorrhages only damage the outer segments in the rabbit and the recovery of vision in patients with thin hemorrhages implies the same level of retinal damage.

It would seem appropriate to attempt to diminish the height of a thick subretinal hemorrhage in the macula as soon as possible after its presentation with a gas displacement or other procedure to allay extensive retinal necrosis and subsequent breakthrough. Reynders et al. in a 2002 study of excised age-related macular degenerative lesions that were complicated by subretinal hemorrhage concurred that the hemorrhage creates a barrier to metabolic exchange and causes degenerative changes in the retina and they recommend pneumatic displacement. They point out, however, that part or all of the hemorrhage may be within fibrovascular stroma on the choroidal side of pigment epithelium and not responsive to pneumatic displacement. Ocular coherence tomography can help to define the disposition of the hemorrhage.

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REFERENCES


ARCHIVES Web Quiz Winner

Congratulations to the winner of our September quiz, Richard Hanson, MRCOphth, Specialist Registrar Oxford Eye Hospital, England. The correct answer to our September challenge was Henoch-Schönlein purpura and bilateral subperiosteal orbital hematomas. For a complete discussion of this case, see the Clinicopathologic Reports, Case Reports, and Small Case Series section in the October ARCHIVES (Ma’luf RN, Zein WM, El Dairi MA, Bashshur ZF. Bilateral subperiosteal orbital hematomas and Henoch-Schönlein purpura. Arch Ophthalmol. 2002;120:1398-1399).

Be sure to visit the Archives of Ophthalmology World Wide Web site (http://www.archophthalmol.com) and try your hand at our Clinical Challenge Interactive Quiz. We invite visitors to make a diagnosis based on selected information from a case report or other feature scheduled to be published in the following month’s print edition of the ARCHIVES. The first visitor to e-mail our Web editors with the correct answer will be recognized in the print journal and on our Web site and will also receive a free copy of the Clinical Eye Atlas, published by AMA Press.

Figure 1. Bilateral upper eyelid ecchymosis with exophthalmos more prominent on the right side.
tion of voriconazole was above the MIC90 (minimum inhibitory concentration) of 0.5 µg/mL for A fumigatus reported in various studies.1-3,5 Voriconazole also has been shown to be effective for endogenous Fusarium endophthalmitis8 and Paecilomyces lilacinus endophthalmitis.9

In larger clinical studies of systemic fungal diseases, the most commonly reported adverse effects of voriconazole were transient visual disturbances, including brightness, blurring, light sensitivity, or altered color perception.4,6 These visual abnormalities were reported in approximately 30% of patients receiving voriconazole, typically began 30 minutes after dosing, and lasted about 30 minutes. Other adverse effects included elevated liver enzyme levels and facial erythema.5,6

The ability to achieve effective intraocular drug concentrations with oral administration, the broad spectrum of antifungal activity, and the relatively low level of systemic adverse effects suggest that voriconazole may have a wider role in the future for treatment of ocular fungal infections.

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