Objective: To evaluate the morphologic outcomes resulting from surgical vitreoretinal separation in young adult primates.

Materials and Methods: Vitrectomy and mechanical separation of the vitreous from the internal limiting lamina (ILL) of the posterior retina and surface of the optic disc were performed on 25 young adult cynomolgus monkey eyes in vivo. Lectin histochemical studies were used to evaluate the vitreoretinal interface. Morphologic outcomes were tabulated.

Results: In 11 of 25 eye regions, residual vitreous remained attached to the ILL in some of the regions. Localized ILL breaks or separation of the ILL from the neural retina was noted in 9 eyes. Retinal tissue loss, including avulsion of the ganglion cell, inner plexiform, or inner nuclear layers, was observed in 7 eyes. Avulsion of axon bundles in the optic disc was noted in 9 eyes. Significantly, partial- or full-thickness foveal tears were noted in 11 eyes. Based on the surgeons' intraoperative observations, small superficial optic disc or retinal hemorrhages were observed in 3 of 25 eyes. None of the eyes on which a vitrectomy alone was performed showed ILL damage, or retinal or optic disc tissue loss.

Conclusion: Damage may occur to the optic disc, fovea, and extrafoveal retina as a result of surgical separation of the vitreous from the retina in young adult primates.

Clinical Relevance: These data support the contention that surgically induced damage at the level of the vitreoretinal interface may help explain the visual field defects noted after surgery to close full-thickness macular holes. These data also support the need for developing additional modalities to assist in vitreous separation, thereby reducing the risk of traumatic complications associated with purely mechanical procedures.


The vitreous is a connective tissue compartment occupying approximately 80% of the volume of the eye and providing structural and metabolic support for ocular tissues. The vitreous is a semisolid, transparent gel composed primarily of water. Approximately 1% of this gel is composed of other components, including collagen and hyaluronic acid. The strongest adherence between the vitreous body and the retina occurs at the vitreous base. Other areas of firm vitreoretinal attachment include the peripapillary region near the optic disc, the posterior vitreous associated with the fovea, and areas along the major retinal vessels. The molecular basis for vitreoretinal adhesion has not been elucidated. However, 2 chondroitin sulfate proteoglycans, each of approximately 240-kd molecular weight, have been isolated from the vitreoretinal interface of the vitreous base, where they have been proposed to participate in adhesion of the vitreous body of the neural retina (G.S.H. and S.R.R., unpublished data, 1994).

Current therapies to treat macular holes, retinal detachments, and many other vitreoretinal abnormalities rely on the premise that traction mediated by the vitreous cortex is the proximate cause of visually threatening disease. For several surgical indications, mechanical removal of the vitreous cortex is performed or has been advocated. Surgical procedures for the management of idiopathic and traumatic full-thickness macular holes have included removal of the posterior hyaloid from macular holes and posterior retinal surfaces. As growing numbers of patients undergo surgical procedures to close macular holes, complications such as profound visual field defects have been reported. Controversy regarding the cause of these visual field defects exists. However, mechanical damage inflicted on the
MATERIALS AND METHODS

SURGICAL PROCEDURE AND TISSUES

Twenty-five experimental and 5 control (sham) procedures were performed in 17 cynomolgus monkeys between the ages of 6 and 10 years. Cynomolgus monkeys were restrained in a squeeze cage and injected in the thigh with 0.7 mL of ketamine hydrochloride (Ketaset, 100 mg/mL; Aveco Inc; Fort Dodge, Iowa) to induce sedation. The monkeys were subsequently transported to an operating room. Mask anesthesia, using 1% to 3% isoflurane (Forane; Baxter PPI, Liberty Corner, NJ) was administered until muscular relaxation was obtained. The monkeys were subsequently intubated using a 3-mm-diameter endotracheal tube. After verifying tube position and adjusting the expandable endotracheal cuff, the monkeys were placed on a heating blanket to maintain their body temperature throughout the procedure. Precordial electrocardiographic electrodes and a blood pressure cuff were applied and vital signs were monitored every 15 minutes throughout the procedure. A 20-gauge intravenous catheter was introduced into the posterior tibial vein and a 0.9% sterile isotonic sodium chloride solution was administered at a rate of 30 mL/h. The monkeys’ necks were hyperextended to allow adequate superior vitreotomy. Lateral tarsorrhaphies were performed to allow adequate globe access and sclerotomy sites were placed 2.25 mm posterior to the inferotemporal limbus. The 2.3-mm cannulas were sewn into place, using 5-0 polygalactin (Vicryl) suture. Additional sclerotomy sites were located 2.25 mm posterior to the limbus, in the superotemporal and inferotemporal quadrants. A vitreous cutter was inserted through the superotemporal sclerotomy site and a fiberoptic illuminator was inserted through the supranasal sclerotomy site. Core vitreous was removed from each eye and exchanged with sterile balanced salt solution (BSS; Alcon, San Antonio, Tex). In the experimental eyes, a silicone-tipped cannula was used to create a posterior vitreous separation encompassing the macula and optic disc. Conscious efforts were made to apply traction that was circumferentially or tangentially positioned to the retina as practicable; vertical elevation of the attached posterior hyaloid face was avoided. The instruments were then removed, the sclerotomy ports were closed using 7-0 polygalactin sutures, and the infusions lines were clamped. The monkeys were killed using 2 mL of 2% pentobarbital hydrochloride (Sleepaway; Fort Dodge Inc, Fort Dodge) administered through the intravenous line. Enucleations were performed and the eyes were immediately processed as described below. Wedges of eyes (retina, retinal pigmented epithelium, optic nerve head, ciliary body, lens, posterior lens capsule, zonules, iris, and cornea) were prepared as described below for histopathological and immunohistochemical analyses.

The eyes were immediately fixed in 4% paraformaldehyde in 100mM sodium cacodylate buffer, pH 7.4. Studies were conducted in accord with Association of Vision Research in Ophthalmology and National Institutes of Health guidelines for the care of laboratory animals. Consent was obtained from the St Louis University Animal Care Committee prior to conducting all procedures.

LIGHT MICROSCOPY AND LECTIN HISTOCHEMISTRY FINDINGS

After enucleation, the anterior segments of the eyes were excised and the eyecups and anterior segments were fixed for 2 to 4 hours by immersion in 4.0% formaldehyde (freshly generated from paraformaldehyde) in 100mM sodium cacodylate buffer, pH 7.4. The eyecups were rinsed for a minimum of 6 hours in 100mM sodium cacodylate buffer, embedded in acrylamide, and sectioned to a thickness of approximately 5 to 6 µm on a cryostat at −20°C. Meridional serial sections from the pars plana to the optic nerve were cut from each eye.

For lectin histochemical studies, the sections were exposed to Agaricus bisporus agglutinin at a concentration of 30 mg/mL in 10mM phosphate-buffered saline solution containing 1mM magnesium chloride to 1mM calcium chloride and 1 mg/mL of globulin-free bovine serum albumin and processed as described previously. Adjacent sections were stained with hematoxylin-eosin. The slides subsequently were rinsed in a combination of a phosphate-buffered solution with magnesium chloride and calcium chloride. Controls included incubation of sections with A bisporus agglutinin in the presence of a competitive hapten. The sections were examined by epifluorescence microscopy using a light microscope (model Vanox VH-2; Olympus America Inc, Melville, NY) and photographed using commercially available film (Kodak Ektachrome ASA 400; Kodak, Rochester, NY) set on an exposure index of 800.

RESULTS

Focal regions of adherent vitreous cortex were observed in the region of the fundus underlying the mechanically induced posterior vitreal detachment in 11 eyes; limited breaks in or elevation of the ILL were observed in 9 eyes (Figure 1, Table). More damage or disruption, including detachment of the ILL, foveal detachment, retinal delamination, retinal tearing, and vascular avulsion were...
observed (Figure 1C, E-F). Foveal tissue was avulsed in 11 eyes. Lesions observed included tearing of the foveal umbo (Figure 2B, D), detachment of the perifoveal ILL (Figure 2C), detachment of the fovea (5 eyes), and avulsion of the foveola (Figure 2E-F) that was histologically indistinguishable from an acute macular hole. Damage to the optic disc, which included loss of axons, was observed in 9 eyes (Figure 3C-D). Small superficial optic disc or retinal hemorrhages were noted in 3 of 25 experimental eyes. No similar pathologic abnormality was noted in any of the control (sham-operated on) eyes (not shown).

<table>
<thead>
<tr>
<th>Condition</th>
<th>No. of Eyes</th>
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<tbody>
<tr>
<td>Vitreous islands</td>
<td>11</td>
</tr>
<tr>
<td>Focal ILL breaks and localized ILLD*</td>
<td>9</td>
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<tr>
<td>Cellular loss and damage</td>
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<tr>
<td>Optic disc</td>
<td>9</td>
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<td>Nonfoveal retina</td>
<td>7</td>
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<tr>
<td>Fovea</td>
<td>11</td>
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*a* ILL indicates internal limiting lamina of the retina; ILLD, ILL detachment.

Figure 1. Epifluorescence photomicrographs depicting binding of fluorescein-conjugated *Agaricus* bisporus agglutinin to residual vitreous (A) following surgical vitreous separation and representative damage sustained to the extrafoveal retina. Mild damage includes retinal internal limiting lamina breaks (B, arrow) or segmental avulsion (C). Substantial avulsion of inner retina results in loss of axons (D) and of ganglion cells (E and F). All figures are positioned with the vitreous at the bottom of the photomicrographs (original magnification ×400 for all panels).
COMMENT

These data show that mechanically induced posterior vitreous detachment can cause immediate damage and neurosensory tissue loss to the posterior fundus of young adult primates. Of 25 eyes, substantial damage to the optic disc, fovea, and extrafoveal retina occurred in 11, 7, and 9 eyes, respectively. Unexpectedly, these lesions are not typically detectable by the operating surgeon. The consequences of intentional removal of the ILL, which would be expected to result in considerably more tissue damage and loss, were not addressed in this study.

Although the significance of these injuries to the vision in young adult primates could not be directly ascertained, visual defects might be expected based on the location and severity of the induced lesions. In contrast to the findings of preceding studies, more recent data on primate retinotopic organization suggest that larger, more peripheral regions of ILL, nerve fiber layer, and ganglion cell avulsion might cause widespread or wedge-
shaped peripheral visual field defects. The visual field defects noted following macular hole surgery are absolute large or wedge-shaped visual field defects.\textsuperscript{18}

Trauma to the optic disc was proposed by Melburg and Thomas\textsuperscript{19} as a possible cause of visual field defects associated with macular hole repair and caution was advised when aspirating directly over the optic disc. In 1 of 8 eyes with visual field loss following vitreous surgery, Kerrison et al\textsuperscript{20} noted a small hemorrhage adjacent to an optic disc vessel after surgically separating the posterior hyaloid from the superotemporal optic nerve, an incidence similar to the finding in our study. The location of the hemorrhage corresponded to the region of the visual field loss. A similar peripheral, absolute visual field defect was noted in another eye that had not received an air-fluid exchange. These findings suggest that in some instances air or gas contact with the retina may not be required for development of visual field defects. These authors considered the visual field loss to be a result of optic disc damage, perhaps due to shearing of peripapillary axons and/or vessels,\textsuperscript{20} a finding noted with high incidence in our study. In another report of visual field defects following macular hole surgery,\textsuperscript{18} 6 of 8 eyes were found to have a statistically significant reduction of peripapillary nerve fiber layer thickness corresponding to the distribution of the visual field defects. Hutton et al concluded that “artificial, abrupt detachment of the cortical vitreous, perhaps in combination with rather prolonged, direct contact of the gas bubble against the posterior pole of the eye”\textsuperscript{18}(p2157) may be causative.

The notion that optic disc or peripheral retinal damage may cause peripheral visual field loss is consistent with the study findings of Cullinane and Cleary,\textsuperscript{21} who demonstrated that 18 (24%) of 75 eyes developed peripheral visual field defects after “complete” surgical posterior vitreous detachment. None of the 20 eyes in which mechanical separation of vitreous was confined to the macula (vitreous not separated from the optic disc or peripheral retina) showed similar peripheral visual field defects.

There is strong evidence that visual field defects following some macular hole surgical procedures may be associated with desiccation of the retina or optic disc.\textsuperscript{22} The posterior hyaloid attached to the optic disc or peripheral retina may act as a hydration reservoir. When removed, desiccation of the optic disc or retina may be facilitated. Denuding the neural retina of its basement membrane may increase susceptibility from desiccation, cold, or other mechanisms. Removal of the ILL directly exposes neural cells to the intraocular environment, placing ganglion cells (if not avulsed) and retinal interneurons at potentially greater desiccation risk.  

Figure 3. Epifluorescence photomicrographs depicting binding of fluorescein-conjugated \textit{Agaricus bisporus} agglutinin to optic nerves that have sustained iatrogenic damage from surgical vitreous disinsertion. Disturbances of optic nerve tissue vary from mild loss of the internal limiting lamina over the optic nerve (A) to separation of nerve fascicles (B) or loss of axonal segments (C and D). All figures are positioned with the vitreous at the bottom of the photomicrographs (original magnification \times400 for all panels).
ough discussions of these and other causes of iatrogenic visual field loss are reviewed elsewhere.9,18

This study documents significant damage to the inner retina, macula, and optic nerve following surgical separation of the vitreous from the ILL. These observations may help to explain cases in which visual field defects result from similar surgical procedures in humans. Our results suggest that other nonmechanical techniques, including enzyme-assisted vitrectomy,23,24 for separating the vitreous cortex from the ILL, could result in lower operative complications and improved visual outcomes.

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REFERENCES