Experimental posterior penetrating eye injury in the rhesus monkey: vitreous-lens admixture

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SUMMARY A standardised experimental posterior penetrating eye injury in the rhesus monkey with the vitreous-lens admixture resulted in limited fibrous ingrowth from the wound and minimal traction on the peripheral retina. Microscopically inflammation was noted in the iris, ciliary body, and as a perivascular infiltrate of the inner retina. Periretinal membranes did not occur, and cellular proliferation within the vitreous was less marked than observed after an identical wound and simulated vitreous haemorrhage in previously reported studies. The lens may be a less important stimulus to intravitreal fibroblastic proliferation than previously assumed.

Penetrating ocular trauma remains an important cause of blindness, particularly among young, productive individuals.1-4 In recent years the visual prognosis has improved markedly for penetrating injuries of the anterior segment of the eye. However, injuries involving the posterior segment continue to result frequently in a poor visual outcome.5-11 The principal reason for loss of vision after posterior injury is the development of retinal detachment,9,10 12 13 which may be rhegmatogenous or tractional.10 14 Posterior penetrating injuries of the eye characteristically develop traction retinal detachment.9,10

The sequence leading to traction retinal detachment after a penetrating injury is characterised histologically by intravitreal fibrocellular proliferation forming cyclitic, transvitreal, epiretinal, and retroretinal membranes.13 15-17 Elements that may contribute to the fibrocellular response include the penetrating wound, vitreous haemorrhage, lens injury, trauma of the ciliary body, infection, or foreign bodies.12 13 15 18 Clinical studies have shown the significance of vitreous incarceration in the scleral wound and vitreous haemorrhage in the development of intravitreal fibrocellular proliferation and traction retinal detachment.4 7 18-20 Other studies have identified injury to the lens with vitreous-lens admixture as a potent stimulus to inflammation and cellular proliferation within the vitreous.19 18 21

Because the laboratory environment allows more precise control of variables than a random patient population, we have developed an experimental animal model of a posterior penetrating eye injury in the rhesus monkey to analyse the importance of specific variables and to elucidate pathogenetic mechanisms. We have found this model useful in confirming the significance of vitreous haemorrhage and vitreous incarceration in the wound in posterior penetrating eye injuries and in demonstrating the pathophysiology of injury-induced traction retinal detachment.21 22 The model has also proved useful in assessing the role and timing of vitrectomy in the treatment of such injuries.24 In the present study we have combined our standard penetrating injury with an injection of autologous lens material into the vitreous to examine, as an isolated variable, the effects of the lens-vitreous admixture.

Material and methods

Five rhesus monkeys weighing 5 to 7 kg, and of either sex, were anaesthetised by intravenous injection of sodium pentobarbital. The pupils were dilated with 1 drop of 1% cyclopentolate hydrochloride and 1 drop of 10% phenylephrine. The eyelids and surrounding skin were scrubbed with povidone-iodine solution, and the eyes were draped with sterile adhesive polyethylene drape. All surgery was performed under sterile conditions under an operating microscope.

In the right eye of each animal an intracapsular lens extraction was performed through a limbal
incision. A muscle hook was used to rupture the zonular fibres and the lens was removed with a lens loop. A sector iridectomy and an anterior vitrectomy by means of the Ocutome were performed. The limbal incision was closed with interrupted 8-0 silk sutures.

The extracted lens was ground with a sterile mortar and pestle, and suspended in sterile Ringer’s lactate solution for a total volume of 0·5 ml.

In the left eye of each animal a standard injury was performed, as previously described.22 An incision 8 mm long and 3·5 mm from the corneal-scleral limbus was made through the pars plana, avoiding the lens and the peripheral retina. Prolated vitreous was excised and the wound carefully closed with interrupted sutures of 8-0 silk by microsurgical techniques. After wound closure the fundus was inspected by indirect ophthalmoscopy, and the areas of the wound and peripheral retina were examined by scleral indentation to exclude any eye with vitreous haemorrhage or damaged retina. 0·5 ml of the previously prepared whole lens suspension from the contralateral eye was injected slowly under low pressure from a 22-gauge needle inserted through the wound into the mid-vitreous under ophthalmoscopic control. Finally, a subconjunctival injection of 20 mg gentamicin was given.

The animals were observed twice in the first week after injury, at 2 weeks after injury, and then at biweekly intervals for 6 months. The eyes were examined by slit-lamp biomicroscopy and by indirect ophthalmoscopy. Eyes with opaque media were examined by B-scan ultrasound. One of the 5 eyes was enucleated at 3 weeks after standard injury and injection of lens material. The other 4 eyes were followed up clinically for 6 months and then enucleated.

For histological examination eyes were fixed in one-half strength Karnovsky’s fixative (2·5% glutaraldehyde and 2% paraformaldehyde in 0·1 M cacodylate buffer, pH 7·2) or 4% paraformaldehyde. Before enucleation 0·2 ml of fixative was injected through the pars plana away from the wound into the vitreous cavity. Immediately after enucleation a central corneal button was excised and the eyes were immersed in cold fixative for 24 hours. The eyes were then sectioned through the optic nerve and midpoint of the wound for gross examination. Each half was examined under the dissecting microscope, and drawings and photographs were made. The eyes were then dehydrated in graded alcohols, embedded in paraffin, and sectioned and stained in the routine manner. Stains used included haematoxylin-eosin, periodic acid Schiff (PAS), and Masson trichrome.

Results

CLINICAL OBSERVATIONS

The anterior chamber contained fibrin in 2 eyes for the first 2 to 3 days after injury. By 1 week the fibrin had cleared. For the duration of the follow-up period anterior segments of all 5 eyes remained free of signs of inflammation on slit-lamp examination.

The vitreous remained clear during the first week after injury and the injected lens particles were visible in the anterior and mid-vitreous. In the second week the lens particles became swollen, white, and flocculent and the surrounding vitreous mildly hazy. At 4 weeks the lens material in the vitreous was more opaque and the vitreous haze had increased so that the fundus view was obscured in all 4 eyes. The vitreous then gradually cleared, so that by 3 months the retina was again visible by indirect ophthalmoscopy. Discrete opacities of the injected lens material remained in the mid-vitreous and anterior vitreous.

Two eyes developed cataracts. In one a posterior subcapsular lens opacity was first observed at 4 months after injury, localised to the quadrant of the penetrating wound. The lens opacity progressed to involve the entire lens, which became intumescent by 6 months. In the other a localised posterior subcapsular cataract occurred but did not progress.

The penetrating wound in the pars plana was visible by indirect ophthalmoscopy. Vitreous fibrils were incarcerated in the wound, and some of the injected lens material was distributed along these vitreous fibrils. In one eye the penetrating wound healed as a flat, white scar in the pars plana. However, in the other 3 a fibrous ingrowth was apparent clinically as early as 9 weeks after injury. The ingrowth extended along the incarcerated vitreous fibrils, along the anterior hyaloid and posterior lens capsule (Fig. 1). In the eye that developed a progressive cataract the fibrous ingrowth invaded through the posterior capsule into the lens cortex.

The retina remained attached in all 5 eyes. None showed clinical signs of vitreoretinal traction or epiretinal membranes. When the fundus was not visible, a B-scan ultrasound was used to confirm that the retina remained attached.

In 1 eye at 4 months after injury small white deposits were noted along the margins of the retinal branch veins (Fig. 2a). Along the peripheral retinal veins some of these deposits became confluent, extending along and sheathing segments of the vein. The clinical appearance simulated a retinal periphlebitis, and the walls of these vessels showed some staining with dye on fundus angiography (Fig. 2b). Another eye had a similar appearance of perivascular sheathing at 3 months after injury, but
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this eye developed a progressive cataract, precluding further clinical observation.

After injection the lens particles were suspended in the anterior and mid-vitreous. In 2 eyes they seemed to migrate anteriorly during the first 4 weeks after injury, but in the other 2 eyes the lens particles were distributed in a funnel-shaped con-

figuration, with the apex of the funnel attached at the optic disc. The clinical appearance and examination by B-scan ultrasound suggested the presence of a posterior vitreous detachment.

The eyes from which the lenses were extracted had an uneventful postoperative course. After the initial inflammatory reaction subsided no eyes showed signs of intraocular inflammation, and the retina remained attached in all.

HISTOLOGY

Gross examination of the enucleated eyes confirmed that the retina was attached in all eyes. One eye showed signs of minimal vitreous traction on the peripheral retina directly posterior to the penetrating wound. Fibrous ingrowth along the anterior hyaloid was present in 3 eyes and was marked in the eye in which the ingrowth invaded the lens.

Condensed vitreous fibrils were visible in the plane of the anterior hyaloid, behind the lens, and also radiating posteriorly from the penetrating wound towards the optic nervehead. Particles of the injected lens material were distributed along these vitreous fibrils.

THE WOUND

On microscopic examination good wound apposition was present in all eyes. In the eye obtained at 3 weeks after injury a round-cell infiltrate was noted in the episclera, and fibrovascular tissue was present

Fig. 1 External photograph of monkey eye focused on anterior vitreous 6 months after standard injury and injection of lens material. The anterior segment shows no signs of inflammation. Fibrous ingrowth from the area of the wound (arrow) extends across the anterior hyaloid and incorporates residual injected lens material.

Fig. 2(a) Fundus photograph shows perivenous infiltrates in rhesus monkey eye 6 months after standard injury and injection of lens material. Infiltrates were clinically apparent along many branch veins in this eye with focal areas of sheathing in the periphery.

Fig. 2(b) Venous phase of fluorescein angiogram, showing same area of fundus as seen in Fig. 2(a). The vessel wall shows some staining in the area of perivascular infiltrates (arrows).
through the full thickness of the wound and at its inner aspect. At 6 months the scleral wound appeared as a remodelled fibrous scar. Pigment granules were profusely scattered throughout the wound; some were intracellular and engulfed in phagocytic cells, but most were lying free in the mesh of the scar tissues. A few fragments of lens cortex were also present in the wound, and occasional foreign body giant cells containing refractile material were observed.

THE VITREOUS

In the eye examined 3 weeks after injury and in 1 eye at 6 months vitreous fibrils were adherent to the posterior retina. This posterior vitreous contained fragments of lens material, macrophages, and chronic inflammatory cells. In the other 3 eyes the posterior retina was entirely free of vitreous attachments.

Condensed vitreous fibrils were incarcerated in the penetrating wound and were attached to the peripheral retina and to the nonpigmented ciliary epithelium in the region of the vitreous base. Fibroblastic proliferation from the penetrating wound extended along these vitreous fibrils, anteriorly along the anterior hyaloid and posteriorly along the vitreous fibrils, and were attached to the peripheral retina and vitreous base posterior to the wound (Fig. 3). In the eye enucleated at 3 weeks after injury the fibrous ingrowth extended as far as the equator of the lens. At 6 months 2 eyes showed a fibrous ingrowth invading the lens cortex through a defect in the posterior lens capsule (Fig. 4). The fibrous ingrowth did not reach the midline in any eye and was limited to the immediate area of the wound in 1 eye (Fig. 5).

The fibroblastic proliferation within the vitreous appeared to be derived mainly from the stroma of

Fig. 3 Vitreous fibrils incarcerated in the wound extend posteriorly to the nonpigmented ciliary epithelium and peripheral retina. Lengthening and possibly proliferation of the nonpigmented ciliary epithelium (single arrow) has occurred. The peripheral retina is displaced anteriorly and shows cystoid degeneration, possibly due to vitreous traction (arrows). (Haematoxylin and eosin, ×55).

Fig. 4 Histology 6 months after injury shows marked fibrous ingrowth extending from the penetrating wound along the anterior hyaloid and posterior surface of the ciliary body (broad arrow). The fibrous ingrowth has invaded the posterior lens capsule adjacent to the wound, and there is hyperplasia of the lens epithelium (double arrow). The fibrous ingrowth contains abundant spindle cells and collagen. Scattered throughout the fibrous tissue are inflammatory cells, macrophages, and occasional foreign body giant cells. (PAS, ×40).

Fig. 5 At 6 months after injury and lens injection a minimal fibrous ingrowth extends from the wound along the anterior hyaloid (arrows). Note that in this case it does not reach even the ciliary processes in this section taken through its maximal extent. (Haematoxylin and eosin, ×60).
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**Fig. 6** Six months after injury the area immediately posterior to the wound shows proliferation of the nonpigmented (broad arrow) and pigmented (double arrows) ciliary epithelium. Cystoid degeneration of the peripheral retina is present. (Haematoxylin and eosin, \( \times 80 \)).

**Fig. 7** Six months after injury the peripheral retina and pars plana 180 degrees away from the wound shows lengthening and probable proliferation of the nonpigmented (broad arrow) ciliary epithelium, orientated towards the attached vitreous fibrils of the anterior hyaloid. Proliferation of the pigment epithelium of the pars plana is also present (double arrows). (Haematoxylin and eosin, \( \times 45 \)).

The ciliary body and choroid at the wound, but possibly also originated from the nonpigmented ciliary epithelial cells posterior to the wound, became greatly elongated in the direction of the vitreous fibrils that were incarcerated in the wound and were attached to these cells (Fig. 6). In 1 eye a mound of cells proliferated at the junction of the ora serrata and pars plana 180° from the penetrating wound (Fig. 7). Most of these proliferating cells had rounded nuclei and abundant clear cytoplasm, but there were several layers of pigment-containing cells at the base of the mound (Figs. 6 and 7). The appearance suggested the possibility of proliferation of both the pigmented as well as the nonpigmented ciliary epithelium.

The fibrous ingrowth contained a few fine capillaries, chronic inflammatory cells, and pigment-containing macrophages. Occasional multinucleated giant cells were present surrounding or engulfing small refractile particles. Remnants of injected lens capsule were also visible. In 2 eyes where the fibrous ingrowth invaded the lens capsule proliferation of the lens epithelium may have contributed to the fibrous ingrowth (Fig. 4).

**RETINA**

In the 4 eyes with a fibrous ingrowth from the wound the peripheral retina posterior to the wound showed a characteristic appearance. This peripheral retina was drawn anteriorly towards the pars plana. Vitreous fibrils radiated between this peripheral retina and the penetrating wound, suggesting the presence of vitreous traction. To a variable extent the pulled-forward peripheral retina showed cystoid degeneration, and a small localised retinal detachment was present in 1 eye (Fig. 3). Also in 1 eye this same appearance of vitreous traction on the peripheral retina was visible at 180° from the penetrating wound (Fig. 7). None of these eyes showed any evidence of epiretinal membranes, despite extensive sectioning.

Macrophages were occasionally present in the inner retina. A few macrophages and particles of injected lens material were usually found scattered along the internal limiting membrane over the peripheral and posterior retina and at the optic nerve head.

In the eye with clinical signs of retinal periphlebitis light microscopy showed cuffing of the retinal veins with inflammatory cells, mostly lymphocytes, but also eosinophils and occasional macrophages (Fig. 8). In the eye enucleated at 3 weeks after injury a precisely similar appearance was found. In addition the retinal blood vessels, both arteries and veins, at the optic nerve head showed perivascular infiltrates composed of lymphocytes and plasma cells in 4 of 5 eyes (Fig. 9). Three of these eyes also showed evidence of a limited fibroglial proliferation at the optic nerve head. The iris and ciliary body were found to contain focal round cell infiltrates in some sections. The choroid showed no histological evidence of inflammation in any of the eyes studied.

The eyes from which lenses were extracted had no evidence of inflammation in the iris, in the ciliary body, or in the choroid. The retinal blood vessels contained no perivascular infiltrates. The optic
nerve head was free of inflammatory cells or fibroglial proliferation. In these eyes the retina was attached without signs of vitreous traction.

Discussion

Clinical studies of penetrating eye injuries have identified vitreous lens admixture as a potent stimulus of intravitreal fibroblastic proliferation and thus as an indication for early vitrectomy.\textsuperscript{10} \textsuperscript{15} \textsuperscript{18} \textsuperscript{21} Moreover, clinical impressions are reinforced by the histopathology of enucleated specimens that indicate the injured lens may contribute to vitreous organisation, either as an inflammatory stimulus or as a source of cellular proliferation.\textsuperscript{15} However, the precise role of the lens in promoting intravitreal fibrocellular proliferation remains difficult to define in clinical situations, because lens injury is so often accompanied by damage to other ocular tissues or vitreous haemorrhage, which may constitute equal or greater stimuli to intraocular cellular proliferation.

It is acknowledged that intravitreal lens fragments after cataract surgery may result in persistent uveitis with vitreous opacification and condensation.\textsuperscript{35} \textsuperscript{36} Such a reaction may result in fibrocellular proliferation within the vitreous and may culminate in traction retinal detachment. The lens has been similarly implicated in the intraocular inflammation occurring in phacoanaphylaxis, sympathetic ophthalmia, and chronic nongranulomatous, lens-induced uveitis.\textsuperscript{27} \textsuperscript{28} However, as with penetrating injuries, these clinical situations are often accompanied by other complications, such as uveal or vitreous prolapse, obscuring the exact role of the lens in the pathogenesis of the ocular response.

A previous report detailed studies which combined a standard posterior penetrating injury with injection of autologous whole blood to simulate vitreous haemorrhage.\textsuperscript{22} Massive cellular proliferation occurred within the vitreous and resulted in nonrhegmatogenous traction retinal detachment in 80\% of injured eyes. An identical wound with injection of an equal volume of balanced salt solution caused little vitreous reaction and no retinal detachment.

In this study, when the standard injury was combined with an intravitreal injection of autologous emulsified whole lens, the response within the vitreous was much more limited than in eyes with blood injection.\textsuperscript{22} \textsuperscript{23} A fibrous ingrowth was present containing spindle cells and abundant collagen and originating mainly from the wound. It also contained moderate numbers of chronic inflammatory cells, macrophages, and foreign body giant cells. In 2 eyes where the posterior capsule of the in-situ lens proved to be ruptured on histological section, the lens epithelium appeared to be hyperplastic and may have contributed to the fibrous ingrowth. It was unlikely that such proliferation was due to rupture of the lens at the time of injury, since all injections were performed under ophthalmoscopic control with the needle tip continuously visible. Moreover, in the earlier series of saline- and blood-
injected eyes, by the same surgeon using identical techniques, similar proliferations of the lens epithelium were not observed, nor was there any inadvertent perforation of the lens. It is tempting to speculate that the injected lens material may have stimulated secondary changes in the in-situ lens.

The extent of the fibrous ingrowth varied from essentially absent to moderate among the eyes in this series. It was most prominent at the inner aspect of the wound and extended along the scaffold of the anterior hyaloid and to a less extent posteriorly along the vitreous base, much as in blood-injected eyes. The most prominent fibrous ingrowth occurred in eyes with rupture of the posterior capsule of the in-situ lens.

It is impossible to determine whether the greater fibrous ingrowth contributed to lens rupture or the reverse situation occurred. One might speculate that either rupture of the lens incited greater vitreous reaction or vitreous inflammation resulted in cataract and lens rupture. The observation of either indicates the need for a further series of animal experiments with rupture of the in-situ lens.

Periretinal membranes were not observed clinically or histologically in this series, unlike previous blood-injected eyes. Although the stimuli to formation of epiretinal membranes are uncertain, it is generally predicted that the integrity of the retina must be breached by mechanisms such as inflammation, retinal tear, or posterior vitreous detachment for proliferation of glial or pigment epithelial elements to occur. In our previous studies of injury with blood injection epiretinal membranes consisting of glial and fibrous elements were observed on the peripheral and posterior retina. A prominent mononuclear response in these eyes occurred, and electron micrographs showed macrophages traversing the internal limiting membrane, possibly leaving defects through which glial elements could gain access to the surface of the retina. Humoral factors elaborated by the intense macrophage response may also have stimulated fibrogial proliferation, both in the vitreous and on the retina. Lens-injected eyes contained considerably fewer macrophages on histological section than blood-injected eyes. Also, posterior vitreous detachment occurred almost universally in blood-injected eyes but in only 2 of the lens-injected eyes, eliminating another possible stimulus to epiretinal membrane formation.

No clinically evident traction retinal detachments occurred in the present series of eyes, though microscopic evidence of vitreoretinal traction occurred posterior to the wound and at the peripheral retina 180° away. The absence of retinal detachment can be attributed to limited fibrous ingrowth from the wound and to the absence of periretinal membranes, which in earlier experiments combined to exert traction on the vitreous base and circumferential retinal traction.

The inflammatory response observed after injury and lens injection were limited to focal round-cell infiltration of the iris, ciliary body, optic nerve head, and a retinal perivasculitis. Scattered macrophages and foreign body giant cells were observed in the wound and fibrous ingrowth. These giant cells and a portion of the inflammatory exudate in the vitreous may have been a response to the refractile material observed in microscopic sections of the wound, fibrous ingrowth, and vitreous. These particles were not observed in the histology of previous series and were probably fragments of the mortar and pestle used to emulsify an injected lens.

Reactions resembling phacoanaphylaxis, sympathetic ophthalmia, or other lens-induced uveitis were not observed in this series. In fact the overall inflammatory response was less marked than would have been expected based on clinical studies of penetrating injuries with lens damage. It is recognised that penetrating injuries without lens damage may cause uveal and retinal inflammation similar to that observed in this series, and, moreover, the histology of animals with a standard wound and blood injection also showed a perivasculitis and uveitis. It is possible, however, in the lens-injected eyes that the inflammatory signs observed were the residua of an earlier, more prominent reaction.

Before concluding that vitreous-lens admixture may not be as serious as previously thought it must be realised that animal models of lens-induced uveitis have been difficult to produce. It is possible that species variability and the lack of presensitisation to lens protein may account for the lesser reaction. The uninjured in-situ lens and intact anterior vitreous face of our model are not analogous to most penetrating injuries with lens damage and may play a role in the minimal ocular response observed. Therefore further experiments with greater numbers and damage to the in-situ lens are indicated.

These experiments indicate that in the rhesus monkey eye vitreous-lens admixture is a much less potent stimulus to intravitreal fibroblastic proliferation and to traction retinal detachment than vitreous haemorrhage. The study reinforces our impression that periretinal membranes are a very important factor in inducing traction retinal detachment.

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