A New Model of Posterior Capsule Opacification in Rodents

Noemi Lois, Rosemary Dawson, Alastair D. McKinnon, and John V. Forrester

PURPOSE. To describe a new model of posterior capsule opacification (PCO) in rodents.

METHODS. An extracapsular lens extraction (ECLE), by continuous curvilinear capsulorhexis and hydrodissection, was performed in 42 consecutive Brown Norway rats. Animals were killed at 0, 6, and 24 hours and 3, 7, and 14 days after surgery. Eyes were enucleated and processed for light microscopy and immunohistochemistry.

RESULTS. In 34 (81%) of the animals the operated eye appeared well healed before death, with a clear cornea and a well-formed anterior chamber. In eight (19%) there was no view of anterior segment structures because of hyphema, fibrin, or corneal opacification. PCO was clinically evident 3 days after ECLE and was present in all animals at 2 weeks. Immediately after ECLE, lens epithelial cells (LECs) were present in the inner surface of the anterior capsule and lens bow. Twenty-four hours after surgery, LECs started to migrate toward the center of the posterior capsule. At 3 days, multilayered LECs, some spindle shaped, were present throughout the lens capsule. Capsular wrinkling was apparent. Lens fibers and Soemmerring’s ring were observed in all animals 14 days after surgery, indicating some degree of cellular differentiation. Activated macrophages were present in greater numbers at 3 and 14 days after surgery (P < 0.05), when proliferation and migration of LECs appeared to be greatest, and lens fiber differentiation was evident, respectively.

CONCLUSIONS. In rodents PCO occurs after ECLE and is associated with low-grade inflammation, mostly of mononuclear macrophages. Although no intracapsular lens implantation was performed, this model appears to be valuable for studying the sequence of events that leads to PCO after cataract surgery and the extracellular matrix cues that promote lens fiber differentiation. (Invest Ophthalmol Vis Sci. 2003;44:3450–3457) DOI:10.1167/iovs.02-1293

Posterior capsule opacification (PCO) remains the most common complication of cataract surgery. Although earlier clinical studies showed very high rates of PCO, as high as 50% in some series, recent data have disclosed a decrease in rates of PCO with new surgical techniques and intraocular lens (IOL) designs. Despite recent decreased rates of PCO, between 14.1% and 18.8% of patients require Nd-YAG posterior capsulotomy. This procedure not only has inherent risks (i.e., retinal detachment, ciliary macular edema, increased intraocular pressure) but also represents a considerable cost burden to national health care systems. Furthermore, rates of PCO and Nd-YAG posterior capsulotomy are expected to be higher in young individuals, particularly children, and in certain groups of patients, such as those with diabetic retinopathy and traumatic cataracts. Thus, a better understanding of the pathogenic mechanisms of PCO is still needed to eradicate this complication.

PCO seems to be caused by postoperative proliferation and migration of lens epithelial cells (LECs), collagen deposition, and lens fiber regeneration. It appears that epithelial cells from the anterior lens capsule, left behind after cataract surgery, can undergo fibrous metaplasia, and it is believed that these cells are predominantly responsible for the fibrosis-type of PCO. This type of PCO may cause significant visual loss by producing folds and wrinkles in the posterior capsule. Cells located at the equatorial lens region (lens bow) have been implicated in the development of the pearl-type form of PCO, responsible for most cases of PCO-related visual loss.

To date, the signals that stimulate LECs to proliferate, migrate, transdifferentiate, and produce collagen after cataract extraction are incompletely understood. Experimental studies using animal models of PCO and “in vitro” studies suggest that some cytokines may play a major role in the pathogenesis of PCO (summarized by Meacock et al. and Nishi). Thus, it appears that transforming growth factor (TGF)-β and basic fibroblastic growth factor (b-FGF) play a central role in the cell biology of PCO, modulating the proliferation of LECs and the deposition and contraction of collagen. Elucidating the role of these and other cytokines involved in the pathogenesis of PCO necessitates further investigation.

In this report, a new model of PCO in rodents is presented. The inflammatory response to the surgical procedure of lens extraction was studied, and its role in the development of PCO was investigated. Clinical, light microscopy, and immunohistochemistry studies were performed, and the findings are described herein.

METHODS

Animals

For this study, 42 Brown Norway adult (200–250 grams) male rats were used. All animal procedures were performed in accordance with Home Office (UK) regulations and the ARVO Statement for the Use of Animals in Ophthalmic and Vision Research.

Surgical Procedure

An extracapsular lens extraction (ECLE) was performed in the right eye of all 42 animals. All surgeries were performed consecutively. Animals were anesthetized with an intraperitoneal injection of ketamine hydrochloride (Vetalar; Pharmacia and Upjohn, Ltd., UK), at a dose of 60 mg/kg, and xylazine (Rompum; Bayer AG, Leverkusen, Germany), at a dose of 5 mg/kg. Pupils were dilated with 1% tropicamide (Chauvin Pharmaceuticals, Essex, UK) and 2.5% phenylephrine (Chauvin Pharmaceuticals). The surgery was performed as follows. A corneal incision...
was made with a keratome (Laseredge; Bausch & Lomb, Heidelberg, Germany; Fig. 1A). After the injection of 1% sodium hyaluronate in the AC, the corneal incision was extended to approximately 120° to 180° (B). An anterior curvilinear continuous capsulorrhexis was made (C) followed by hydrodissection and lens removal (D, E). Saline solution was then injected into the AC and capsular bag to assure adequate cleaning, and the AC was filled with sodium hyaluronate. The surgery was completed by suturing the corneal wound (F). At the end of the procedure, the eye appeared well healed, and fundus examination showed that the choroid and retina had remained flat (G). Clinically evident PCO was observed in all animals 14 days after ECLE (H).

Figure 1. Anterior segment photographs obtained through the operating microscope during ECLE in a Brown Norway rat. First, a corneal incision was made (A). After the injection of 1% sodium hyaluronate in the AC, the corneal incision was extended to approximately 120° to 180° (B). An anterior curvilinear continuous capsulorrhexis was made (C) followed by hydrodissection and lens removal (D, E). Saline solution was then injected into the AC and capsular bag to assure adequate cleaning, and the AC was filled with sodium hyaluronate. The surgery was completed by suturing the corneal wound (F). At the end of the procedure, the eye appeared well healed, and fundus examination showed that the choroid and retina had remained flat (G). Clinically evident PCO was observed in all animals 14 days after ECLE (H).

was made with a keratome (Laseredge; Bausch & Lomb, Heidelberg, Germany; Fig. 1A). After the injection of 1% sodium hyaluronate (Microvisc; Bohus BioTech, Stromstad, Sweden) in the anterior chamber (AC), the corneal incision was extended to approximately 120° to 180° with fine Vannas scissors (Storz, Heidelberg, Germany; Fig. 1B). An anterior curvilinear continuous capsulorrhexis was made with a capsulorrhexis forceps (Storz; Fig. 1C), followed by hydrodissection and lens removal (Figs. 1D, 1E). Saline solution was then injected in the AC and capsular bag to assure adequate cleaning, and the AC was filled with sodium hyaluronate. The surgery was completed by suturing the corneal wound with interrupted 9-0 nylon sutures (Ethicon, Brussels, Belgium; Figs. 1F, 1G). Topical chloramphenicol (Chauvin Pharmaceuticals) and cyclopentolate 1% (Chauvin Pharmaceuticals) were administered at the end of the surgery.

Animals were killed at 0 (after the surgery was completed), 6, and 24 hours, and at 3, 7, and 14 days after surgery, with a lethal dose of CO₂. Seven animals were killed at each time point and the right eye was enucleated and processed for light microscopy (n = 1) or immunohistochemistry (n = 6) studies.

Clinical Examination

Before death, animals were anesthetized and the anterior segment was evaluated by using the operating microscope. The status of the posterior capsule (presence or absence of opacification), cornea (clear or...
number of cells per eye was recorded. Section per eye was counted in a masked fashion, and the average

ED1, ED2, ED3, ED7, ED8, Ox8, Ox19, Ox34, W3/25, R73 Detects an antigen present in T-cell receptor

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animals. In seven of these animals, a few anterior synechiae were observed in the wound, and in five, very small amounts of fibrin were present in the AC but did not obscure the view of the iris or capsular bag. In one other animal, posterior synechiae were present at the pupillary margin, which prevented full dilation of the pupil.

In the remaining eight (19%) animals, postoperative complications were noted, including marked neovascularization in the wound (n = 6), corneal opacification (n = 6), hyphaema (n = 3), and large amounts of fibrin in the AC (n = 1; Table 2). In five eyes (two from the 3-day experiment, two from the 1-week experiment, and one from the 2-week experiment), the cornea was cloudy enough to obscure the view of the anterior segment structures.

PCO was noted first 3 days after ECLE. All eyes showed development of clinically evident PCO 2 weeks after surgery (Fig. 1H).

Histopathologic Findings

Light Microscopy. Immediately after ECLE, a monolayer of LECs was observed in the inner surface of the anterior capsule and at the lens bow (Fig. 2A). Scarce residual lens matter was present inside the capsular bag. The capsular bag appeared open, and there was no contact between the inner surface of the anterior and posterior lens capsule.

Six hours after ECLE, the anterior and posterior lens capsules were in contact around the site of the capsulorrhexis (Fig. 2B). No LECs were present in the center of the capsular bag, where only the posterior capsule was present. Scarce residual lens matter and some inflammatory cells were observed inside the capsular bag.

Twenty-four hours after surgery the space between the anterior and posterior lens capsules was filled with LECs (Figs. 2C, 2D). LECs migrated toward the center of the capsular bag, where only the posterior capsule was present, although no LECs were found at that location (Figs. 2E, 2F).

Three days after lens extraction, multiple layers of LECs filled the capsular bag, including its center (Fig. 2G). Some cells were spindle shaped. Wrinkling of the posterior capsule was evident. Inflammatory cells were present around the capsular bag and over the iris (Fig. 2H).

At 7 days, further changes had occurred in LEC morphology. Thus, cells lining the inner aspect of the posterior lens capsule appeared to show early changes of lens fiber differentiation, with oval nuclei lining up anteroposteriorly and migrating away from the basement membrane (Fig. 2I). Meanwhile, extensive vacuolation had taken place in the cells lining the

TABLE 1. Monoclonal Antibodies Used for Immunostaining of Ocular Cells

<table>
<thead>
<tr>
<th>Antibody</th>
<th>Specificity</th>
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<tbody>
<tr>
<td>ED1</td>
<td>Recognizes a single chain glycoprotein expressed by the majority of tissue macrophages and weakly by peripheral blood granulocytes</td>
</tr>
<tr>
<td>ED2</td>
<td>Reacts with a membrane antigen expressed by resident macrophages</td>
</tr>
<tr>
<td>ED3</td>
<td>Recognizes a membrane antigen (sialoadhesin) expressed by macrophages in autoimmune diseased tissues but not expressed in healthy tissues</td>
</tr>
<tr>
<td>ED7, ED8</td>
<td>Recognizes a membrane antigen (CR3) on macrophages, monocytes, dendritic cells, and granulocytes</td>
</tr>
<tr>
<td>Ox8</td>
<td>Recognizes an antigen present in a subset of T cells (CD8+) and NK cells</td>
</tr>
<tr>
<td>Ox19</td>
<td>Recognizes an antigen expressed by peripheral T cells and weakly by a subset of B cells</td>
</tr>
<tr>
<td>Ox34</td>
<td>Detects an antigen expressed by thymocytes and mature T cells</td>
</tr>
<tr>
<td>W3/25</td>
<td>Recognizes a membrane antigen (CD8+) and macrophages</td>
</tr>
<tr>
<td>R73</td>
<td>Detects an antigen present in T-cell receptor</td>
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Histology and Immunohistology

For light microscopy studies, eyes were fixed in 2.5% glutaraldehyde and processed to glycol methacrylate (GMA) resin (Histocryl; London Resin Company, Reading, UK). Then 2.5-μm sections were cut at three levels through the midpoint of the eye and stained with hematoxylin and eosin (H&E). For immunohistochemistry, eyes were embedded in optimal cutting temperature (OCT) compound, snap frozen, and stored at −80°C. Cryostat sections (8–10 μm) of tissues were placed on poly-l-lysine-coated slides at −20°C, air dried, and fixed in acetone. They were then rehydrated in Tris-buffered saline (TBS) and incubated in the primary antibody. The following primary antibodies were used: ED1, ED2, ED3, ED7, ED8, Ox8, Ox19, Ox34, W3/25, R73, vimentin, and α-smooth muscle actin (α-sma; Table 1). After two 5-minute washes, a secondary biotinylated rabbit anti-mouse antibody (Dako, Glostrup, Denmark) was added for 30 minutes, followed by further washes. Sections were then incubated with streptavidin avidin-biotin complex alkaline phosphatase (AP) for 30 minutes at room temperature, washed in TBS, and rinsed briefly in distilled water. This was followed by the addition of the substrate and further rinsing in distilled water. Sections were then counterstained with hematoxylin. Washed sections were mounted and viewed under a microscope. Positive-stained cells were counted in three 40× fields per eye studied. One section per eye was counted in a masked fashion, and the average number of cells per eye was recorded.

Statistical Analysis

Student’s t-test was used to evaluate differences in number of cell at all time points. For statistical analysis, the number of cells at 0 hours was compared with that at 6 and 24 hours and 3, 7, and 14 days. Differences were considered to be statistically significant at P < 0.05.

RESULTS

Surgical Procedure

The surgical procedure was uncomplicated in 35 (83%) of the animals. In seven (17%) some bleeding from the iris occurred at the time of the wound closure. In three of these, the bleeding was very limited, and in all it was successfully controlled during the surgery. In no case did posterior capsule rupture, vitreous loss, hyphema, or corneal edema occur. The eye appeared well-formed at the end of the procedure in all animals.

Postoperative Clinical Course

In 34 (81%) animals the eye appeared well healed, with a clear cornea and a deep AC before death (Table 2). The wound appeared variably opaque, with no or mild neovascularization. In seven of these animals, a few anterior synechiae were observed in the wound, and in five, very small amounts of fibrin were present in the AC but did not obscure the view of the iris or capsular bag. In one other animal, posterior synechiae were present at the pupillary margin, which prevented full dilation of the pupil.

In the remaining eight (19%) animals, postoperative complications were noted, including marked neovascularization in the wound (n = 6), corneal opacification (n = 6), hyphaema (n = 3), and large amounts of fibrin in the AC (n = 1; Table 2). In five eyes (two from the 3-day experiment, two from the 1-week experiment, and one from the 2-week experiment), the cornea was cloudy enough to obscure the view of the anterior segment structures.
inner aspect of the anterior capsule, suggesting that extensive cell death had occurred. Only occasional inflammatory cells were seen.

By 2 weeks, the early lens fibers on the posterior capsule had elongated extensively, and their nuclei were now positioned in the center of the cell, which had become a well-differentiated lens fiber (Fig. 2J). A Soemmering’s ring had developed in the periphery of the lens. Evidence of vacuolation was still present in the anterior LEC and at both ends of the lens fibers.

LECs were not observed over the outer surface of the anterior capsule in any of the specimens studied, and it seemed that migration of the cells stopped at the edge of the capsulorrhexis, where they contacted the anterior lens capsule (Fig. 2F, 2G).

**Immunohistochemistry.** Inflammatory cells were evaluated by immunohistochemistry, with a wide range of cell markers (Table 1).

The number of blood-borne (ED1-positive) and activated (ED7- and ED8-positive) macrophages was significantly higher ($P = 0.02$, $P = 0.01$, $P = 0.01$, respectively) 3 days after ECLE (Fig. 3A). A statistically significant increase in the number of ED8-positive activated macrophages was also detected at 7 and 14 days after ECLE ($P = 0.04$; Fig. 3A). Similarly, the number of ED7-positive activated macrophages was also significantly higher 14 days after surgery ($P = 0.03$). Very scarce sialoadhesive-positive macrophages (ED3-positive) were present (Fig. 3A).

Although few T cells were found at all time points studied, the number of T-cells seemed to peak also at 3 days after ECLE (Fig. 4A). However, there was no statistically significant change in the number of T cells (Fig. 4A).

When eyes with hyphema and large amounts of fibrin in the AC (animals 20, 30, 33, and 40) were removed from the analysis,
the findings remained unchanged, with the exception of the increased number of ED8-positive activated macrophages at 7 days, which was not statistically significant (Figs. 3B, 4B).

Vimentin was immunolocalized to all cells at all time points. Immunoreactivity to α-sm actin was not observed at any of the time points studied.

DISCUSSION

Despite advances in surgical techniques of cataract extraction and in IOL material and design, PCO remains the most common complication of cataract extraction and the most common cause of visual failure after successful surgery. Further knowledge of the pathogenic mechanisms involved in its occurrence is still needed, and better ways to prevent or treat this complication should be sought. In this regard, the rodent model of PCO would be valuable as a rapid screening method for possible pharmacological preventive treatments that could be applied during or after the surgery. Furthermore, and despite the small size of the eye, this model could be used also to evaluate new materials and designs for IOL and new surgical maneuvers that could reduce or prevent the occurrence of PCO.

The experimental model of PCO in rodents has several advantages in comparison with others currently available.
These include the shorter time required for the development of PCO, the low cost and easy handling of these animals, and the possibility of studying in great detail all the tissue available, given the small size of the eye. The marked fibrinous reaction usually observed in the cataract and rabbit models of PCO is infrequently seen in rodents (Table 2). In contrast to “in vitro” models of LEC proliferation, in which new lens material is generally not observed, new lens fibers and new lens formation were evident in the rodent model of PCO only 2 weeks after ECLE. Because lens regeneration reproducibly occurred in all animals in this study, it seems that this model could be used to mimic some of the events that take place during the embryonic development of the lens.

In the present study, IOL implantation was not performed. Thus, it is not clear whether findings described herein also would be observed if an IOL had been placed in the capsular bag. Migration and proliferation of LECs as well as other later events (i.e., new lens fiber formation) occur in eyes with IOLs. It seems, however, that PCO occurs more frequently after ECLE in eyes without IOLs. Furthermore, the proliferative LEC response appears to be exaggerated in the empty capsular bag. In this respect, it has been proposed that an IOL in the bag would reduce the space available for growth and migration of LECs. Thus, it would be expected that IOL implantation in the rodent model of PCO would have diminished, but not eliminated, the risk of development of PCO. Further studies using IOL implantation in this rodent model of PCO are warranted.

It appears that LECs can migrate into the posterior capsule, acquire a spindle-shaped appearance and produce basement membrane and collagen. It is believed that these cells undergo a process of epithelial-mesenchymal transition (EMT), acquiring a myofibroblastic phenotype and expressing α-smooth muscle actin (α-smactin). Myofibroblastic cells expressing α-smactin have been observed in the adhesive region (where the incised edge of the anterior capsule adheres to the posterior capsule) of the capsular bag 5 days after cataract extraction in rabbits with aphakic eyes. Myofibroblastic cells have been associated with wrinkling and opacification of the posterior capsule. In the present study, spindle-shaped cells were observed in the adhesive region and in areas where posterior capsular wrinkling was evident. However, immunostaining for α-smactin was negative in the capsular bag at all time points studied. It is unlikely that this was the result of a technical problem, because positive staining for α-smactin was detected in the sphincter and dilator muscles of the iris. Given that these cells are only seen transiently, it is still possible that in this study the time of death did not coincide with the time at which most α-smactin-positive cells are present.

During lens development, the LECs present in the posterior half of the lens vesicle elongate and differentiate into lens fibers, whereas those located in the anterior half of the lens differentiate into the anterior lens epithelium. The signals involved in the process of cell differentiation are at present unclear. It seems that the close relationship of the LEC with the AC or vitreous cavity may modulate this differentiation. Thus, when the lens is inverted 180° and the anterior LECs face the vitreous cavity, these cells elongate and differentiate into lens fibers. Growth factors, such as FGF, may play a role in lens fiber differentiation. It has been shown that FGF induces lens fiber differentiation in vitro, and FGF seems to be present at higher concentrations in the vitreous than in the aqueous humor. In the rodent model of PCO, lens differentiation was observed in all animals only 2 weeks after ECLE. Hence, it would be possible to use this model to study the signals involved in the process of lens fiber differentiation.

There is controversy in the literature regarding the possible role of inflammation in the development of PCO. Indirect evidence suggests that an active inflammatory process may occur. Thus, children and youngsters, who usually have a marked inflammatory-fibrin response after cataract extraction, have higher rates of PCO than adults. Moreover, a chronic inflammatory reaction, signaled by the presence of monocytes and macrophages, has been detected after successful cataract extraction and IOL implantation. Furthermore, anti-inflammatory drugs, including diclofenac and indomethacin, have been shown to inhibit LEC proliferation in vitro and in experimental animal models of PCO. Other studies, how-

**Figure 3.** Macrophagic response after ECLE in a rat model of PCO. (A) The number of ED1-, ED7-, and ED8-positive cells appeared to peak 3 days after ECLE. Very few ED3-positive cells were present at all time points. Histograms correspond to the mean number of cells counted in three ×40 fields per animal (n = 6) at each time point. (B) When eyes with hyphema and large amounts of fibrin in the AC (animals 20, 30, 33, and 40) were removed from the analysis, findings remained unchanged, with the exception of the increased number of ED8-positive activated macrophages at 7 days, which was not statistically significant. *Statistically significant, P < 0.05.

**Figure 4.** (A) T-cell response after ECLE in a rat model of PCO. Although the number of Ox8-, Ox34-, and Ox19-positive cells appeared to peak at 5 days after ECLE, differences were not statistically significant. Plots correspond to the mean number of cells counted in three ×40 fields per animal (n = 6) at each time point. (B) Findings were not modified by removing from the analysis those eyes with large amounts of fibrin in the AC and hyphema (animals 20, 30, 33, and 40).
ever, have not supported these findings. Thus, sustained administration of indomethacin failed to reduce the development of PCO in rabbits.39,40

A low-grade inflammatory response, mostly of mononuclear macrophages, was observed in the rodent model of PCO. This inflammatory response appeared to be at its maximum at 3 days after ECLE, coinciding with maximum proliferation and migration of LECs in the capsular bag and capsular wrinkling. Furthermore, the number of activated macrophages (ED7- and ED8-positive) was also higher 14 days after surgery, when lens fiber differentiation was evident. Although few T cells were detected, their number seemed similarly higher 3 days after cataract surgery, but the difference was not statistically significant.

It is possible that macrophages and T cells play a role in the formation of PCO. Thus, activated macrophages can synthesize TGF-β.11 Experimental studies have supported the role of TGF-β in the development of cataracts and PCO. It has been shown that TGF-β induces cataractous changes in the lens, including anterior lens opacities, formation of spindle-shaped cells, and capsule wrinkling.42,43 It seems that TGF-β inhibits proliferation of LECs,15 but stimulates the production of collagen by LECs,21 increases α-sm expression in LECs,17 and promotes the contracture of collagen.14,17 When added to an in vitro capsular bag model, TGF-β promotes the contraction of collagen.14,41 Experimental studies have supported the role of TGF-β in the development of PCO, further studies in which these cells are selectively inhibited are under way.

The feasibility of a successful removal of the lens in rodents may allow the use of these animals in other areas of eye research previously restricted by the large size of the lens in this species.

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References


