Vivo Rodent Model of Posterior Capsule Opacification

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Purpose. This study evaluated the effect of transforming growth factor (TGF-β2) and anti-TGF-β2 antibody in a rodent model of posterior capsule opacification (PCO).

Methods. An extracapsular lens extraction (ECLE) was performed in 72 Sprague-Dawley rats. At the end of the procedure, 10 μL TGF-β2 (TGF-β2-treated group), fetal calf serum (FCS)/phosphate-buffered saline (PBS; FCS/PBS-treated control group), a human monoclonal TGF-β2 antibody (anti-TGF-β2-treated group), or a null control IgG4 antibody (null antibody-treated control group) was injected into the capsule. Animals were killed 3 and 14 days postoperatively. Eyes were evaluated clinically prior to euthanization, then enucleated and processed for light microscopy and immunohistochemistry afterward. PCO was evaluated clinically and histopathologically. Student’s t-test and χ² were used to assess differences between groups.

Results. There were no statistically significant clinical or histopathological differences in degree of PCO between the TGF-β2- and FCS/PBS-treated groups at 3 and 14 days after ECLE. Nor were there differences between the anti-TGF-β2- and the null antibody-treated groups, with the exception of the histopathology score for capsule wrinkling 3 days after ECLE (P = 0.02). α-Smooth-muscle actin staining was observed in the lens capsular bag only in areas where there was close contact with the iris.

Conclusions. No sustained effect of TGF-β2 or anti–TGF-β2 antibody on PCO was found in rodents at the dose and timing administered in this study. Iris cells may play a role in the process of epithelial mesenchymal transition linked to PCO. (Invest Ophthalmol Vis Sci. 2005;46:4260–4266) DOI:10.1167/iovs.05-0168

Posterior capsule opacification (PCO) is the most common cause of visual loss after successful cataract surgery. To restore vision in these cases, Nd:YAG laser posterior capsulotomy is required. This latter procedure has inherent risks, including retinal detachment, cystoid macular edema, and increased intraocular pressure, and it is expensive. Furthermore, in most underdeveloped countries this treatment is not available, and PCO is now becoming the most common cause of visual loss, only surpassed by the cataract itself. Lens epithelial cell (LEC) proliferation, collagen deposition and contraction, and new lens fiber formation have been considered the major events that lead to PCO.

Previous experimental studies support the role of transforming growth factor (TGF-β) in the development of cataracts and PCO. Thus, it has been shown that TGF-β induces cataractous changes in the lens, including anterior subcapsular lens opacities, accumulation of extracellular matrix, formation of spindle-shaped cells, and capsule wrinkling. It has been demonstrated also that TGF-β inhibits LEC proliferation in vitro, but stimulates the production of collagen by LEC, increases the expression of the major extracellular matrix proteins and proteoglycans, and increases α-smooth-muscle actin expression in LEC. Furthermore, when added to an in vitro capsular bag model, TGF-β stimulated the contraction of the capsular bag. Since most of the above cellular phenomena are known to take place during the development of PCO, it is likely that TGF-β may play a role in the occurrence of this complication.

The purpose of the present study was to evaluate the effect of TGF-β2 and of an anti–TGF-β2 antibody in a new in vivo rodent model of PCO.

Methods

Animals

Sprague-Dawley male adult rats (200–250 g; Harlan, Oxon, UK) were used.

Surgical Procedure and Interventions

All animal procedures were performed in accordance to Home Office (UK) regulations and the ARVO Statement for the Use of Animals in Ophthalmic and Vision Research. Institutional guidelines regarding animal experimentation were followed.

An extracapsular lens extraction (ECLE) was performed in the right eye of all animals. All surgeries were done consecutively. Animals were anesthetized using intraperitoneal injection of 60 mg/kg ketamine hydrochloride (Vetalar; Pharmacia & Upjohn Ltd., Southampton, UK) and 5 mg/kg xylazine (Rompun; Bayer, Kiel, Germany). Pupils were dilated using 1% tropicamide (Chauvin, Essex, UK) and 2.5% phenylephrine (Chauvin). The surgery was performed as previously described. Briefly, a corneal incision was made followed by injection of 1% sodium hyaluronate (Micravis; Bohus BioTech, Bjorko, Sweden) into the anterior chamber (AC). Once the corneal incision was extended, an anterior curvilinear continuous capsulorrhexis was done, followed by hydrodissection and lens removal. The AC was then filled with 2.3% sodium hyaluronate (Healon 5; Alcon, London, UK) and 5 mg/kg xylazine (Rompun; Bayer, Kiel, Germany). Pupils were dilated using 1% tropicamide (Chauvin, Essex, UK) and 2.5% phenylephrine (Chauvin). The surgery was performed as previously described. Briefly, a corneal incision was made followed by injection of 1% sodium hyaluronate (Micravis; Bohus BioTech, Bjorko, Sweden) into the anterior chamber (AC). Once the corneal incision was extended, an anterior curvilinear continuous capsulorrhexis was done, followed by hydrodissection and lens removal. The AC was then filled with 2.3% sodium hyaluronate (Healon 5; Pfizer, Tadworth, Surrey, UK) and the corneal wound was sutured using interrupted sutures. Then, through a small gap in the corneal wound, a bubble of air was injected into the capsular bag, as described by Holmen and colleagues (Holmen JB, et al. JOVS 2003;44:ARVO E-Abstract 283) to achieve an adequate opening of the bag before the injection of different solutions (see below). Topical 2.5% phenylephrine (Chauvin), 1% tropicamide (Chauvin), and 1% atropine (Chauvin) were administered at the end of the surgery.

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Effect of TGF-β2 and Anti–TGF-β2 Antibody in a New In Vivo Rodent Model of Posterior Capsule Opacification

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The following solutions (10 μL) were injected into the capsular bag: TGF-β2 (recombinant human TGF-β2) diluted in 2% fetal calf serum (FCS)/phosphate buffered saline (PBS) to 1 ng/mL (TGF-β2–treated group); 2% FCS/PBS (FCS/PBS-treated control group); a human monoclonal TGF-β2 antibody (CAT-152, lerdelimumab; Cambridge Antibody Technology, Cambridge, UK) (anti–TGF-β2–treated group), 1 mg/mL; or a null control IgG4 antibody (CAT-001; Cambridge Antibody Technology) (null antibody–treated control group), 5.2 mg/mL.

Six animals each from the TGF-β2– and FCS/PBS–treated groups were killed at 3 and 14 days postoperatively. Twelve animals each from the anti–TGF-β2– and null antibody–treated groups were killed at 3 and 14 days after ECLE.

Clinical Evaluation
Before the animals were killed, the degree of PCO was assessed clinically under the operating microscope. In the first group of experiments (TGF-β2– and FCS/PBS–treated groups) only the presence or absence of PCO was recorded. In the second group of experiments (anti–TGF-β2– and null antibody–treated groups), PCO was graded with regard to the presence of a central area of opacification in the capsular bag and wrinkles in the posterior capsule, on a four-point scale: none, mild, moderate, and severe. In both sets of experiments, the status of the cornea (clear/opaque), corneal wound (presence/absence of neovascularization and iris synechiae into the wound), iris (presence/absence of posterior synechiae), and pupil (good/poor dilation) was also recorded. The clinical evaluation was done, in all animals, by the same investigator (NL) in a masked fashion.

Light Microscopy Studies
Animals were killed using a lethal dose of CO₂. For light microscopy studies and in the first group of experiments (TGF-β2– and FCS/PBS–treated groups) only the presence or absence of PCO was recorded. In the second group of experiments (anti–TGF-β2– and null antibody–treated groups), PCO was graded with regard to the presence of a central area of opacification in the capsular bag and wrinkles in the posterior capsule, on a four-point scale: none, mild, moderate, and severe. In both sets of experiments, the status of the cornea (clear/opaque), corneal wound (presence/absence of neovascularization and iris synechiae into the wound), iris (presence/absence of posterior synechiae), and pupil (good/poor dilation) was also recorded. The clinical evaluation was done, in all animals, by the same investigator (NL) in a masked fashion.

Histopathologic Evaluation
Qualitative Assessment. In the first group of experiments (TGF-β2– and FCS/PBS–treated groups), the degree of PCO with respect to capsular wrinkling, LEC proliferation (as detected in the center of the capsular bag, where no anterior capsule was present), and Soemmerring’s ring formation was graded as mild (1), moderate (2), or severe (3) (Fig. 1). In the second group of experiments (anti–TGF-β2– and null antibody–treated groups), in addition to the above categories, the degree of PCO was graded as none (0) whenever capsular wrinkling, LEC proliferation, or Soemmerring’s ring formation was less than that shown in Figure 1.

Quantitative Assessment. Using a custom-designed computer program (written using Aphelion ActiveX objects; ADCIS SA, France), a quantitative analysis of LEC at the center of the capsular bag was undertaken. Sections were visualized using a microscope (Zeiss Axioskop), and images were captured using a digital camera (Progress C14 CCD) linked to an image analysis workstation. Cell counts were determined semiautomatically using the custom program. First, the area of interest was manually outlined on the image. Then, cell nuclei were identified using a combination of gray-level thresholding and identification of local minima in the image, followed by logical filtering of the resulting objects on the basis of size and shape. The resulting object set was displayed overlaid on the original image for verification by the operator (Fig. 2).

The qualitative and quantitative evaluation of PCO was done by a single observer (NL) in a masked fashion.

Immunohistochemistry
In the second group of experiments (anti–TGF-β2– and null antibody–treated groups), six eyes in each group and at each time point were processed as explained above. Three sections per eye were obtained through the midpoint of the eye (taking the optic nerve as a reference point), stained with toluidine blue, and assessed qualitatively and quantitatively (see below) for the degree of PCO.
14 days) were processed for immunohistochemistry. Eyes were embedded in optimal cutting temperature compound, snap frozen, and stored at −80 °C. Cryostat sections (8 to 10 μm) of tissues were taken onto 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, ED7, ED8, OX8, W3/25, and α-sma. After two 5-minute washes, a secondary biotinylated rabbit anti-mouse antibody, E0354 (Dako, Glostrup, Denmark), at a dilution of 1:200 and 10% rat serum was added for 30 minutes, followed by further washes. Sections were then incubated with streptavidin (ABComplex AP; Dako) 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 the microscope. For α-sma, the antibody was used at a dilution of 1:150.

Positive stained inflammatory cells were counted in three 40× fields per eye studied. One section per eye was counted. The average number of cells per eye was recorded. Staining for α-sma was graded as none (0), mild (1), moderate (2), or severe (3). Cell counts and α-sma grading were done by a single observer (NL) in a masked fashion.

Statistical Analysis

Degree of PCO. PCO scores (on clinical and histopathological evaluation) were compared between TGF-β2- and FCS/PBS-treated groups and between anti-TGF-β2- and null antibody-treated groups using χ². Cell counts were compared between TGF-β2- and FCS/PBS-treated groups and between anti-TGF-β2- and null antibody-treated groups using Student’s t-test. Differences were considered significant at values of P < 0.05.

Inflammatory Response and α-sma Immunoreactivity. Cell counts were compared between anti-TGF-β2- and null antibody-treated groups using Student’s t-test. Grading for α-sma was compared between anti-TGF-β2- and null antibody-treated groups using χ². Differences were considered significant at values of P < 0.05.

RESULTS

Surgical Procedure

The surgery was uncomplicated in 63 animals (87%). In nine animals (12%), a small self-contained bleeding occurred, either after lens removal or at the end of the procedure, when suturing the wound. In no case was there any hyphema observed at the end of the surgery.

Postoperative Clinical Evaluation

The status of the cornea, AC, iris, and pupil before the animals were killed is summarized for all groups in Table 1. In all cases, some degree of neovascularization into the wound was observed.

TGF-β2- and FCS/PBS–Treated Groups. Three days after ECLE, PCO was detected in the form of wrinkles or thickening in the posterior capsule in three animals in the control group and in one in the TGF-β2–treated group. This difference was not significant (P = 0.29). No adequate view of the posterior capsule was achieved in two animals in the TGF-β2–treated group (Table 1).

At 2 weeks, PCO was present in all animals, with no significant differences between groups.

Anti–TGF-β2– and Null Antibody–Treated Groups. No significant difference in the clinical scores for central opacification of the posterior capsule was observed between groups at 3 or 14 days postoperatively (P = 0.66 and P = 0.09, respectively; Fig. 3). Similarly, there was no significant difference in the clinical scores for capsular wrinkling between the groups at 3 or 14 days after ECLE (P = 0.38 and P = 0.27, respectively; Fig. 3). In three animals in the anti-TGF-β2–treated group and in two animals in the control group, there was no view of the posterior capsule 3 days after ECLE (Table 1).

Postoperative Histopathologic Evaluation

Qualitative Evaluation. No significant differences in the histopathology scores for capsular wrinkling, LEC prolifera-
TABLE 1.
Postoperative Clinical Evaluation in a Rodent Model of PCO after Treatment with TGF-β2, FCS/PBS, Anti–TGF-β2 Antibody and Null Antibody

<table>
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<tr>
<th>View of PC</th>
<th>Cornea</th>
<th>AC Pupil Dilation</th>
<th>iris Synechiae</th>
<th>Postoperative Hyphema</th>
<th>Complete Ectropion</th>
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<th>Poor (n)</th>
<th>Complete Ectropion (n)</th>
<th>Incomplete Ectropion (n)</th>
<th>Hyphema Deep (n)</th>
<th>Hyphema Peripheral (n)</th>
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**Inflammatory Response and α-sma Staining**

No significant differences in the number of inflammatory cells (stained with ED1, ED2, ED7, ED8, OX8, and W3/25) were detected at 3 days ($P = 0.69$, $P = 0.75$, $P = 1$, $P = 0.83$, $P = 0.63$, and $P = 0.86$, respectively) and 14 days ($P = 0.24$, $P = 0.20$, $P = 0.28$, $P = 0.26$, $P = 0.36$, and $P = 0.29$, respectively) between the anti–TGF-β2- and null antibody–treated groups.

No significant differences in the scoring for α-sma staining were found at 3 or 14 days between the anti–TGF-β2-treated and the null antibody-treated control groups ($P = 0.4$ and $P = 0.1$, respectively).

**Discussion**

TGF-β is a member of a superfamily of growth and differentiation factors. There are several TGF-β isoforms, of which TGF-β1, TGF-β2, and TGF-β3 have been identified in mammals. TGF-β1 and TGF-β2 have been detected in primary human LEC cultures, using RT-PCR techniques and in situ hybridization. TGF-β2 is the predominant form in aqueous humor. TGF-β exists in precursor, latent, and bioactive forms. Once active TGF-β is released from the latent complex, it can be bound by various extracellular matrix components and serum proteins, which may protect TGF-β from degradation and function as a long-term reservoir, sustained release mechanism, or TGF-β clearance system.

Levels of active TGF-β decrease in aqueous humor immediately after cataract extraction and return to normal values a few days after the procedure. Since TGF-β2 inhibits LEC proliferation, at least in vitro, it could be hypothesized that the decreased levels of this growth factor postoperatively could facilitate LEC proliferation after cataract surgery and PCO formation. If this were the case, a reduction in the number of LECs in the capsular bag and in the degree of PCO would be expected after intracapsular injection of TGF-β2 immediately after lens removal, as was done in the present study. However,
we failed to find a significant difference in the number of LECs and the degree of PCO between control and TGF-β2–treated groups at 3 and 14 days after ECLE. Alternatively, it might be possible that the effects of TGF-β2 previously reported (inducing accumulation of extracellular matrix, formation of spindle-shaped cells, and capsule wrinkling)⁴⁻⁶ could promote the development of PCO. Then TGF-β2–treated animals should have increased PCO formation. This was not observed in the present study.

It has been shown that the effect of TGF-β of inducing cataract-like changes in rat lens epithelial explants is dependent on the age of the donor animal.⁶⁻¹¹ Adult rat explants developed cataract-like changes after being exposed to TGF-β alone, whereas this was not the case in explants obtained from 10-day-old rats. In the present study, adult rats were used; thus, a response to TGF-β would be expected. The lack of effect of TGF-β2 observed in the rodent model of PCO could be related to an insufficient availability of this molecule to LECs. This latter possibility seems unlikely, however, since the capsular bag was well inflated with a bubble of air before the injection of TGF-β2, and the AC was filled with very viscous sodium hyaluronate (2.3%), which helped to maintain the TGF-β2 inside the capsular bag. It seems improbable also that the lack of response to TGF-β2 could be related to an insufficient dose of these molecules, since LEC proliferation in vitro occurred with a dose of 1 ng/mL TGF-β2⁷⁻⁸ similar to that used in the present study. In vitro, however, LECs would have been exposed continuously to these molecules, whereas in vivo degradation or inactivation of TGF-β2 could have occurred. This could explain the different responses observed in vivo and in vitro. It is also possible that TGF-β2 alone may not have a significant effect on LEC behavior in rodents. In this regard, Garcia and colleagues (Garcia CM, et al. IOVS 2004;45:ARVO E-Abstract 2331) have recently reported that signaling through TGF-βRII is not required for normal lens development or myofibroblastic formation in mice.

Despite the lack of effect of TGF-β2 on PCO formation, an effect of anti–TGF-β2 antibody on the development of capsular

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**FIGURE 3.** Histograms of clinical scores for central opacification of the posterior capsule and capsular wrinkling at 3 and 14 days in the anti–TGF-β2– and null antibody–treated groups.

**FIGURE 4.** Light microscopy specimens obtained 3 days (top) and 14 days (bottom) after ECLE from animals from the TGF-β2– (top and bottom, right) and FCS/PBS–treated groups (top and bottom, left) (toluidine blue, ×10).

**FIGURE 5.** Light microscopy specimens obtained 3 days (top) and 14 days (bottom) after ECLE from animals from the anti–TGF-β2– (top and bottom, right) and null antibody–treated groups (top and bottom, left) (toluidine blue, ×10).
wrinkling was observed 3 days after ECLE in the present study. The development of wrinkles in the posterior capsule appears to be the result of LEC/myofibroblast migration or LEC/myofibroblast-induced collagen contraction. In this regard, it has been shown that anti-TGF-β2 antibody inhibits TGF-β2-stimulated fibroblast-mediated contraction, proliferation, and migration.22 Furthermore, it has been shown that anti-TGF-β2 antibody inhibits the TGF-β2-stimulated wrinkling of the capsular bag in an in vitro capsular bag model of PCO.13 The lack of sustained effect of TGF-β2 antibody on capsular wrinkling 14 days after ECLE in the rodent model of PCO may have been related to a decreasing levels of this molecule over time.

Epithelial mesenchymal transition (EMT) has been implicated in the development of PCO. It has been proposed that through a process of EMT, LECs transdifferentiate into myofibroblasts, which express α-sma. Myofibroblasts are able to contract and secrete extracellular matrix components; thus, these cells could be responsible for the production of wrinkles and thickening in the posterior capsule, both features of PCO. Only mild α-sma staining was found in the center of the capsular bag or at the site of the capsulorrhexis in the rodent model of PCO. This confirms our previous findings.13,25 However, marked α-sma staining was detected in the iris and in those areas of the capsular bag that were in close apposition with the iris. Usually, this occurred at the site of the capsulorrhexis, where the iris seemed to form synechiae or synechiae-like structures with the incised anterior lens capsule. To our knowledge, this observation has not been previously reported.24-25 Therefore, it could be hypothesized that iris cells may play a role in the process of EMT, stimulating LECs to undergo transdifferentiation into myofibroblasts. Alternatively, the positive α-sma cells observed in the capsular bag may represent iris cells that have migrated into the anterior surface of the posterior capsule, contributing to PCO.

In all eyes treated with anti-TGF-β2 antibody and anti-TGF-β2 null antibody, there was some degree of corneal edema 3 days after surgery, although it persisted 14 days postoperatively in only one case. It is possible that this transient corneal edema could be related to an increased intraocular pressure (IOP) after the injection of anti-TGF-β2 antibody or anti-TGF-β2 null antibody and 2.3% sodium hyaluronate at the time of the surgery. Subsequent normalization of the IOP, a result of the decreasing levels of these molecules over time, would have been followed by a parallel decrease and disappearance of the corneal edema. A transient increase in IOP would also explain the frequent presence of posterior synechiae in anti-TGF-β2 antibody and anti-TGF-β2 null antibody groups, since posterior synechiae can be observed in eyes with acutely high intraocular pressure related to a breakdown in the blood–ocular barrier and subsequent intraocular inflammation. Since both corneal edema and iris synechiae seemed to occur in both anti-TGF-β2 antibody and anti-TGF-β2 null antibody groups, it is unlikely that they were caused by the antibody molecule itself, but rather by the vehicle in which these molecules were prepared. Despite the high concentration of anti-TGF-β2 used in this study, no statistically significant difference in the inflammatory response was detected between the anti-TGF-β2 treated group and the null antibody-treated control group in this rodent model of PCO. This finding may be of importance clinically, given the potential applications of this molecule to treat other eye diseases, such as glaucoma and proliferative vitreoretinopathy.

In summary, no sustained effect of TGF-β2 or anti-TGF-β2 antibody on PCO formation was found in rodents at the dose and timing administered in this study. This is, to our knowledge, the first time that the short- and long-term effects of these molecules (administered in a single injection inside the lens capsular bag at the end of the cataract surgery) on capsular wrinkling and LEC proliferation have been studied in vivo, and may have implications in the development of potential treatments for PCO.
References