Effects of dexamethasone on posterior capsule opacification-like changes in a rat lens explant model

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Purpose: Many patients whose sight is initially restored by cataract surgery eventually suffer secondary loss of vision because of posterior capsule opacification (PCO; after-cataract), a condition in which lens epithelial cells left behind at surgery become aberrant and migrate into the light path. The aim of this study was to determine whether dexamethasone (DEX), an anti-inflammatory agent widely used before and after cataract surgery, influences the behavior of lens cells under conditions relevant to PCO development.

Methods: An established rat PCO model was used in which explanted epithelial cells attached to the lens capsule are exposed sequentially to TGFβ2 and FGF-2. Cultures with or without DEX (100 nM), and appropriate controls, were maintained for up to 30 days and assessed by light and scanning electron microscopy or immunolocalization of PCO markers (α-smooth muscle actin or fibronectin) or a marker for lens epithelial cell phenotype (Pax-6).

Results: In the absence of DEX, explants become multilayered and plaques that express PCO markers form. Cells tend to gather up into the plaques, leaving the surrounding lens capsule denuded. Changes in lens cell behavior with addition of DEX included rapid formation of long, needle-like cells, less extracellular matrix deposited on explant surface, and plaques surrounded by a monolayer of migratory cells. Immunolocalization confirmed that the latter were not normal lens epithelial cells.

Conclusions: Lens cell behavior in this PCO model was significantly affected by inclusion of DEX, highlighting the possibility that its use as an anti-inflammatory at the time of cataract surgery may influence PCO development.

Cataract, or loss of lens transparency, is a leading cause of blindness. Cataract associated with aging is by far the most common form, with prevalence rising from about 25% to 100% between the ages of 60-69 and 90+ years [1,2]. At present the only treatment available is surgery. The preferred procedure involves removing a circular, anterior portion of the thick capsule that encloses the lens, breaking up and removing the fiber mass it contains, and placing a synthetic lens implant (IOL) into the empty capsular “bag”. However, lens epithelial cells that are left behind during this procedure can become abnormal, multiply, and spread forming opacities that consist of multilayered fibrotic plaques, strands of cells containing abnormally deposited extracellular matrix (ECM), or aggregations of enlarged cells (Elschnig’s pearls). This condition is known as posterior capsule opacification (PCO) or after-cataract [3-7]. Within five years of cataract surgery, some 20-40% of patients suffer further loss of vision because of PCO [8]. Vision can be restored by Nd:YAG laser treatment, but the procedure is not without risk to sight and adds substantially to the costs of treating cataract [4]. New technology IOLs, state of the art equipment and highly skilled surgeons are helping to reduce the incidence of PCO, but these are not available to everyone, especially in the developing world where cataract is a major health problem [4]. Prevention or reduction of the severity of PCO thus remains an important goal.

Many studies now point to transforming growth factor-beta (TGFβ) as a key player in early events associated with the development of PCO and certain forms of naturally occurring human cataract, a possibility first highlighted by the findings of Liu et al. [9]. As reviewed elsewhere [10], both in vitro and in vivo, TGFβ rapidly induces lens epithelial cells from humans, rats and other mammalian species to undergo epithelial-mesenchymal transition. The resulting fibroblastic and spindle-like myofibroblastic cells express proteins not normally found in lens cells, such as α-smooth muscle actin, types I and III collagen, fibronectin, desmin, and tenascin. Such changes characteristically occur during human PCO development.

Results obtained recently using a rat lens epithelial explant model suggest that fibroblast growth factor (FGF) may also play a role in PCO development [10]. In this model, explants were exposed sequentially to TGFβ2 and FGF-2, both of which are potentially available to lens cells in situ following cataract surgery [10]. Addition of FGF-2 not only promoted the long term survival of aberrant TGFβ affected cells, which otherwise rapidly undergo apoptosis [11], but also induced a variety of responses typically associated with PCO development. Many of these had not previously been generated in a PCO model in vitro.
PCO-like changes in this model [10] included the retraction of cells into multilayered strands or plaques with abnormal deposition of ECM, a feature of the fibrotic form of PCO, and the formation of plaques of cells that resemble the Elschng’s pearls that are a feature of “pearl-like” PCO. In addition, these cells expressed α-smooth muscle actin, type I collagen, and fibronectin, as in PCO. Such responses were generated using concentrations of TGFβ2 and FGF-2 ranging from 25-100 pg/ml and 20-100 ng/ml, respectively. Other growth factors tested (insulin-like growth factor, platelet-derived growth factor, epidermal growth factor, and hepatocyte growth factor), did not promote survival of TGFβ affected cells [10].

Previously, FGF had been shown to stimulate proliferation and spreading of lens cells across the posterior capsule towards the visual axis in cultured human lens “capsular bags” [12], obtained by carrying out sham cataract surgery on eyes post mortem, and to exacerbate TGFβ induced subscleral cataract formation in cultured rat lenses [13].

Surgery for cataract generates short term inflammation, which is routinely controlled by applying anti-inflammatory drugs both before surgery and for 2-6 weeks after surgery. Glucocorticoids are widely used for this purpose [14,15]. Human, rabbit, bovine, mouse, and rat lens epithelial cells have been shown to express glucocorticoid receptors [16-19]. Further, even a single application of a glucocorticoid containing eye drop is sufficient to generate potentially effective concentrations of these drugs in the aqueous [20,21], the ocular fluid to which residual lens epithelial cells are directly exposed after cataract surgery. It is thus important to establish whether the use of this drug influences PCO development.

Using the rat lens explant model described above, we have investigated the effects of including dexamethasone (DEX), a widely used glucocorticoid, in the medium at a clinically relevant concentration during the development of PCO-like changes. Such changes were generated by culturing for one day with TGFβ2 then with FGF-2 for up to 30 days. The effects of including DEX throughout the culture period were assessed by light and scanning electron microscopy and by immunolocalization of two proteins that typically appear in lens cells undergoing epithelial-mesenchymal transition during PCO development and Pax-6, a marker for lens epithelial phenotype.

METHODS

Recombinant human TGFβ2 (Genzyme, Cambridge, MA) and FGF-2 (PeproTech, Rock Hill, NJ) were used as described previously [10]. Cell culture grade DEX was purchased from Sigma (St Louis, MO). The following antibodies were used; mouse α-smooth muscle actin antibody (Sigma; clone 1A4), goat anti-α-fibronectin (Calbiochem, La Jolla, CA), Pax-6 antibody (Covance, Berkeley, CA), Alexa 488 conjugated anti-mouse antibody (Molecular Probes, Eugene, OR), and Cy3 conjugated anti-goat or anti-rabbit antibody (Zymed, San Francisco, CA).

Lens epithelial explants were prepared from 19-21 day-old Wistar rats using serum free medium M199 containing bovine serum albumin and antibiotics (control medium), as described [10,11]. In the basic model (TGFβ2/FGF treated group), explants were exposed to TGFβ for one day then to FGF throughout culture. When all explants had been set up, medium was replaced with control medium, 50 pg/ml TGFβ was added, and explants were cultured at 37 °C in 5% CO2. One day later, the TGFβ-containing medium was discarded and replaced with 1 ml control medium containing 20 ng/ml FGF. Explants were maintained in culture for up to 30 days, with change of medium and re-addition of FGF every 4-6 days. DEX treated explants received DEX at a final concentration of 100 nM on day 0 immediately prior to the addition of TGFβ and again on day 1, then at each change of medium unless otherwise indicated. TGFβ and/or FGF and DEX were omitted from controls.

Explants were monitored by phase contrast microscopy during culture then fixed in 100% ethanol (for Pax-6 localization) or Carnoy’s fixative (ethanol:acetic acid, 3:1 v/v) and stored in 70% ethanol. Some explants were embedded in paraffin and sectioned serially perpendicular to the lens capsule and central sections were stained with hematoxylin and eosin. Whole mounts were used for immunolocalization of markers for PCO-like change (α-smooth muscle actin and fibronectin, double labeling) or Pax-6, visualized using appropriate Alexa 488 or Cy3 conjugated secondary antibodies, counterstained with Hoechst dye and mounted using Vectashield, as described [10,13]. Under these conditions, immunoreactivity was absent from controls in which antibodies were replaced with corresponding non-immune IgGs. For SEM, whole mounts were dehydrated with 100% ethanol, critical point dried, gold coated, and viewed using a Philips XL30 scanning electron microscope.

RESULTS

Morphological changes induced by DEX: As described previously [10,11], in the absence of FGF, TGFβ at the concentration used in the present study induced the formation of spindle-like cells beginning on about day 2 of culture. These cells, and surrounding cells, subsequently undergo apoptotic cell death. They exhibit extensive cell surface blebbing, then round up and die leaving the underlying lens capsule denuded [10,11,22]. A typical TGFβ treated explant and a corresponding control explant cultured without growth factors are shown in Figure 1A,B. Loss of cells makes it difficult to discern the presence of spindle-like cells at this stage of culture. In contrast, TGFβ/FGF treated explants remained well covered with viable cells including occasional spindle-like cells (Figure 1C) [10]. In TGFβ/FGF/DEX treated explants, however, numerous spindle-like or needle-like cells were present in parallel arrays through the explant (Figure 1D). These cells, which were much more regularly arranged than the spindle-like cells observed in explants treated with TGFβ/FGF in the absence of DEX (compare Figure 1C and Figure 1D), were clearly distinguishable between days 4-6 but not at later stages of culture. Further, the spindle-like cells in TGFβ/FGF treated explants, with or without DEX, differed from spindle-like cells induced by TGFβ alone in that, generally, they did not de-
velop cell surface blebbing or other morphological changes associated with TGFβ induced cell death (Figure 1B-D). Cells in corresponding explants cultured in control medium only or with FGF or DEX alone tended to remain in the cobblestone array typical of the normal lens epithelium (as in the explant depicted in Figure 1A), although some cell loss was noted under these conditions in explants that were initially poorly covered with cells (not shown).

Inclusion of DEX also influenced the outcome in TGFβ/FGF treated explants cultured for a longer period (Figure 2). In the absence of DEX, where cell coverage was initially patchy, cells quickly began to gather up into plaques or thickened mounds, while in regions that remained well covered with cells, retraction of cells into plaques and mounds began on about day 10-15, as described previously [10]. Thus, at the end of the culture period, patches of denuded lens capsule were visible between cellular regions, which were multilayered and contained swollen cells (Figure 2A,C,E). In contrast, when DEX was included, retraction of cells from the capsule in well-covered regions did not occur and such explants remained covered with cells at the end of the culture period (Figure 2B,D,F). While generally arranged in multilayers, these cells were sometimes present in a monolayer (Figure 2D) and appeared to be much smaller than in corresponding explants that did not receive DEX (Figure 2E,F). Whether or not DEX was included, explants showed more extensive thickening in the peripheral region than in the central region (Figure 2C-F). In the presence of DEX, in regions where cells were initially present in isolated patches, plaques formed surrounded by a monolayer of cells. In corresponding explants cultured with DEX alone (Figure 2G,H), and in explants cultured in control medium only (not shown), cells were present in a cuboidal monolayer as in the normal lens epithelium.

Similarly, SEM revealed marked differences between

![Figure 1](http://www.molvis.org/molvis/v10/a88)

**Figure 1.** Effect of dexamethasone on cell morphology: phase contrast microscopy. Explants were cultured in control medium (A) or with 50 pg/ml TGFβ2 for 1 day (B-D), then medium was replaced and 20 ng/ml FGF-2 was added to some cultures (C,D). In D only, 100 nM DEX was included on day 0 and day 1. Explants were cultured for a total of 5 (A,B) or 6 (C,D) days, then photographed. Cells in control explants, cultured without growth factors or DEX, tended to remain in cobblestone arrays typical of the normal lens epithelium, as shown in A. Treatment with TGFβ only (B) resulted in extensive cell surface blebbing (arrowheads) associated with loss of cells from the lens capsule (asterisk) and wrinkling of the lens capsule (arrow). Adding FGF on day 1 largely prevented these changes (C,D). Under the latter conditions, in the absence of DEX (C), explants remained well covered with cells, which included occasional spindle-like cells (arrows). However, inclusion of DEX (D) resulted in the formation of numerous parallel arrays of spindle-like cells (arrows) throughout the explant. The bar represents 30 µm in A and B and 75 µm in C and D.
TGFβ/FGF treated explants cultured for 30 days in the presence or absence of DEX (Figure 3). Explants cultured without DEX or with DEX are shown at relatively low magnification in Figure 3A and Figure 3B, respectively. In each case, plaque formation occurred; however, only the explant exposed to DEX contained migratory cells extending in a monolayer from the base of the plaque. In some explants exposed to DEX this monolayer of cells was quite extensive (Figure 4D, Figure 5A,C). The surface of the explant cultured without DEX shown in Figure 3A, was covered by a thick web of ECM-like material, which obscured the cells (Figure 3C,E). In another explant from the latter treatment group, ECM-like material was present only in occasional patches on the surface of the cells, many of which were flattened and elongated and exhibited tongue and flap processes, like cells undergoing FGF induced fiber differentiation (Figure 3G) [10,23]. In contrast, in explants cultured with DEX, there was little or no deposition of ECM-like material on the surface of the cells, which exhibit-

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Figure 2. Effect of dexamethasone on cell morphology: hematoxylin and eosin stained sections. Lens epithelial explants were treated with TGFβ then FGF without addition of DEX (A,C,E) or with addition of DEX (B,D,F), as described in the legend to Figure 1, or treated in parallel with DEX alone (G,H). Medium was replaced 5 days after addition of FGF and every 4-6 days thereafter, with re-addition of FGF (A-F) and/or re-addition of DEX (B,D,F-H). At the end of the culture period explants were fixed, embedded and sectioned transversely. Low power images of sections are shown in A and B, with labeled boxes to indicate the regions shown at higher magnification in C,D,E, and F. In TGFβ/FGF treated explants in the absence of DEX, explants exhibited multilayering and extensive enlargement of cells (C,E) and, between cellular aggregates, regions of denuded lens capsule were visible (C, arrow). When DEX was included, multilayering also occurred but there appeared to be less cell enlargement and most of the lens capsule remained covered with cells (B,D,F), although in some regions only a thin monolayer of cells was present (D, arrow). With or without DEX, the explants were thicker in the peripheral region (E,F) than in the central region (C,D). In contrast, explants cultured in parallel with DEX alone generally remained covered with a monolayer of cuboidal cells in both the central (G) and peripheral (H) region, as in the normal lens epithelium. The bar represents 250 µm in A and B and 50 µm in C-H. The peripheral region (Per) and the central region (Cn) of the explants are labeled.
ited a variety of unusual profiles (Figure 3D,F,H,I). These were quite different from the cobblestone arrays of cells observed in lens epithelial explants cultured in control medium (not shown) [10,23].

**Immunolocalization studies:** Immunolocalization was carried out to determine whether the monolayers of cells present around the base of plaques in TGFβ/FGF/DEX treated explants were normal lens epithelial cells or whether they were aberrant cells. First, explants cultured for 30 days with TGFβ then FGF, with or without inclusion of DEX, were assessed by immunolocalization of α-smooth muscle actin and fibronectin (Figure 4). These proteins, which are not present in normal lens epithelial cells, are found in PCO and are induced by exposing lens cells to TGFβ. Cells present in the multilayered plaques that formed in TGFβ/FGF treated explants exhibited reactivity for both α-smooth muscle actin and fibronectin, whether DEX was absent or whether it was included throughout the entire culture period (Figure 4A-F), although reactivity for α-smooth muscle actin appeared weaker when DEX was included (Figure 4B,E). However, no reactivity for either of these proteins was detectable in the continuous monolayer of attenuated cells surrounding the plaques in

Figure 3. Effect of dexamethasone on cell morphology: SEM. Lens epithelial explants were treated with TGFβ then FGF without addition of DEX (A,C,E,G) or with addition of DEX (B,D,F,H,I), as described in the legend to Figure 2, then processed for SEM. Asterisks indicate regions of denuded lens capsule. In these TGFβ/FGF treated explants, both in the absence of DEX (A) and the presence of DEX (B), thick plaques of cells formed during culture. In the presence of DEX, however, a monolayer of attenuated cells formed, extending from the base of the plaque across the lens capsule (B, arrowhead). The general topography of these explants was also markedly different (C,D). Examination at higher magnification revealed that the explant cultured without DEX was covered with a thick web of ECM-like material, which completely obscured the cellular surface (E). In another TGFβ/FGF treated explant cultured without DEX (G), ECM-like material was present in scattered patches (arrow), and flattened, elongated cells were visible, which exhibited occasional tongue and flap-like, interdigitating processes at their margins (G, arrowheads). In contrast, the DEX treated explant shown in B and D exhibited only very sparse deposition of ECM-like material (F), while extensive regions of another explant in this treatment group appeared completely devoid of surface ECM (H,I). The cells in the DEX treated explants exhibited diverse and unusual morphologies (F,G,H). The bars represent 50 µm in A-D and 20 µm in E-I.
the DEX treated explants (Figure 4E,F).

Removing DEX from the culture medium during the last 20 days of culture resulted in markedly enhanced reactivity for α-smooth muscle actin in the plaques (Figure 4H), compared with cultures that received DEX continuously (Figure 4E), and reactivity became detectable in the surrounding monolayer of cells (Figure 4H). However, reactivity for fibronectin in the plaques was similar whether DEX was removed (Figure 4I) or present continuously (Figure 4F) and remained undetectable in the monolayer of cells (Figure 4I). The only exogenous growth factor the explants were receiving over the last 20 days of culture in this case was FGF. It has previously been shown that culturing freshly prepared rat lens epithelial explants with FGF alone, at the concentration used in the present study, tends to suppress α-smooth muscle actin expression rather than induce it [10]. Thus it is highly likely that the cells in the monolayer that formed in TGFβ/FGF/DEX treated explants were already abnormal prior to the removal of DEX on day 10.

Immunolocalization was also used to assess whether Pax-6 was expressed by cells in TGFβ/FGF/DEX treated explants, particularly in the monolayered regions. This transcription factor, which is required for proper eye development, is expressed by normal lens epithelial cells but not mature fiber

Figure 4. Immunolocalization of α-smooth muscle actin and fibronectin in cell cultures. Lens epithelial explants were cultured with TGFβ then FGF, without DEX (A-C) or with addition of DEX on day 0 and at each change of medium throughout the entire culture period (D-F), as described in the legend to Figure 2. Other explants (G-I) were treated with TGFβ/FGF/DEX as for D-F but DEX treatment was discontinued from day 10 of culture onwards. Explants were fixed as whole mounts and processed for immunolocalization of α-smooth muscle actin (B,E,H; green) and fibronectin (C,F,I; red) by a double labeling technique, with Hoechst counterstaining of nuclei (A,D,G). In all treatment groups, reactivity for both α-smooth muscle actin (B,E,H) and fibronectin (C,F,I) was detected wherever cells had become multilayered and formed into plaques (asterisks in A,D,G). Reactivity for α-smooth muscle actin was relatively weak in plaques in explants treated continuously with DEX (E) but particularly strong in explants in which DEX treatment was discontinued at 10 days (H). Monolayered regions of cells adjacent to plaques were present only in explants that received DEX, whether DEX was present continuously (D, arrowheads) or discontinued at 10 days (G, arrowheads). In the former treatment group, no reactivity for the PCO markers was present in the monolayered cells (E,F), while in the latter group, reactivity for α-smooth muscle actin was readily detectable in these cells (H, arrowheads) but reactivity for fibronectin was not (I). The bar represents 80 µm.
cells [24-26] and thus serves as a marker for lens epithelial cell phenotype. It has been shown previously that, in the absence of DEX, cells in TGFβ/FGF treated explants lose Pax-6 expression within 5 days [10]. In TGFβ/FGF/DEX treated explants cultured for 30 days, there was no detectable Pax-6 expression either in the plaques or in the monolayer of cells surrounding them (Figure 5). This represents further evidence that the cells, even those that remained in a monolayer, were not normal lens epithelial cells. Frequent monitoring of TGFβ/FGF/DEX treated explants by phase contrast microscopy during culture revealed that many of the cells at the leading edge of flattened, monolayered regions exhibited the characteristics of actively migrating cells. They became attenuated and developed lamellae (Figure 5C) and appeared to spread out across the adjacent bare lens capsule as culture proceeded.

Thus, in TGFβ/FGF treated explants, whether DEX was included or not, all cells lost their epithelial cell phenotype, as evidenced by loss of Pax-6 expression, while cells in thickened regions of the explants began expressing the myofibroblast/PCO marker α-smooth muscle actin. However, a striking feature of TGFβ/FGF/DEX treated explants that was not observed in corresponding TGFβ/FGF treated explants was the monolayer of aberrant, apparently migratory, cells that formed at the base of thickened plaque-like regions and spread out across the lens capsule. Moreover, the cells in this monolayer were “PCO-like” in that they were capable of expressing α-smooth muscle actin, which became detectable upon removal of DEX.

**DISCUSSION**

In the rat lens explant model used in the present study explants cultured with TGFβ then FGF without addition of DEX exhibited many features of PCO development over the 30 day culture period, as reported previously [10]. Cells became multilayered and tended to gather up into plaques, leaving the surrounding lens capsule denuded. These plaques, which arose quite abruptly from the lens capsule, were covered with a thick web of ECM-like material. In addition, cells underwent epithelial-mesenchymal transition, as evidenced by expression of α-smooth muscle actin and fibronectin, non-lens proteins that are typically associated with human PCO.

In this model, significant changes in lens cell behavior occurred when DEX was included throughout the culture period. These included rapid formation of long, needle-like cells early in the culture period and a reduction in the overall extent of thickening of the explant over time, which was associated with a decreased tendency for the lens capsule to become denuded as plaques formed. However, when plaques did form adjacent to regions of denuded lens capsule, they were generally surrounded by a monolayer of migratory cells. These did

![Figure 5. Lack of Pax-6 expression in explants cultured with TGFβ, FGF, and DEX. Explants were cultured with TGFβ then FGF with inclusion of DEX throughout the entire culture period (A,B,C), as described in the legend to Figure 2. They were then photographed by phase contrast microscopy (C) or fixed as whole mounts and processed for immunolocalization of Pax-6 (B) with Hoechst counterstaining of nuclei (A). An explant cultured without addition of growth factors for 7 days and processed in parallel provided a positive control for Pax-6 expression (D, Hoechst dye; E, Pax-6). In TGFβ/FGF/DEX treated explants multilayered plaques formed (A, asterisk indicates plaque); these were associated with a monolayer of cells radiating out from the base of the plaque onto the lens capsule. Pax-6 reactivity was not detectable in either the plaque or the surrounding monolayer of cells (B), whereas cells in the positive control exhibited strong nuclear reactivity for Pax-6 under these localization conditions (D,E). A phase contrast micrograph (C) of an explant analogous to that depicted in A and B shows a monolayer of cells spreading out from the base of a plaque (asterisk); cells at the leading edge have developed lamellae (arrowheads), a characteristic of actively migrating cells. The bar represents 50 µm in A, B, D, and E and 40 µm in C.](http://www.molvis.org/molvis/v10/a88)
not appear to be normal lens epithelial cells in that they did not express the lens epithelial phenotype marker Pax-6. Further, although α-smooth muscle actin reactivity could not be detected in the monolayered cells when DEX was included throughout the entire culture period, reactivity for this marker for PCO-like change did become detectable when DEX was removed late in the culture period. The mechanism(s) underlying these effects of DEX have not yet been elucidated. Of interest in this context, however, is the finding that TGFβ2 enhances synthesis of prostaglandin E2 by explanted human lens epithelial cells obtained at cataract surgery [27], whereas an established general mechanism of action of DEX is inhibition of the synthesis of the prostaglandin precursor arachidonic acid [28].

The concentration of DEX used in the present study, 100 nM (39 ng/ml), has been shown to be effective in inducing biological effects in many studies in vitro of cells from the rat and other species [29-32], including lens epithelial cells [33]. It also approximates concentrations of DEX achieved in the aqueous in the anterior chamber of the eye following administration of DEX eye drops as at cataract surgery. After application of a single drop of DEX alcohol (Maxidex™) to human subjects, mean peak concentrations in the aqueous of 31 ng/ml [20] and 60 ng/ml [34] have been reported. A mean concentration in the aqueous of 31 ng/ml was also reported in a study in which patients received repeated doses of DEX disodium phosphate [35].

In previous studies, DEX has been shown to influence lens epithelial cells in vitro in various ways. It enhances αB-crystallin expression of epithelial cells in rat lens explants [36] and alters expression of basement membrane proteins in cultured bovine cells [33]. These effects were achieved using 100-500 nM DEX. In other studies, which showed altered cadherin expression in cultured rat lenses [37] and inhibition of lens epithelial cell proliferation [38] in response to DEX treatment, higher concentrations were used, 5 µM and 250 µM, respectively. In vivo, treatment of rabbits with DEX eye drops as used for cataract surgery has been shown to induce metabolic changes and reduce glutathione levels in the lens [39].

The causative relation between long term corticosteroid therapy and the induction of cataractous changes in the intact lens is well established, albeit not well understood [40]. Less attention has been paid to the possibility that glucocorticoids such as DEX, when administered at the time of cataract surgery, may influence the behavior of the residual lens cells from which PCO arises. There are two studies in which the effect of DEX on PCO development has been investigated in normal rabbits subjected to a cataract surgery procedure. In the study of Wallentin et al. [41], rabbits received DEX eye drops or no treatment for one month post surgery. When PCO total wet weight was measured four weeks after ceased treatment, there was no difference between DEX treated and untreated groups. A similar result was obtained when rabbits were treated with large doses of prednisolone and DEX prior to and for 12 h after surgery [42]. Note that this method of PCO assessment did not provide any information about possible between treatment differences in specific responses of cells and their localization relative to the light path. Furthermore, the assessment was carried out at only one time point at a relatively late stage in PCO development, which occurs very rapidly in the rabbit [43]. In another such study that reported reduction of PCO by DEX [44], lens cells were exposed directly during surgery to a much higher dose of DEX than is normally applied to the eye surface to control inflammation after cataract surgery.

In the present study, DEX at a clinically relevant dose was shown to influence the behavior of lens cells undergoing PCO-like changes in ways that could influence visual outcome if they occurred in a human patient. For example, it could be argued that a reduction in the thickness of the cellular layer and prevention of deposition of ECM-like material, as observed in the presence of DEX in the rat model, would reduce opacification and hence improve visual outcome. Formation of opaque multilayered plaques and aggregations of cells and abundant deposition of ECM by aberrant cells are thought to contribute significantly to visual impairment in human PCO [3-5]. On the other hand, if DEX induced the formation of a monolayer of viable, aberrant cells spreading out from the base of PCO-like plaques, as noted in the present study, it could be argued that visual outcome would be worsened because of an increased risk that abnormal cells would reach the visual pathway. Interestingly, regions of monolayered cells expressing α-smooth muscle actin occur in human PCO [45,46], reminiscent of the monolayer of cells in Figure 4H. In the latter case, the explant received DEX together with FGF for the first 10 days of culture then FGF only, a treatment that mimics discontinuation of DEX treatment 1-2 weeks post cataract surgery.

Because of the remarkable similarities between lens epithelial cells from rats and humans in terms of their responses to the cataractogenic effects of TGFβ, useful information about human PCO can be obtained using rat models. However, direct extrapolation from the results reported in this study to the development of human PCO under clinical conditions, along the lines suggested above, is problematic. First, it cannot be assumed that dexamethasone will produce cellular responses in human lens cells identical in every respect to those observed in the rat model, although it is likely that it will have some effect on them, given the presence of glucocorticoid receptors on human lens cells [16-18] and the plethora of known effects of dexamethasone on cells in general. More importantly, the behavior of lens cells during human PCO development in situ and their responses to the presence of DEX may be significantly affected by the complex assortment of growth factors, cytokines and other effector substances present in their environment post cataract surgery. Clinical trials designed to test the effects of DEX (or other glucocorticoids) on PCO development in vivo, under conditions typically encountered during and after cataract surgery, thus offer a distinct advantage over studies in vitro in either rat or human models.

While the literature contains reports of numerous clinical trials of the effectiveness of DEX in prevention of inflammation [15], follow-up assessment of PCO development has rarely been included. Only a few relevant reports appear in the literature, all published recently [47-49]. In the first of these
[47], three treatment groups were compared; patients received DEX, diclofenac (a non-steroidal anti-inflammatory drug), or placebo (saline). No significant difference between the groups was detected 2 years after surgery, in terms of the proportion of patients requiring laser capsulotomy for PCO (15-18%). After 4 years, however, capsulotomy rates for DEX, diclofenac and placebo were 47, 63, and 32%, respectively. Only the difference between diclofenac and placebo reached statistical significance, however. This study is thus noteworthy in that it indicates that at least one drug used to control inflammation at the time of cataract surgery may be associated with an increased incidence of PCO requiring laser treatment. In a second study by this group [48], the same anti-inflammatory drugs were used at lower dosages and PCO development was assessed by both image analysis and capsulotomy rate. The three treatments again gave similar results at 2 years, but PCO evaluation beyond 2 years was not included. In these studies, the number of patients per group was relatively small, less than 60 [47] or 40-45 [48].

In another study, in which no placebo group was included, the effects of the glucocorticoid betamethasone were compared with diclofenac and PCO development was assessed 3 years post surgery by grading of photographs as well as capsulotomy rate [49]. Here PCO outcome appeared to be worse in betamethasone treated patients. For example, in the betamethasone and diclofenac groups, respectively, the overall incidence of PCO was 70% and 55%, with a 30% and 15% incidence of severe PCO. However, again only a small number of patients was evaluated (20-30 per group) and apparent differences did not reach statistical significance.

In summary, using a rat lens explant model for PCO, the effects of DEX have been investigated at a concentration and over time periods that lens epithelial cells are likely to encounter following cataract surgery. Aspects of lens cell behavior highly relevant to PCO development were clearly affected by the presence of DEX. As this rat model closely mimics cellular events in PCO development in humans and other species [10] and as human lens epithelial cells express transcriptionally active glucocorticoid receptors [18,19], it seems likely that glucocorticoids exert effects on residual lens cells left behind at cataract surgery.

Although the results of the present study in the rat model point to various ways in which PCO development may be influenced by DEX, it is not clear whether analogous effects occur in the clinical situation. Limited clinical trials carried out to date have provided equivocal results, leaving this question unresolved. The present study thus highlights both the possibility that anti-inflammatory drugs used routinely at the time of cataract surgery may influence PCO development and the need for more detailed clinical evaluation of this issue.

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