Dexamethasone-Induced Ocular Hypertension in Perfusion-Cultured Human Eyes

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Purpose. Glucocorticoid administration can lead to the development of ocular hypertension and corticosteroid glaucoma in a subset of the population through a decrease in the aqueous humor outflow facility. The purpose of this study was to determine whether glucocorticoid treatment can directly affect the outflow facility of isolated, perfusion-cultured human eyes.

Methods. The anterior segments of human donor eyes from regional eye banks were placed in a constant flow, variable pressure perfusion culture system. Paired eyes were perfused in serum-free media with or without 10^-7 M dexamethasone for 12 days. Intraocular pressure was monitored daily. After incubation, the eyes were morphologically characterized by light microscopy, transmission and scanning electron microscopy, and scanning laser confocal microscopy.

Results. A significant increase in intraocular pressure developed in 13 of the 44 pairs of eyes perfused with dexamethasone with an average pressure rise of 17.5 ± 3.8 mm Hg after 12 days of dexamethasone exposure. The contralateral control eyes, which did not receive dexamethasone, maintained a stable intraocular pressure during the same period. The outflow pathway of the untreated eyes appeared morphologically normal. In contrast, the dexamethasone-treated hypertensive eyes had thickened trabecular beams, decreased intertrabecular spaces, thickened juxtacanalicular tissue, activated trabecular meshwork cells, and increased amounts of amorphogranular extracellular material, especially in the juxtacanalicular tissue and beneath the endothelial lining of the canal of Schlemm. The dexamethasone-treated nonresponder eyes appeared to be morphologically similar to the untreated eyes, although several subtle dexamethasone-induced morphologic changes were evident.

Conclusion. Dexamethasone treatment of isolated, perfusion-cultured human eyes led to the generation of ocular hypertension in approximately 30% of the dexamethasone-treated eyes. Steroid treatment resulted in morphologic changes in the trabecular meshwork similar to those reported for corticosteroid glaucoma and open angle glaucoma. This system may provide an acute model in which to study the pathogenic mechanisms involved in steroid glaucoma and primary open angle glaucoma.


A major obstacle impeding progress in glaucoma research is the lack of good model systems in which to understand the pathogenic mechanisms involved in the development of primary open angle glaucoma (POAG). Insight has been gleaned through epidemiologic studies as well as through the examination of glaucomatous trabeculectomy samples and donor cadaver eyes. Epidemiologic studies have suggested that important risk factors for the development of POAG are: family history, ocular hypertension, myopia, race, untreated systemic hypertension, diabetes mellitus, and steroid responsiveness.1-18 Morphologic studies of the trabecular meshwork (TM) from glaucomatous eyes have shown a variety of changes including: decreased TM cellularity compared to age-matched controls, increased deposition of plaquelike extracellular material, decreased giant vacuoles on the inner wall of the canal of Schlemm, collapse of regions of Schlemm's canal, increased deposition of long spacing collagen, and thickening of trabecular beams.9-16

Numerous studies have suggested that glucocorti-
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Accordingly, we have directly tested whether GCs can alter the aqueous humor outflow using iso-
cellular matrix (ECM) deposition, secreted glyco-proteins, cytoskeletal organization, and TM cell function. All the current evidence suggesting an association of GCs with the pathogenesis of ocular hypertension and POAG has been indirect. In vivo administration of GC can lead to numerous alterations and secondary effects that may modify aqueous dynamics. Likewise, one cannot directly correlate the in vitro effects of GCs on cultured TM cells with a physiological function. Accordingly, we have directly tested whether GCs can alter the aqueous humor outflow pathway remain viable for up to 4 weeks in culture. After stabilization for 2–3 days, 10^{-7} M dexamethasone (Sigma Chemical Co., St. Louis) was added to the culture media of one eye while the contralateral eye served as the control and received an equivalent volume of the ethanol vehicle (> 0.1% ethanol). The dexamethasone concentration used is equivalent to that obtained in the aqueous humor of the eye after topical administration of a single 0.1% dose. The eyes were subsequently perfused for 10–12 days and the IOPs were recorded at least once but usually several times a day in the morning and/or afternoon. Statistical analyses were done using the Student's t-test with a P value of less than 0.05 considered statistically significant.

The morphologic consequences of perfusion in the absence and presence of dexamethasone were examined. The eyes were fixed by perfusion at 15 mm Hg constant pressure with either: 1) a fixative consisting of 4% paraformaldehyde in Sorenson's buffer, followed by immersion fixation in 2.5% glutaraldehyde and 2% paraformaldehyde in 0.1 M Sorenson's phosphate-buffered solution (pH 7.3); or 2) a fixative consisting of 1% paraformaldehyde, 1% glutaraldehyde, 0.5% acrolein in 0.1 M sodium cacodylate, pH 7.3. Wedge-shaped sections of the iridocorneal angle from four quadrants were removed and processed for morphologic analysis by light microscopy and transmission and scanning electron microscopy. The tissue prepared for transmission electron microscopy was osmicated, dehydrated through an ascending series of alcohols, and embedded in a Polybed 812/araldite mixture. Semithin sections were cut from randomly selected blocks from each quadrant and stained with toluidine blue for light microscopy. Thin sections were taken from each quadrant in the TM and examined in a Zeiss CEM-902 transmission electron microscope. Dissection and tissue preparation for scanning electron microscopy of the inner wall of Schlemm's canal was done as previously described. Inner wall sections were examined using a JEOL JSM 35 or JEOL 6400 SEM (JEOL USA, Inc., Peabody, MA).

Samples of TM for immunohistochemical study were taken immediately after constant pressure perfusion fixation in 4% paraformaldehyde. Frozen sections (20 μm) were cut from segments of control and dexamethasone responder eyes and were labeled with a monoclonal antibody to human fibronectin (Cappel, West Chester, PA) followed by FITC-labeled rabbit anti-mouse IgG (Cappel, West Chester, PA) using standard immunohistochemical procedures. A Bio-Rad MRC-600 confocal laser scanning microscope (Bio-Rad Laboratories, Hercules, CA) was used to obtain the IOP within each eye. In this perfusion system, the perfusate exits the eye through the conventional aqueous humor outflow system. The TM cells of the outflow pathway remain viable for up to 4 weeks in culture.
serial digital images of the inner wall of Schlemm's canal and juxtacanalicular tissue (JCT). Stacks of 0.5 μm optical sections were reconstituted three-dimensionally with BioRad software combined with the image restoration techniques of Dr. Fred Farquhar on a Silicon Graphics 4D/240 GTX system (Silicon Graphics, Mountain View, CA).

RESULTS

Sixty pairs of human donor eyes were evaluated in the ocular perfusion organ culture system. Sixteen pairs of eyes did not have stable baseline pressures after several days of perfusion and were excluded from the study. Therefore, only 75% of the eyes received were suitable for use in this study. The 44 four pairs of eyes that remained stable were perfused with media ± 10⁻⁷ M dexamethasone for 10-14 days. In each case the IOP of the control eyes, receiving media without dexamethasone, was constant (±2 mm Hg) during the perfusion time. The average beginning and final IOPs and outflow facilities for the nonresponder and dexamethasone responder sets of perfusion-cultured eyes are shown in Figures 2A and 2B. The daily change in IOP for the dexamethasone responder group is shown in Figure 2C. A dexamethasone responder eye is defined as a greater than 5 mm Hg rise in pressure after 10 days of dexamethasone exposure. Thirty-one of the eyes perfused with dexamethasone had relatively stable pressures that were not significantly different from control eyes (Fig. 2A). However, 13 of the dexamethasone-treated eyes had significant and progressive increases in IOP (Figs. 2A, 2C) and a significant decrease in outflow facility (Fig. 2B). A progressive rise in IOP was seen after 5–6 days of dexamethasone perfusion in these steroid responder eyes (Fig. 2C). The average increase in IOP was 17.5 ± 3.8 mm Hg after 12 days of treatment. The contralateral control eyes remained stable during this same period of time. The IOPs of the dexamethasone-treated eyes were statistically different from control eyes on days 4–6 (P<0.05) and 7–12 (P<0.01) of treatment. The average initial IOP of the dexamethasone responder group was higher than the dexamethasone-treated nonresponder group (21.9 versus 15.0, P<0.01) and the outflow facility was lower (0.103 versus 0.142, P<0.02). The average age of the dexamethasone responder population was 79.2 ± 9.2 years and consisted of 8 men and 5 women. The nonresponder population consisted of 16 men and 15 women with an average age of 71.1 ± 13.5 years.

Four separate quadrants each of 9 pairs of eyes perfused for 10 days in the presence or absence of dexamethasone (5 dexamethasone responder and 4 nonresponder) were examined histologically and ultrastructurally. In the control eyes, the aqueous humor outflow pathway appeared relatively normal with abundant TM cells, intertrabecular spaces and separate beams (Figs. 3A, 4A) similar to what has been described previously. In contrast, marked changes were evident in the responder eyes perfused with dexamethasone: An apparent loss of cells from the trabecular beams and from the endothelial lining of the inner wall of Schlemm's canal was noted (Figs. 3C and 4C). Throughout the meshwork, TM cells appeared "activated" as indicated by increased levels of smooth endoplasmic reticulum, rough endoplasmic reticulum, ribosomes, and mitochondria (Figs. 4C, 5B). A marked accumulation of fibrillar and amorphogranular extracellular material was seen in both the intertrabecular spaces and in the JCT of dexamethasone-perfused responder eyes (Figs. 3C, 4C, 5D, 5F, 6B). The trabecular beams appeared to be thickened due to an increase in trabecular basement membrane material including long spacing collagen. The intertrabecular spaces were decreased and occasionally the trabecular beams were fused (Figs. 3C, 4C, 5D). These results were consistent in at least three of the four quadrants.
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The ultrastructural differences in the dexamethasone-treated nonresponder eyes compared to the untreated control eyes were subtle (Figs. 3B, 4B). These changes consisted of varying degrees of loss of TM cells lining the trabecular beams, a moderate thickening of the beams, and in one eye, a modest accumulation of ECM-like material beneath the canal of Schlemm and in the trabecular beams (Fig. 4B). While differences between control and nonresponder eyes were noted, the morphologic changes were not of the magnitude of those detected in the dexamethasone responder eyes.

Three dexamethasone-induced ocular hypertensive eyes and their paired controls were processed for immunohistochemical analysis of fibronectin using immunofluorescent light microscopy and scanning laser confocal microscopy. The region showing the most obvious differences between these two groups was the juxtacanalicular region just beneath the inner wall of the canal of Schlemm. The fibronectin distribution in the JCT of the untreated control eyes was patchy (Fig. 7A). The fibronectin labeling appeared to be associated with the inner wall cells and their underlying basement membrane as well as with the cells in the JCT matrix. In contrast, the patchy distribution of fibronectin seen in the untreated eyes was replaced with a uniform fibronectin distribution just beneath the inner wall endothelial cells in the dexamethasone responder eyes (Fig. 7B). In comparison to control eyes, it appeared that the amount of fibronectin was increased in all of the dexamethasone responder eyes examined. There did not appear to be detectable changes in fibronectin distribution in the nonresponder eyes. A summary of the morphologic alter-
FIGURE 3. Light photomicrographs of the outflow pathway of perfusion-cultured human eyes. Control eye (A) perfused for 10 days in the absence of dexamethasone has an open network of cell-lined trabecular beams with giant vacuoles (arrowheads) in the inner wall endothelial cell layer lining Schlemm’s canal (S). The dexamethasone nonresponder eye (B) perfused for 10 days with media containing $10^{-7}$ M dexamethasone, without developing a change in intraocular pressure, is very similar in appearance to the control eye. Dexamethasone responder eye (C) (contralateral eye to 3A) perfused for 10 days with media containing $10^{-7}$ M dexamethasone developed ocular hypertension and showed increased beam fusion, a loss of cells lining the trabecular beams, and increased density in the juxtacanalicular region. Bar = 50 μm.

DISCUSSION

Perfusion-cultured human eyes provide a useful system in which to directly evaluate the effects of various agents on the aqueous outflow pathway. It appears that the IOPs (averaging 15–24 mm Hg) are slightly higher and the outflow facilities (0.10 to 0.14 ml/min/mm Hg) lower than one would expect compared to intact eyes using this constant flow, variable pressure perfusion culture system. However, the eyes used in this study were from older donors (average age 73.5 years). There have been several reports of a significant decrease in outflow facility with age, and the average outflow facilities in our older perfusion-cultured eyes agrees with tonographic outflow facilities of one of these reports. We therefore believe that this perfusion culture model is a physiologically relevant system in which to study the human outflow pathway. The trabecular cells in this perfusion system remain viable up to 4 weeks in culture, enabling us to examine the effects of chronic exposure to agents, such as GCs, on the human aqueous outflow facility.

Many patients treated with GCs for prolonged periods can develop significant elevations in IOP. Clinical studies on the propensity of the general population to respond to 4–6 weeks of topical ocular administration of a potent GC have indicated that there are differences in steroid responsiveness. Approximately 4–6% of the population tested had significant rises in IOP (an IOP of > 31 mm Hg or a pressure rise of > 15 mm Hg) and another 30–33% had a moderate ocular hypertension (an IOP of > 20 mm Hg or pressure increase of > 5 mm Hg). Patients treated systemically with GCs can also experience steroid-induced ocular hypertension although the responder rate does not appear to be as high as with topical administration of GCs. In contrast to the general population, certain groups of persons, such as patients with POAG, POAG descendants and siblings of POAG patients, persons with diabetes, and high myopes are at greater risk for being steroid responders.

In our study using an ex vivo organ culture system,
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13 of the 44 pairs of eyes treated with dexamethasone for 10–12 days showed a significant rise in IOP, which corresponds to a responder rate of 30%. This rate of steroid responsiveness approximates the responder rate shown in numerous clinical studies after topical ocular GC administration. The time frame for the development of ocular hypertension appeared to be similar, although perhaps slightly accelerated in the perfusion-cultured eyes. Steroid-induced ocular hypertension reported in many of the clinical studies generally required several weeks of topical ocular administration. The perfusion-cultured human eyes were treated for only 10–12 days with $10^{-7}$ M concentrations of the potent GC dexamethasone, and those eyes that responded to dexamethasone exposure had a lag period of 5–6 days before the onset of ocular hypertension. The fixed concentration of dexamethasone used in our perfusion studies was based on the aqueous humor concentration of dexamethasone found in the aqueous humor of human eyes topically administered with a single drop of 0.1% dexamethasone. However, the aqueous humor level of dexamethasone in patients dosed 3 to 4 times a day with a topical ocular GC is undoubtedly variable and not the constant concentration achieved in our perfusion study. Whether the constant level of dexamethasone used in the present study had any effect on steroid responsiveness is not known. That 30% of the perfused eyes treated with $10^{-7}$ M dexamethasone developed a significant rise in IOP after 10 days is remarkable. In a smaller study, Johnson and colleagues reported one out of nine human eyes perfused with $5 \times 10^{-7}$ M dexamethasone for 3 weeks developed ocular hypertension. It is quite possible that exposure of the perfused eyes to the steroid for longer periods might lead to a higher degree of responsiveness. The modest ultrastructural changes seen in some of the dexamethasone perfused nonresponder eyes would tend to support this hypothesis. It is also quite possible that all persons if treated long enough would develop some degree of ocular hypertension.

The dexamethasone-induced ocular hypertension in perfusion-cultured human eyes is associated with significant ultrastructural changes in the TM. Because the vast majority of eyes used in this study were from elderly donors (the average age for eyes used in this study was 73.5 years), it is important to control for possible age-related changes in the TM. A number of age-related changes in the TM have been previously reported including: thickening of the trabecular beams, increased deposition of extracellular plaque material beneath the inner wall endothelial cells and within the JCT, a decrease in vacuoles and pores in the inner wall, as well as loss of TM cells in the corneoscleral and uveal portion of the meshwork. Age-related versus dexamethasone-induced changes

FIGURE 4. Transmission electron micrographs of the trabecular meshwork from human eyes perfused for 10 days with media lacking (A) or containing (B,C) $10^{-7}$ M dexamethasone. Control eye (A) contains open network of trabecular beams lined with cells. Many of the trabecular cells contain intracellular pigment granules. Dexamethasone nonresponder eye (B) contains an open network of trabecular beams. Dexamethasone responder eye (C) has thick trabecular beams and increased deposition of extracellular material in the juxtacanalicular region (*), as well as small regions of cellular debris (arrows). S = Schlemm's canal. Bar = 10 μm.
FIGURE 5. Transmission electron micrographs of regions of the trabecular meshwork from control (A, C, E) and dexamethasone responder (B, D, F, G) eyes. Trabecular cells lining beams from control (A) and dexamethasone responder (B) eyes. Note the stacked layers of endoplasmic reticulum and extensive ribosomes in the trabecular cells of the dexamethasone responder eye. Trabecular beams from control (C) and dexamethasone responder (D) eyes. Beams appear to be thicker with less intertrabecular space in the dexamethasone responder eye. Inner wall endothelial cells and juxtacanalicular region of trabecular meshwork from control (E) and dexamethasone responder (F, G) eyes. Note the abundance of extracellular material (*) (F, G) just beneath the inner wall endothelial cells and the "activated" cell (arrow) (F) in the dexamethasone responder eyes. Bar = 0.5 µm (A, B) and 1 µm (C, D, E, F, G).
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FIGURE 6. Scanning electron micrographs of inner wall endothelial lining as visualized from inside Schlemm’s canal in control (A) and dexamethasone responder (B) eyes. Amorphogranular material (arrows) can be seen through a break in the endothelial lining of the dexamethasone responder eye. Bar = 5 μm.

FIGURE 7. Immunofluorescent scanning laser confocal photomicrographs of fibronectin distribution in juxtacanalicular/inner wall endothelial interface in control (A) and dexamethasone responder (B) eyes. The patchy fibronectin distribution in the control eye is altered to a more uniform and denser distribution in the dexamethasone responder eye. S = Schlemm’s canal. Bar = 1 μm.

in our perfusion-cultured eyes were controlled for through the use of paired eyes. Only those changes not observed in the contralateral control eye were deemed attributable to the dexamethasone treatment. The average age and initial IOP was higher in the dexamethasone responder group compared to the nonresponder group. It is possible that, in general, the older eyes with higher initial pressures and lower outflow facilities would be more susceptible to the pressure elevating effects of dexamethasone in this system.

Morphologic studies have been conducted on trabeculectomy samples from patients with corticosteroid-induced glaucoma. There are striking similarities as well as some differences in the morphologic consequences of corticosteroid glaucoma and the changes associated with dexamethasone perfusion-cultured responder eyes. Many of these differences are in the degree of change. An apparent thickening of trabecular beams, decreased intertrabecular space, deposition of extracellular material in the cribriform layer, and activation of TM cells was seen in our perfusion-cultured dexamethasone responder eyes. Similarly, in the steroid glaucomatous eyes there was a marked increase in the density of the cribriform layer, massive deposition of amorphous and fibrillar material in the intertrabecular spaces and cribriform pathways, thickened and enlarged trabecular lamellae, clumps of long spacing collagen, and enlarged trabecular cells. There was a decrease in cellularity in the TM of corticosteroid glaucoma patients. In the dexamethasone responder perfused eyes there also appeared to be decreased TM cellularity and evidence of cellular debris in the TM. The cell debris may be from the TM cells and is easily differentiated from the...
FIGURE 8. Summary of morphologic characteristics of control (A) and dexamethasone responder (B) perfusion-cultured human eyes showing narrowing of intertrabecular space (P), thickening of trabecular beams (B), and deposition of extracellular material (*) in the dexamethasone responder eye. S = Schlemm’s canal.

extracellular amorphogranular material seen in the dexamethasone responder eyes. Although some cell loss and death of TM cells is expected in this long-term culture system, the loss appears more apparent in the dexamethasone responder eyes compared to the contralateral control eyes and is therefore most likely due to the dexamethasone exposure. In steroid glaucomatous eyes there were extensive fibrillar extracellular deposits in the JCT and intertrabecular spaces \( ^{22,24} \) while in the dexamethasone responder perfused eyes there were only modest fibrillar deposits. There were differences in the degree of change between steroid glaucomatous eyes and the dexamethasone responder perfused eyes. The patients with steroid glaucoma who were receiving steroid therapy for a number of years, so it may not be so surprising that the degree of these morphologic changes was much more extensive than those seen in the perfusion-cultured eyes exposed to dexamethasone for only 10–12 days.

The dexamethasone-induced morphologic changes in the TM of perfusion-cultured responder eyes also appeared to be similar to many of the morphologic changes attributed to POAG. Several groups have found a decrease in cellularity in the TM compared to age-matched controls. \( ^{9-10} \) A number of laboratories have reported increased deposition of extracellular plaque material in the cribiform layer and beneath the inner wall of the canal of Schlemm. \( ^{10-15,50-59} \) There is hyalinization of the trabecular beams of POAG eyes, a thickening of the basement membrane lining the beams, the loss of trabecular cells covering the beams, and fusion of denuded beams. \( ^{10,12} \) The remaining trabecular cells appear to enlarge, spread, and show signs of activation with enlarged nuclei and increased free ribosomes. \( ^{12} \) POAG is also reported to involve the collapse of the canal of Schlemm, \( ^{50} \) decreased vacuolization of the inner wall, \( ^{57} \) and a decrease in inner wall pore density. \( ^{16} \) The deposition of extracellular material in the JCT, thickening and fusion of trabecular beams, apparent loss of cells from beams, and activation of TM cells were also found in the dexamethasone perfusion-cultured ocular hypertensive eyes included in this study.

There are a number of possible causes for the development of dexamethasone-induced increase in pressure in these perfusion-cultured human eyes. Human TM cells in situ and grown in culture have been shown to contain GC receptors and are therefore likely to be direct targets for GC action. Dexamethasone treatment of cultured human TM cells can lead to altered deposition of the ECM molecules fibronectin, \( ^{35} \) laminin (Steely et al., submitted for publication), collagen, \( ^{37} \) and elastin. \( ^{36} \) GC treatment can also change the glycosaminoglycan composition of the TM. \( ^{38,55} \) The dexamethasone-induced alteration in the TM ECM could be due to direct effects on the synthesis of ECM components and/or due to effects on the turnover of these molecules. Matrix metalloproteinase activity in cultured human TM cells is inhibited in a dose-dependent manner by GCs and TM tissue plasminogen activator activity is inhibited by dexamethasone treatment. \( ^{34} \) Additional studies have shown the induction of a major secreted sialoglycoprotein on GC treatment of cultured human and porcine TM cells. \( ^{28,30} \) Cultured human TM cells exposed to GCs have also been shown to become enlarged, \( ^{30-32} \) and the TM cells appear to be activated as seen by increased amounts of endoplasmic reticulum and enlarged Golgi complexes. \( ^{32} \) There appears to be a specific, dexamethasone-induced alteration in TM fibronectin deposition in the dexamethasone responder perfused eyes that is again similar to GC effects on in vitro cultured human TM cells. \( ^{35} \) The increased deposition of extracellular amorphogranular and fibrillar material in the dexamethasone-responsive, perfusion-cultured eyes may reflect these reported dexamethasone-induced in vitro changes. The turnover of...
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the TM ECM is much more rapid than in other ocular tissues, which may account for the relatively rapid changes (10–12 days) in the TM beam thickness and ECM deposition in the dexamethasone responder perfused eyes.

GCs have also been shown to induce a major reorganization of actin microfilaments in cultured human TM cells, which is both time and dose dependent. The GC-induced alteration in TM cell cytoskeleton and ECM may account for the reported effects of GCs on TM cell size and function. Several weeks of treating cultured human TM cells with hydrocortisone or dexamethasone can cause a dramatic increase in TM cell size, nuclear size, and total DNA content. GCs also inhibit certain TM cell functions including phagocytosis, proliferation, and migration. The steroid-induced changes in TM cell structure and function may have a direct impact on the resistance of aqueous humor outflow, leading to the development of ocular hypertension.

The GC-induced elevation in IOP observed in patients treated topically or systemically with GCs and in the TM of perfusion-cultured human eyes is most likely due to a combination of effects. The time course of treatment and degree of any GC-induced changes probably determines whether the eye will develop increased resistance to aqueous outflow and a concomitant rise in IOP. The dexamethasone-induced changes in the TM cytoskeleton and ECM may dramatically compromise TM cell function through one or more of the following mechanisms: (1) The dexamethasone-induced inhibition of TM cell phagocytosis may lead to the accumulation of pigment and extracellular debris which physically occludes the meshwork and increases outflow resistance; (2) The dexamethasone-induced alteration in the TM cytoskeleton and ECM molecules, particularly fibronectin and laminin (Steely et al., submitted for publication), could result in alterations in integrin expression and organization thus changing the adhesive properties of TM cells to the trabecular lamellae as well as the inner wall endothelial cell adhesion to the basement membrane of the JCT. The resulting loss of TM cells could then compromise the aqueous outflow pathway, possibly leading to beam fusion, and an inability of the TM cells to adequately regulate the trabecular environment; (3) A dexamethasone-induced change in the TM cytoskeleton and ECM may compromise the ability of the inner wall endothelial cells to form giant vacuoles and pores, which are thought to be major routes of aqueous humor egress through the lining endothelium. The TM cytoskeleton may play an active role in regulating the cell shape change, which is part of giant vacuole formation. Previously reported changes in the TM cytoskeleton have been found to alter aqueous humor outflow resistance; (4) A GC-induced increase in TM cell size may lead to a decrease in TM cell porosity. Human TM cells grown on filters and treated with dexamethasone have decreased hydrostatic fluid flow (possibly through decreased porosity) through this meshwork monolayer. (5) Dexamethasone-induced changes in TM cytoskeleton and ECM composition may seriously compromise TM cell function. Protein synthesis, cell migration, cell proliferation, and protein secretion can be regulated by changes in the cytoskeleton and ECM.

We have shown that a subset of isolated, perfusion-cultured human eyes treated with the GC dexamethasone developed ocular hypertension. Many of the dexamethasone-induced morphologic changes in these eyes were similar to the changes reported in the TM of patients with steroid glaucoma and POAG. This system may therefore prove useful as an acute model in which to study the cellular, biochemical, and molecular changes responsible for the generation of ocular hypertension.

Key Words

trabecular meshwork, glucocorticoids, ocular hypertension, glaucoma, perfusion culture

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