Increased Intraocular Pressure in Mice Treated with Dexamethasone

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PURPOSE. Glucocorticoids are potent modulators of the immune system and are useful in treating systemic and ocular diseases, but they can increase intraocular pressure (IOP) in susceptible persons. Steroid-induced ocular hypertension resembles several characteristics observed in primary open angle glaucoma (POAG). Elucidating genetic and environmental mechanisms impacting steroid-induced ocular hypertension may provide important insight into pathophysiological drivers of POAG. The purpose of this study was to create a mouse model of steroid-induced ocular hypertension.

METHODS. Osmotic mini-pumps delivering dexamethasone or PBS were implanted into C57BL/6j-Tyrε-red × 129S5/SvEvBrd (B6.129) mice. Repeated IOP measurements were obtained over a 4-week study using a tonometer before and after pump implantation. Body weights, complete blood counts (CBCs), and blood pressure were obtained to further characterize the model. Pharmacologic effects of timolol, latanoprost, and Y-39983 were studied in hypertensive mice.

RESULTS. Administration of dexamethasone to B6.129 hybrid mice resulted in significant increases in IOP in most animals compared with baseline or mice treated with PBS. No significant change in IOP was observed in PBS-treated mice. Interestingly, dexamethasone failed to increase IOP in a subset of mice, though steroid delivery was successful as measured using CBC analysis. Moreover, topical agents that lower IOP in nonmotensive mice also produced significant decreases in mice exhibiting elevated IOP in response to dexamethasone.

CONCLUSIONS. Systemic treatment with dexamethasone significantly increased IOP in most genetically heterogeneous mice used in this study. This mouse model should facilitate studies aimed at understanding mechanisms affecting steroid-induced ocular hypertension in humans. (Invest Ophthalmol Vis Sci. 2010;51:6496 – 6503) DOI:10.1167/iovs.10-5430

Administration of glucocorticoids, regardless of delivery route, results in intraocular pressure (IOP) elevations in certain persons.1,2 Increased IOP is an important risk factor for glaucoma, which is defined as optic nerve damage and visual field loss. Primary open angle glaucoma (POAG) is the most common form of glaucoma and is the second leading cause of blindness worldwide.3 In a relatively small number of patients, prolonged administration of steroids resulted in secondary OAG, leading investigators to suggest that mechanisms underlying steroid-induced secondary angle glaucoma are also important in the much larger population of POAG patients.1,2

Armaly et al.4 demonstrated that in otherwise normal eyes, repeated (4 weeks) topical administration of 0.1% dexamethasone (DEX) resulted in large IOP increases (>15 mm Hg compared with baseline) in approximately 5% of subjects. The remaining subjects exhibited either an IOP increase ranging from 6 to 15 mm Hg (~29% of subjects) or ≤5 mm Hg change compared with baseline (~66% of eyes). Therefore, high responders constitute the minority of healthy eyes, whereas the majority exhibits no IOP effect or a modest increase in IOP after steroid treatment.

Unlike the heterogeneous steroid response observed in the normal eye, IOP in glaucomatous patients increased dramatically in nearly every patient subject after topically applied steroids.5 Mean IOP increased approximately 47% in glaucoma patients (baseline IOP averaged 16.9 mm Hg and increased to 32.1 mm Hg). Relatives of POAG patients also exhibit a higher risk for steroid-induced ocular hypertension.6–8 These observations led to the supposition that common genetic factors affect both POAG and steroid responder status in humans. Although suggestive evidence exists for heritable factors in glaucoma and steroid-induced ocular hypertension, the genetics are likely to be complex and influenced by quantitative trait loci.9,10

Animal models of steroid-induced ocular hypertension have been created in several species. Elevation of IOP after topical administration of DEX has been reported in cows, cats, monkeys, rabbits, and, most recently, sheep.11–18 These models are appropriate to examine physiological mechanisms underlying steroid-induced ocular hypertension and its relationship to POAG. However, they are not easily amenable to direct testing of environmental and genetic contributions affecting ocular steroid responsiveness. The present study describes a model of steroid-induced ocular hypertension in mice, the ideal species for genetic investigations.

METHODS

Animals

All animal experiments were performed in accordance with the ARVO Statement for the Use of Animals in Ophthalmic and Vision Research, and all protocols were reviewed and approved by Lexicon Pharmaceuticals internal Institutional Animal Care and Use Committee. Male F2 generation hybrid mice from crosses between C57BL/6j-Tyrε-red and 129S5/SvEvBrd (referred to as B6.129 mice) were used for all IOP studies. The mice were housed in a 12-hour light/12-hour dark cycle with full access to food and water. The mice used for these studies were 10 to 13 weeks of age.
Osmotic Minipump Surgery

Micro-osmotic pumps (Alzet, model 1004; DURECT Corp., Cupertino, CA) were filled with either PBS or PBS containing water-soluble DEX (Sigma, St. Louis, MO). DEX was formulated at a concentration of 34.5 mg/mL (wt/vol). The flow rate for the micro-osmotic pumps was 0.11 μL/h, which delivers 0.09 mg DEX/day.

Animals were anesthetized with isoflurane during implantation of osmotic mini-pumps delivering DEX or PBS-filled pumps (controls). Loss of consciousness was determined by a toe pinch. After animals were anesthetized, they were dosed with 0.1 to 0.15 mg/kg buprenorphine analgesic. A second dose of buprenorphine was given 18 to 24 hours after surgery. Surgical instruments were sterilized before use. The surgical area was shaved to remove excess fur and was sanitized with 70% isopropyl alcohol and iodine. A small incision was made midline at the base of the scapula. Using a sterile hemostat, a small pocket was made subcutaneously along the side of the animal, and pumps were inserted with the flow moderator pointed posteriorly away from the surgical site. The tissue was pulled together and blotted dried. Adhesive (Tissue Bond; 3M, St. Paul, MN) was placed on the surgical site and allowed to dry. Mice were then housed singly and placed on a heating pad to recover.

Measurement of DEX in Serum and Aqueous Humor

A cohort of mice was implanted with osmotic mini-pumps containing DEX in the manner described. After 21 days of DEX treatment, plasma samples (20 μL/mouse) and aqueous humor (10 μL/mouse; pooled from both eyes) were analyzed with high-performance liquid chromatography and mass spectrometry (LC/MS-MS). The lower limit of quantification for DEX was 0.2 ng/mL, and the higher limit of quantification was 10,000 ng/mL. Analyst software version 1.4.0 was used for calculation purposes, and linear regression was applied with a 1/x × x weighting.

IOP Measurements

Mice were lightly sedated with isoflurane anesthesia, and their intraocular pressures (IOPs) were recorded with a tonometer (TonoLab; Colonial Medical Supply, Franconia, NH). Briefly, mice were placed into a warmed isoflurane chamber for up to 5 minutes until they lost consciousness. The mice were then moved to a heated pad with an isoflurane nosecone for the IOP measurement. The isoflurane was only used to immobilize the mice for the IOP measurements. This was deemed necessary because it was difficult to scrub conscious mice once the osmotic pumps were implanted on their backs. Appropriate sedation was confirmed by the lack of a blink reflex. Baseline IOPs were obtained between 8 and 11 am the day before pump implantation surgery. Weekly IOP measurements were made on several cohorts in the study to determine the onset of DEX-induced elevation in IOP. The final IOP was obtained at comparable times during the fourth week after surgery. For each animal, IOP was presented as the average of three measurements taken from the right eye.

Evaluation of IOP-Lowering Drugs

DEX-hypertensive and nonimplanted naive mice were treated topically with a single dose of either timolol (Falcon Pharmaceuticals, Fort Worth, TX; 3 μL of a 5-mg/mL solution), latanoprost (Xalatan; Pfizer, New York, NY; 4 μL of a 10-ng/mL solution), or Y-39983 (3 μL of a 1-ng/mL solution). The 10-ng/mL formulation of latanoprost was achieved by diluting the clinical formulation (50 ng/mL) 1:5 using PBS containing 0.02% benzalkonium chloride (Sigma). The vehicle control for timolol and latanoprost was PBS containing 0.01% or 0.02% benzalkonium chloride (Sigma), respectively. The vehicle control for Y-39983 was PBS. Baseline IOP measurements were taken with a tonometer on all mice before topical dosing. Posttreatment IOP recordings were taken at 2, 4, and 6 hours. All IOP measurements were taken under isoflurane sedation.

Blood Pressure and Blood Chemistry

Average daily systolic blood pressures were determined on days 22, 23, and 24 after pump implantation in conscious mice using a tail cuff system (BP-2000; Visitech Systems, Apex, NC). Each mouse was measured in the same chamber, and measurements were taken at the same time each day. The three daily systolic blood pressure values were averaged together to determine a mean systolic blood pressure for each mouse. Complete blood cell count (CBC) analysis was performed on blood isolated from the retro-orbital sinus (CellDyn 3500; Abbott Diagnostics, Abbott Park, IL).

Statistical Analysis

For each study cohort, IOP comparisons between the DEX and PBS treatment groups 4 weeks after surgery were based on analysis of covariance (ANCOVA) general linear models. Averages of three baseline IOP measures were used as covariates, and averages of the three posttreatment (at week 4) IOP values were used as the dependent variables. To evaluate the time course of IOP elevation in the DEX- and PBS-treated groups, repeated-measures ANOVA (RMANOVA) was conducted on the daily averaged IOP measurement at baseline and through 4 weeks after minipump implant, with Dunnett’s adjustment applied for multiple comparisons between treatment groups at each time point. Changes in body weight obtained immediately after surgery and 3 weeks after surgery were compared between treatment groups for each study using the Satterthwaite t-test, adjusted for unequal sample sizes and variances.

RESULTS

Four independent experiments using mini-pumps to deliver DEX resulted in significantly increased IOP in male B6.129 F2

### Table 1. Mean IOP of Separate Cohorts of Mice Treated with PBS or DEX

<table>
<thead>
<tr>
<th>Study (n)</th>
<th>Treatment</th>
<th>Baseline IOP*</th>
<th>Final IOP*</th>
<th>ΔIOP*</th>
<th>% ΔIOP</th>
<th>P†</th>
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<tbody>
<tr>
<td>1 (17)</td>
<td>PBS</td>
<td>14.6 (1.3)</td>
<td>13.6 (1.2)</td>
<td>−1.0 (2.1)</td>
<td>−6.8</td>
<td></td>
</tr>
<tr>
<td>1 (32)</td>
<td>DEX</td>
<td>13.7 (1.7)</td>
<td>17.0 (2.2)</td>
<td>+3.3 (2.4)</td>
<td>+24.1</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>2 (20)</td>
<td>PBS</td>
<td>13.0 (1.1)</td>
<td>13.2 (1.5)</td>
<td>+0.2 (1.6)</td>
<td>+1.5</td>
<td></td>
</tr>
<tr>
<td>2 (15)</td>
<td>DEX</td>
<td>12.6 (0.6)</td>
<td>16.1 (2.3)</td>
<td>+3.5 (2.8)</td>
<td>+27.8</td>
<td>0.0002</td>
</tr>
<tr>
<td>3 (10)</td>
<td>PBS</td>
<td>14.8 (1.5)</td>
<td>14.8 (1.1)</td>
<td>0.0 (1.6)</td>
<td>+0.0</td>
<td></td>
</tr>
<tr>
<td>3 (44)</td>
<td>DEX</td>
<td>14.8 (1.2)</td>
<td>18.7 (2.4)</td>
<td>+3.9 (2.7)</td>
<td>+26.4</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>4 (9)</td>
<td>PBS</td>
<td>14.5 (1.1)</td>
<td>14.8 (1.0)</td>
<td>+0.3 (1.2)</td>
<td>+2.1</td>
<td></td>
</tr>
<tr>
<td>4 (20)</td>
<td>DEX</td>
<td>14.9 (1.1)</td>
<td>18.3 (2.2)</td>
<td>+3.4 (2.7)</td>
<td>+22.8</td>
<td>0.0002</td>
</tr>
</tbody>
</table>

IOP values are given in mm Hg.
* Parentheses indicate standard deviation.
† Significance results from ANCOVA comparing final IOP by treatment with baseline IOP as a covariate.
hybrid mice (Table 1; \( P < 0.0001 \) for studies 1 and 3; \( P = 0.0002 \) for studies 2 and 4). A 23% to 28% increase in IOP was observed in each cohort of mice treated with DEX regardless of starting baseline values (Table 1). The absolute increase in IOP averaged 3.3, 3.5, 3.9, and 3.4 mm Hg in the steroid-treated cohorts, whereas control mice receiving PBS exhibited no significant increases in IOP at completion of the in-life study. In two separate cohorts, IOP measurements were recorded weekly up to 4 weeks to determine the DEX-induced time course of IOP elevation (Figs. 1A, 1B). For the cohort in study 2, shown in Figure 1A, the RMANOVA results indicated a significant within-subjects time effect (\( F = 7.46; P = 0.0002 \)) and a significant time by treatment interaction effect (\( F = 6.53; P = 0.0005 \)). The test of between-subjects effects in this cohort indicated significant treatment effects on repeated measures of IOP at weeks 1 (\( P < 0.0001 \)), 3 (\( P = 0.0002 \)), and 4 (\( P < 0.0001 \)). A similar result was observed in the cohort in study 1, shown in Figure 1B. The RANOVA results indicated a significant within-subjects time effect (\( F = 4.22; P = 0.0034 \)) and a significant time by treatment interaction effect (\( F = 17.57; P < 0.0001 \)). The test of between-subjects effects in this cohort indicated significant treatment effects on the repeated measures of IOP at weeks 1 (\( P = 0.0089 \)), 2 (\( P < 0.0001 \)), 3 (\( P < 0.0001 \)), and 4 (\( P < 0.0001 \)).

Individual IOP measurements for mice in study 1 (Table 1) are shown in Figure 1C. Mice treated with PBS exhibited a mean IOP of 13.53 mm Hg \( \pm 1.2 \) (SD; \( n = 17 \)) after 3 weeks of study. IOP was not statistically different from their baseline IOP (Table 1) measured at the time of pump implantation. In contrast, 3 weeks of DEX treatment increased IOP to an average of 17.01 mm Hg (SD; \( n = 32 \)), representing a 24% increase from baseline IOP (\( P < 0.0001 \); paired \( t \)-test; Dunnett’s posttest). (C) Individual IOP measurements during week 4 from the cohort of mice shown in (B) (**\( P < 0.0001 \); Student’s \( t \)-test). Changes in IOP and body weight for each animal were plotted, and no relationship was found between weight loss and IOP (D; Pearson’s \( r = -0.11; P = 0.45; n = 49 \)).

### Table 2. Mean Body Weight of Separate Cohorts of Mice Treated with PBS or DEX

<table>
<thead>
<tr>
<th>Study (n)</th>
<th>Treatment</th>
<th>Baseline Weight*</th>
<th>Final Weight*</th>
<th>( \Delta ) Weight*</th>
<th>( P )†</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 (17)</td>
<td>PBS</td>
<td>36.3 (5.6)</td>
<td>35.1 (5.8)</td>
<td>-1.2 (1.1)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>1 (32)</td>
<td>DEX</td>
<td>33.6 (2.6)</td>
<td>29.2 (2.2)</td>
<td>-4.4 (1.2)</td>
<td></td>
</tr>
<tr>
<td>2 (20)</td>
<td>PBS</td>
<td>46.8 (4.9)</td>
<td>44.9 (5.4)</td>
<td>-1.9 (3.0)</td>
<td></td>
</tr>
<tr>
<td>2 (15)</td>
<td>DEX</td>
<td>35.2 (2.6)</td>
<td>32.3 (4.0)</td>
<td>-2.9 (2.7)</td>
<td>0.2915</td>
</tr>
<tr>
<td>3 (10)</td>
<td>PBS</td>
<td>33.4 (3.6)</td>
<td>32.4 (4.1)</td>
<td>-1.0 (4.1)</td>
<td></td>
</tr>
<tr>
<td>3 (42)</td>
<td>DEX</td>
<td>33.1 (2.4)</td>
<td>30.0 (2.9)</td>
<td>-3.2 (2.1)</td>
<td>0.1387</td>
</tr>
</tbody>
</table>

Body weights are given in grams.

* Parentheses indicate SD.

† Significance results from Satterthwaite’s \( t \)-test comparing delta weight between treatment groups.

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**FIGURE 1.** Systemic treatment with DEX increases IOP and does not correlate with decreased body weight. Repeated IOP measurements were recorded on two separate cohorts (A, B) of mice implanted with osmotic minipumps containing either PBS (A, \( n = 9 \); B, \( n = 17 \)) or DEX (A, \( n = 20 \); B, \( n = 32 \)). A significant increase in IOP was noted in the DEX group (\( P < 0.01 \), **\( P < 0.001 \), and ***\( P \leq 0.0001 \); RMANOVA with Dunnett’s posttest). (C) Individual IOP measurements during week 4 from the cohort of mice shown in (B) (**\( P < 0.0001 \); Student’s \( t \)-test). Changes in IOP and body weight for each animal were plotted, and no relationship was found between weight loss and IOP (D; Pearson’s \( r = -0.11; P = 0.45; n = 49 \)).
treatment. The scatter plot for study 1 (summarized in Tables 1 and 2 and Fig. 1B) is shown in Figure 1D (Pearson’s r = −0.11; P = 0.43; n = 49).

Figure 2A shows the IOP of individual mice (Table 1, study 4) treated for 3 weeks with either PBS (n = 9) or DEX (n = 20). No significant change from baseline IOP was observed in PBS-treated mice (Table 1). In DEX-treated mice, IOP increased from a baseline of 14.9 mm Hg ± 1.1 (SD) to 18.3 mm Hg ± 2.2 (P < 0.0001; paired t-test; n = 20), representing a 23% increase in IOP. The difference in IOP between the DEX-treated mice and the PBS-treated mice was also significantly different at 4 weeks (P = 0.001; Student’s t-test). Blood pressure was measured in these mice at the conclusion of the in-life study. Mice receiving PBS exhibited blood pressure ranges within the historical range for this B6.129 strain.19 Mice treated with DEX for 3 weeks exhibited a significant increase in blood pressure compared with PBS-treated animals (Fig. 2B; P < 0.0001; Student’s t-test). The mean systolic blood pressure in the DEX group (n = 20) was 141.5 mm Hg ± 15.5 (SD) compared with 113.9 mm Hg ± 10.3 (SD) in the PBS group (n = 9). Therefore, DEX therapy increased blood pressure approximately 20% during the 3-week treatment. Similar to IOP distribution, the range of blood pressure measurements overlapped in PBS- and DEX-treated groups. In Figure 2A, the IOP measurements of four mice in the DEX treatment group are clearly within the IOP range of PBS-treated mice. Their IOP values were 15.7, 15.3, 15.0, and 14.7 mm Hg, suggesting these mice could be classified as steroid nonresponders. Their blood pressures were of 134.8, 133.3, 173.8, and 150.6 mm Hg, respectively. Although increased blood pressure could contribute to elevated IOP, there was no statistical correlation (Pearson’s r = −0.39; P = 0.093) between IOP change and blood pressure in the mice treated with DEX (Fig. 2C).

A separate cohort of mice (n = 12) was implanted with DEX-containing pumps to directly measure levels of steroid in the plasma and aqueous humor after 21 days of exposure. IOP in this cohort was significantly elevated over baseline IOP (P < 0.0001; paired t-test). The IOP increase averaged 5.8 mm Hg ± 1.4 (SD) after 3 weeks of DEX exposure (Fig. 3A). Levels of DEX in plasma averaged 61 nM ± 29 (SD; Fig. 3B). The range of DEX levels in plasma was 24 nM to 109 nM, and no correlation was observed between plasma DEX levels and change in IOP (Pearson’s r = 0.04; P = 0.89; Fig. 3C). DEX was not detectable in the aqueous humor of 8 of 12 mice with the current analytical methods. The remaining four mice had very low DEX levels in the aqueous humor, with a range of 0.4 to 1.8 nM (data not shown).

The pharmacologic effects of known IOP-lowering agents were determined in mice exhibiting increased IOP on days 21 to 25 in response to DEX treatment. Mice were treated with a single dose of either timolol (15 μg/eye), latanoprost (40 ng/eye), or Y-39983 (3 μg/eye). All three agents decreased IOP by >3 mm Hg in the DEX model (Figs. 4A–C). An increase in pharmacologic response was observed with respect to ΔIOP in DEX mice compared with normotensive naïve mice.

Throughout these studies, we observed that most B6.129 hybrid mice responded to DEX with increased IOP. However, a subset of mice exhibited relatively modest to no discernible change in IOP over 4 weeks of DEX treatment. At least two possibilities could explain this observation. First, technical issues associated with the mini-pump might have prevented adequate DEX levels to accumulate. Second, the hybrid mice might have exhibited different degrees of sensitivity to DEX-induced ocular hypertension. To further explore these possibilities, we performed several experiments examining systemic effects of DEX in B6.129 male hybrid mice. DEX is an immunosuppressive agent that decreases the absolute number of lymphocytes in systemic circulation. CBC analysis was performed on mice receiving PBS or DEX therapy for 3 weeks. The lymphocyte count in peripheral blood in mice receiving PBS was 4.74 × 10³/μL (n = 3), which is consistent with previous CBC analysis in the B6.129 hybrid background.20 In contrast, mice receiving DEX for 3 weeks exhibited a statistically significant decrease in average lymphocyte count (0.07 × 10³/μL blood) compared with the PBS-treated mice (Fig. 5A; P < 0.0001; Student’s t-test). Comparisons of individual lymphocyte counts and IOP changes revealed no correlation between lymphocyte number and IOP response to DEX treatment (Fig. 2).

**Figure 2.** Systemic treatment with DEX increases systolic blood pressure. Both IOP (A) and systolic blood pressure (B) were significantly increased after 4 weeks of treatment with DEX (n = 20) compared with PBS-treated mice (n = 9; *P = 0.001 and P < 0.0001 respectively; Student’s t-test). No significant relationship was determined between increased IOP and increased blood pressure (C; Pearson’s r = −0.39; P = 0.093; n = 20).
The decreased lymphocyte count was observed in those mice that exhibited minimal IOP responses (<2 mm Hg change from baseline) after treatment. This line of evidence argues strongly against a steroid nonresponder in the B6.129 hybrid background arising as a result of a technical issue with the DEX pump.

These data suggest that the C57:129 hybrid mice segregate polymorphic alleles in their genetic background and, therefore, likely exhibit a steroid responder status similar to that observed for IOP in primates and humans.12,21 To further define this observation, we compared frequency distributions of IOP in PBS- and DEX-treated eyes. At baseline, there was no significant difference in mean IOP across treatment groups (Fig. 6A). The mean baseline IOP of mice (n = 161) used in these studies was 14.6 mm Hg ± 1.4 (SD). There was no effect on IOP in mice exposed to PBS for 4 weeks (Fig. 6B). The final IOP measurement in 100% (46 of 46) of PBS-treated mice was within 1 SD of the baseline mean. After 3 weeks of DEX exposure, there was a significant shift in both the mean and the variability of IOP measurements (Fig. 6C). The mean IOP (n = 115) was 18.1 mm Hg ± 2.4 (SD). Approximately 64% (74 of 115) of mice treated with DEX exhibited IOP measurements greater than 17.4 mm Hg or 2 SD from the population study mean at baseline. Approximately 36% (41 of 115) of mice receiving DEX exhibited a final IOP at the completion of the study that fell within 2 SD of the baseline mean.

**DISCUSSION**

In this study, we describe a mouse model of steroid-induced ocular hypertension in healthy, normotensive mice. A reproducible increase in IOP of approximately 25% was observed in C57:129 mice after 3 weeks of systemic DEX treatment. In a controlled clinical study, Becker and Mills5 administered topical steroids to healthy, nonglaucomatous humans for a minimum of 2 months. In the treated eye, the mean baseline IOP at the start of the study was 13.6 mm Hg (n = 30). The IOP increased to 18.2 mm Hg in the treated eye, whereas the IOP in the contralateral eye remained at baseline levels. This magnitude of IOP increase over baseline (25%) is similar to what was observed in this mouse model of ocular hypertension. In the human model, the outflow facility in the steroid-treated eye decreased from a mean of 0.30 to 0.21 μL/min/mm Hg.5

The current studies do not definitively explain the mechanism behind this steroid response in mice. Long-term administration of DEX did not cause any gross obstruction in the outflow pathway (data not shown), but further examination at the ultrastructure level may be required to identify subtle differences in the trabecular meshwork and Schlemm’s canal. Treatment of trabecular meshwork cells with DEX has been shown to result in morphologic changes such as increased actin stress fiber formation and development of cross-linked actin networks (CLANS).22–26 Such morphologic changes have also been identified in cross-sections of trabecular meshwork from DEX-perfused human eye anterior segment cultures and human glaucomatous eyes and may be responsible for reduced outflow facility.22,27–29 Changes in the composition of extracellular matrix may also be a causative factor. Treatment with DEX has been shown to alter the expression and deposition of numerous extracellular proteins.27–31 Detailed characterization of the in situ cellular response to chronic DEX treatment in the mouse model is being investigated.

Systemic treatment with glucocorticoids has been shown to increase blood pressure in humans as well as in various animal models,32–35 and could be another possible explanation of ocular hypertension after DEX treatment. In this model, however, the increase in systolic blood pressure did not correlate with the observed increase in IOP. This is evidenced by the fact that several animals in the study had increases in systolic blood pressure but did not show any increase in IOP. Furthermore, normotensive mice dosed systemically with an agent known to increase blood pressure in mice (10 mg/kg phenylephrine)56 did not experience any effect on IOP (data not shown).

One of the most compelling observations from these studies is that a certain population of our hybrid mice did not exhibit increased IOP in response to DEX treatment. The creation of...
this model in mice will enable an understanding of the relative heritable contributions to steroid-induced ocular hypertension. Further studies using recombinant inbred mouse lines and specific gene knockout lines will be necessary to fully explore and identify genes contributing to steroid responsiveness and resistance. Given the correlation between steroid-induced ocular hypertension and susceptibility to glaucoma in the human population, the identification of such genes may have a major impact on the early diagnosis of glaucoma and the development of novel treatments. This mouse model of open-angle ocular hypertension represents a valuable tool for evaluating new ocular therapies aimed at lowering IOP in humans.

**FIGURE 4.** IOP in naive normotensive 129.B6 mice (dotted line) and DEX-induced ocular hypertensive mice (solid line) treated with IOP-lowering agents. IOP measurements were recorded at \( t = 0 \) to determine baseline IOP values and at \( t = 2 \) and 4 hours for timolol (A) and Y-39983 (C) and \( t = 2, 4, \) and 6 hours for latanoprost (B). \( \Delta IOP \) is expressed as mean ± SEM (*\( P < 0.05 \), **\( P < 0.01 \), and ***\( P < 0.001 \)). \( P \) values were determined by comparing \( \Delta IOP \) between compound- and vehicle-treated animals using RMANOVA with a Bonferroni posttest.

**FIGURE 5.** Systemic treatment with DEX leads to lymphopenia. CBCs were determined on blood samples taken from mice after 4 weeks of treatment with either DEX (\( n = 14 \)) or PBS (\( n = 3 \)). DEX treatment resulted in a significant decrease in lymphocyte number (A; *\( P < 0.0001 \); Student’s \( t \)-test). There was no significant correlation between lymphocyte number and IOP response to DEX (B; Pearson’s \( r = -0.33; P = 0.115; n = 24 \)).
A. Distribution of IOP changes after systemic treatment with DEX. Baseline IOP measurements of mice treated with either PBS or DEX exhibited similar frequency distributions (A). There was no change in the distribution of IOP measurements in PBS-treated mice between baseline and week 4 (B). An increase in IOP measurements was observed for the DEX-treated mice between baseline and week 4 (C). However, some mice failed to exhibit an increase in IOP and remained at baseline levels throughout the study.

Acknowledgments

The authors thank Damaris Diaz for her expert help in analyzing the mouse plasma and aqueous humor samples to determine dexamethasone exposure levels.

References


