Pharmacological Validation of a Feline Model of Steroid-Induced Ocular Hypertension

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Objective: To validate pharmacologically the feline model of steroid-induced ocular hypertension.

Methods: Serial studies were conducted in domesticated adult female cats trained to accept topical ocular drug administration and pneumotonometry. To establish intraocular pressure (IOP) values for each study, measurements were performed at the same time of day for 6 consecutive days. Beginning on day 7, cats received either steroid or vehicle administered topically to both eyes three times a day for approximately 28 days. The IOP measurements were performed daily.

Results: After 5 to 7 days of treatment with 0.1% dexamethasone or 1.0% prednisolone acetate, IOP began to increase, reaching peak values within 2 weeks. These values were sustained throughout dosing but declined rapidly to baseline upon cessation of treatment. Maximum IOPs for the dexamethasone- and prednisolone-treated groups averaged 4.5 ± 0.3 mm Hg (n = 12) greater than the mean IOP value obtained in vehicle-treated cats. Cats treated with 0.25% flurometholone, 1.0% loteprednol etabonate, and 1.0% rimexolone exhibited increases of 0.6, 1.2, and 1.7 mm Hg, respectively. These values were significantly lower than those observed following treatment with dexamethasone or prednisolone.

Conclusions: The ocular hypertensive effects of selected anti-inflammatory topical ocular steroids in this model are consistent with clinical findings.

Clinical Relevance: This feline model is a useful tool for assessing the potential IOP liability of novel anti-inflammatory steroids.


GLUCOCORTICOIDs are widely used for the treatment of inflammatory diseases of the eye and other organs. In the 1950s and early 1960s, it was discovered that topical treatment of the eye with glucocorticoids induced ocular hypertension both in persons with normal intraocular pressure (IOP) and in patients with glaucoma.1-4 Steroid-induced ocular hypertension was greater in patients with primary open-angle glaucoma than in normal individuals5 because of decreased aqueous humor outflow facility6,7 associated with biochemical and ultrastructural changes in trabecular meshwork.8,9

A number of investigators have attempted to develop a useful experimental model of steroid-induced ocular hypertension, with the rabbit most frequently the test animal of choice.7-12 However, the response of IOP to topical corticosteroid treatment in this species was found to be inconsistent.7,9 Some animals failed to develop ocular hypertension, whereas others exhibited only a transitory elevation of IOP despite continued administration of steroids. Undesirable adverse effects were also noted in this species.7 While some investigators10,11 reported similar problems using subconjunctival instillation of glucocorticoids, Hester et al12 reportedly achieved consistent elevation of IOP in young rabbits using subconjunctivally injected dexamethasone, cortisone acetate, and, particularly, triamcinolone. However, it should be emphasized that rabbits have a collection of outflow channels but lack the well-defined canal of Schlemm found in primates and humans.13

In 1992, Zhan and coworkers14 described a feline model of ocular hypertension induced by topical ocular administration of dexamethasone or prednisolone. This model reproduced the course and reversibility of steroid-induced ocular hypertension in humans.1,2,4,15 In the present studies, we have pharmacologically validated the feline model of steroid-induced ocular hypertension using preparations of numerous clinically tested topical ocular...
MATERIALS AND METHODS

EXPERIMENTAL ANIMALS

Before the initiation of all studies, adult female cats (ages 4 years ± 2 months) were socialized to human contact for 8 to 12 weeks. Unrestrained animals were introduced in a stepwise manner to topical ocular drug administration by corneal instillation of saline or local anesthetic (Alcaine, 0.5% proparacaine, Alcon, Fort Worth, Tex.), then trained to accept a pneumotonometer tip to measure IOP. Animals that were not domesticated and/or did not respond (9%) to dexamethasone were excluded from the study. Of a total of 32 cats, 24 animals with no visible signs of ocular abnormality were selected for use. Studies were performed in compliance with the Association for Research in Vision and Ophthalmology (ARVO) Statement for the Use of Animals in Ophthalmic and Vision Research.

MEASUREMENT OF IOP

The IOP was measured using a pneumotonometer (model 30 classic; Mentor O&O, Inc, Norwell, Mass). Animals received a drop of local anesthetic applied topically to the cornea immediately before measurement of IOP. Initially, the IOP of all cats was measured twice a day for 2 to 3 weeks until the IOP stabilized. During experiments, IOP was measured at 2 PM, Monday through Friday.

EXPERIMENTAL DESIGN

Individual experiments conducted during this study were parallel designed. No crossover design was used. On completion of each experiment, the cats were pooled and randomly reassigned to steroid or vehicle treatment groups for the next experiment in the series described. At the beginning of each experiment, cats were randomly divided into groups (n = 6–7 per group) using the GraphPad Prism Randomizer (GraphPad Software, Inc, San Diego, Calif). Baseline IOP was established during the week before treatment by a daily reading at 2 PM. Mean baseline IOP values for all treatment groups within an individual experiment varied by less than 1.0 mm Hg. Beginning on day 7, 1 drop (approximately 35 µL) of the appropriate vehicle or drug preparation was instilled in the lower sac of both eyes of each cat 3 times per day, at 9 AM, 3 PM, and 9 PM. In each experiment, all animals received either test drug or appropriate vehicle. Test drug or vehicle was administered for 27 to 29 days. After cessation of dosing, IOP measurements were continued until values returned to predosing baseline levels. A minimum interval of 4 weeks was maintained between studies to allow the cats to recover from the previous treatment.

TEST MATERIALS

Dexamethasone, prednisolone, and fluorometholone were obtained from Sigma-Aldrich Corp., St Louis, Mo. Loteprednol was obtained from the Center for Drug Discovery in Gainesville, Fla. All drug preparations were formulated on a weight/volume basis using a carbopol ophthalmic suspension vehicle. A commercially available preparation of rimexolone (VEXOL) was used.

DATA ANALYSIS

All data were analyzed for statistical significance between the groups using the unpaired t test. Graphs were generated by the GraphPad Prism program (GraphPad Software, Inc, San Diego, Calif). Data given as mean ± SEM.

corticosteroids. Results obtained correlate closely with observations in humans and indicate this model is a reliable predictor of the ocular hypertensive liability of anti-inflammatory steroidal agents for ophthalmic use.

RESULTS

The effects on IOP of the topical ocular treatment of cats with 1.0% prednisolone acetate or 0.1% dexamethasone administered three times a day for 29 days were examined in several studies. Vehicle-treated animals demonstrated a mean change of 0.34 ± 0.21 mm Hg from baseline (n = 10 studies). These changes were not statistically significant (P = 0.39). Consequently, all drug effects were assessed by comparison with the appropriate vehicle control. Between days 4 and 5 of treatment with either steroid, IOP began to increase and reached a maximum by day 15. Mean increase above vehicle controls was 4.6 ± 0.5 mm Hg (n = 3 studies) for prednisolone and 4.4 ± 0.3 mm Hg (n = 7 studies) for dexamethasone. The IOP of the steroid-treated groups remained at this elevated level for the duration of the treatment period. The increased IOP values were significantly greater than pre-study baseline measurements and vehicle-treated controls from day 7 onward. The IOP returned to baseline values within 5 to 7 days after drug treatment was discontinued. Representative data from a single study are presented in Figure 1.

The kinetics of IOP during prolonged topical ocular administration of 1% loteprednol etabonate, 0.25% fluorometholone, and 1% rimexolone are summarized in Figure 2. A, B, and C, respectively. Cats treated with each of these steroids exhibited increased IOP levels that peaked within 9 to 11 days after initiation of dosing. Mean IOP values for the steroid- and vehicle-treated groups during the period of maximum pressure elevation (days 10-28 of dosing) are provided in Table 1. In these studies, the elevated IOP values due to loteprednol, fluorometholone, and rimexolone administration were significantly less than those produced by treatment with dexamethasone or prednisolone. Treatment with loteprednol and fluorometholone resulted in mean IOP increases over vehicle values of only 1.2 and 0.6 mm Hg, respectively, in contrast to the 3.0-mm Hg increase observed in the prednisolone group in concurrent testing (study A). In a separate study (study B), rimexolone-treated cats exhibited a mean IOP increase of 1.7 mm Hg, whereas dexamethasone produced a mean increase of 3.5 mm Hg over vehicle values. Thus, the net increases due to fluorometholone, loteprednol, and rimexolone were less than 50% of those induced by dexamethasone and prednisolone in simultaneous testing.
Results obtained in these experiments confirm that topically administered dexamethasone and prednisolone, known for their ocular hypertensive effects in humans, elevate IOP in cats. The course of development, duration, and reversibility of the steroid response in cats presented in this report are similar to those observed by Zhan et al. There are, however, differences between the 2 studies. The maximum increase in IOP in response to dexamethasone reported by Zhan et al was approximately 7.0 mm Hg compared with approximately 4.5 mm Hg observed in the present study. This quantitative difference may be due to the fact that the investigations by Zhan et al included animals that were used only once for steroid treatment, while cats in the present study were treated with various steroids and vehicles during the experiments. In addition, those investigators used a higher concentration of dexamethasone (1.0%). In our laboratories, the maximum IOP increase induced by dexamethasone and prednisolone appeared to lessen with repeated experimentation (Table 2). This occurred despite a minimum washout period of 6 weeks between studies. These findings suggest that repeated steroid treatment induces some degree of refractoriness in feline IOP response. Nevertheless, IOP was significantly increased on a consistent basis in all dexamethasone- and prednisolone-treated animals compared with vehicle-dosed controls.

Zhan et al have demonstrated that the cat model exhibits the onset, duration, and reversibility of the human ocular hypertensive response to steroids. This occurs in mature animals. However, minor differences are apparent. Humans and cats differ in the kinetics of their IOP response to steroids. For example, in the feline model, within 2 weeks of steroid treatment, IOP reached maximum values, whereas, in humans, IOP increased progressively during a 6-week period. Our investigations confirm these findings.

However, our work extends the findings of Zhan et al and demonstrates another relevant similarity between the feline model and clinical findings. The magnitude of IOP response in cats clearly discriminates between steroids that exhibit varying degrees of IOP liability in humans. This conclusion is supported by the fact that loteprednol, fluorometholone, and rimexolone, which have lower propensity to cause ocular hypertension in humans, did not raise IOP as much as either dexamethasone or prednisolone. The results obtained with these 3 steroids in cats are consistent with those reported for humans. In clinical studies, treatment with 0.25% fluorometholone for 6 weeks produced significantly (P<.001) smaller increases (8.1 mm Hg) in IOP than did 0.1% dexamethasone (11.6 mm Hg) in steroid-responsive individuals. Smaller increases (<50% of those due to dexamethasone) were obtained using 0.1% fluorometholone. Comparative clinical studies indicated that in steroid responders treated with loteprednol (0.5%), mean IOP in-
was rimexolone. Although the increases observed in the studies using either prednisolone acetate or dexamethasone are statistically significant and are consistent with clinical observations, the feline model appears to be suitable for studying the ocular hypertensive potential of novel anti-inflammatory steroids.

Table 1. The Effect of Topical Ocular Corticosteroids on Intraocular Pressure (IOP) in Cats

<table>
<thead>
<tr>
<th>Test Compound</th>
<th>IOP, Mean ± SEM, mm Hg (Days 10-29 of Dosing)</th>
<th>ΔIOP Compared With Vehicle, mm Hg</th>
</tr>
</thead>
<tbody>
<tr>
<td>Study A</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Vehicle</td>
<td>19.9 ± 0.06</td>
<td>Not applicable</td>
</tr>
<tr>
<td>1% Prednisolone acetate</td>
<td>22.8 ± 0.5*‡</td>
<td>3.0</td>
</tr>
<tr>
<td>1% Loteprednol etabonate</td>
<td>21.0 ± 0.5*‡</td>
<td>1.2</td>
</tr>
<tr>
<td>0.25% Fluorometholone</td>
<td>20.4 ± 0.11*‡</td>
<td>0.6</td>
</tr>
<tr>
<td>Study B</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Vehicle</td>
<td>17.9 ± 0.04</td>
<td>Not applicable</td>
</tr>
<tr>
<td>0.1% Dexamethasone</td>
<td>21.4 ± 0.23*</td>
<td>3.5</td>
</tr>
<tr>
<td>1% Rimexolone</td>
<td>19.6 ± 0.15†</td>
<td>1.7</td>
</tr>
</tbody>
</table>

*Significantly different from the respective vehicle group (P<.05).
†Significantly different from the respective prednisolone-dexamethasone group (P<.05).
‡Significantly different from the respective vehicle group (P<.02).

Table 2. Intraocular Pressure (IOP) Increases in Sequential Studies Using Either Prednisolone Acetate or Dexamethasone

<table>
<thead>
<tr>
<th>Study Sequence</th>
<th>ΔIOP Compared With Vehicle, mm Hg</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0.1% Dexamethasone</td>
</tr>
<tr>
<td>First</td>
<td>5.6</td>
</tr>
<tr>
<td>Second</td>
<td>4.4</td>
</tr>
<tr>
<td>Third</td>
<td>3.8</td>
</tr>
</tbody>
</table>

Each of the 6 studies represented consisted of a 29-day, 3-times-a-day dosing regimen, with a minimum washout of 6 weeks between studies. Values represent the difference between the mean IOP of the drug-treated and vehicle control groups for days 10 to 29 of dosing.

Increased from 17.4 to 21.5 mm Hg by day 42 of treatment, a statistically insignificant increase of 4.1 mm Hg (24%). In contrast, in the prednisolone-treated group, mean IOP increased significantly from 18.1 to 27.1 mm Hg—an increase of 9.0 mm Hg (50%) over baseline values. Similarly, 1.0% rimexolone exhibited a significantly lower IOP-elevating potential than 0.1% dexamethasone sodium phosphate and 1.0% prednisolone acetate in steroid-responsive individuals. Prednisolone was as much as 8 times more likely to induce a significant (>10 mm Hg) increase in IOP than was rimexolone. Although the increases observed in the cat model are numerically smaller than those noted clinically, the values obtained in the cat are nevertheless statistically significant and are consistent with clinical observations. Thus, the feline model appears to be suitable for studying the ocular hypertensive potential of novel anti-inflammatory steroids.

Ocular hypertensive response to steroids in humans is believed to be genetically linked. In cats, such a link has not yet been established, but the fact that almost all cats responded to steroids does not suggest a genetically linked steroid effect. Steroid-induced ocular hypertensive response in humans is due to the reduced outflow facility. Because the outflow pathway of aqueous humor drainage in cats is anatomically similar to that in humans, it can be conjectured that changes in the feline outflow pathway account for the increases in IOP. The feline model needs further study to determine the precise mechanism of steroid-induced ocular hypertension. Nevertheless, the feline model of steroid-induced ocular hypertension is an appropriate model for studying the ocular hypertensive potential of novel anti-inflammatory steroids.

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REFERENCES