Effects of ocular administration of ophthalmic 2% dorzolamide hydrochloride solution on aqueous humor flow rate and intraocular pressure in clinically normal cats

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Objective—To determine the effects of ocular administration of ophthalmic 2% dorzolamide hydrochloride solution on aqueous humor flow rate (AHFR) and intraocular pressure (IOP) in clinically normal cats.

Animals—20 clinically normal domestic shorthair cats.

Procedures—Following an acclimation period, IOP was measured in each eye of all cats 5 times daily for 3 days to determine baseline values. Fifteen cats received 1 drop of 2% dorzolamide solution and 5 cats received 1 drop of control solution in each eye every 8 hours for 5 days (treatment phase). The IOP of each eye was measured 5 times during each day of the treatment phase. Prior to and after the treatment phase, AHFR in both eyes of each cat was measured via fluorophotometry.

Results—Prior to treatment, AHFR or IOP did not differ between the treatment and control groups. In dorzolamide-treated cats, mean AHFR after the treatment phase (3.47 ± 1.5 µL/min) was significantly lower than the value prior to treatment (5.90 ± 2.2 µL/min) and mean IOP during the treatment phase (11.1 ± 1.0 mm Hg) was significantly lower than the baseline mean IOP (14.9 ± 1.0 mm Hg). In the control group, IOP values did not differ before or during the treatment phase and AHFRs did not differ before and after the treatment phase.

Conclusions and Clinical Relevance—Ocular administration of 2% dorzolamide solution significantly decreased AHFR and IOP in clinically normal cats. Application of 2% dorzolamide solution may be an effective treatment in cats with glaucoma. (Am J Vet Res 2012;73:1074–1078)

Carbonic anhydrase inhibitors are used for the clinical management of glaucoma and ocular hypertension in humans and other animals. The enzyme carbonic anhydrase catalyzes a reversible reaction involving the hydration of carbon dioxide and the dehydration of carbonic acid. The activity of carbonic anhydrase produces bicarbonate; the bicarbonate produced by the enzyme in the ciliary body epithelium is excreted into the posterior chamber of the eye. This bicarbonate, along with actively secreted sodium ions, creates an osmotic diffusion gradient, drawing water into the posterior chamber to form aqueous humor. Carbonic anhydrase inhibitors contain a sulfonamide group that attaches to the carbonic anhydrase enzyme, thereby preventing the attachment of carbonic acid and suppressing the production of bicarbonate. In the ciliary body epithelium, carbonic anhydrases II, III, and possibly IV have been detected; however, carbonic anhydrase II is considered the most important target for CAIs. To decrease IOP, 98% inhibition of carbonic anhydrase II needs to be achieved in the ciliary body epithelium. Oral administration of CAIs can be associated with anorexia, gastrointestinal tract disturbances, increased respiratory rate secondary to metabolic acidosis, hypokalemia, blood dyscrasias, and neurologic abnormalities in humans and other species. Cats appear to be more susceptible to the adverse effects of oral administration of CAIs, and use of such drugs by that route of administration in this species is not advised. Because of the adverse effects associated with systemically administered CAIs, topical formulations have been developed. The current commercially available ophthalmic CAIs for ocular ad-
administration are 2% dorzolamide hydrochloride solution and 1% brinzolamide suspension.

Previous studies have revealed that ocular administration of dorzolamide solution has IOP-lowering effects in several species,12–16 including cats17–19. Ocular administration of dorzolamide solution also decreases aqueous humor flow in dogs,12 rabbits,13 and humans.20,21 The mechanism of action of 2% dorzolamide solution following application in the eyes of humans,22 monkeys,23 and dogs13 is to decrease aqueous humor production. Fluorophotometry is an accurate and noninvasive method for measuring AHFR.24,25 Anterior segment fluorophotometry has been used to determine the AHFR in clinically normal dogs26 and cats27 and in dogs after ocular tomometry has been used to determine the AHFR in the eyes of humans,22 monkeys,23 and dogs12 is to decrease aqueous humor production. Fluorophotometry is an accurate and noninvasive method for measuring AHFR.24,25 Anterior segment fluorophotometry has been used to determine the AHFR in clinically normal dogs26 and cats27 and in dogs after ocular and systemic administration of CAIs.12,28 The purpose of the study reported here was to investigate AHFR and IOP following ocular application of 2% dorzolamide hydrochloride solution in clinically normal cats by use of fluorophotometry and rebound tonometry, respectively. We hypothesized that ocular application of 2% dorzolamide solution 3 times daily in clinically normal cats would significantly decrease AHFR and IOP.

Materials and Methods

Animals—Twenty (8 neutered males and 12 sexually intact females) domestic shorthair cats that weighed 2.5 to 5.2 kg were used in the study. The cats ranged in age from 8 to 19 months (mean ± SD, 11 ± 3 months). The cats were obtained from the Kansas State University Department of Diagnostic Medicine and Pathobiology, and following the completion of the study, they were returned for eventual adoption. Prior to inclusion in the study, each cat underwent a physical examination and an ophthalmic examination, which included slit-lamp biomicroscopy, fluorescent staining of the cornea, rebound tonometry, and indirect ophthalmoscopy. Results of the prestudy physical and ophthalmic examinations of all cats were considered normal. The cats were housed in a temperature-controlled environment and exposed to an automated 12-hour light-dark cycle (light phase from 7 AM to 7 PM; dark phase from 7 PM to 7 AM). The study was approved by the Institutional Animal Care and Use Committee at Kansas State University.

Experimental procedures—During a 1-week acclimation period, IOP was measured 3 times daily in all cats by use of a rebound tonometer; no ocular anesthetic agent was administered topically. At the initiation of the experiment, the cats were randomly allocated to a control (n = 5 cats) and treatment (15) group. The duration of the study was 10 days, which included a pretreatment phase (days 1 to 3), performance of pretreatment fluorophotometry (day 4), a treatment phase (days 5 to 9), and performance of posttreatment fluorophotometry (day 10).

For each cat, a single IOP measurement was performed at 7 AM, 10 AM, 1 PM, 5 PM, and 9 PM by 1 investigator (AJR) on days 1 to 3 (pretreatment phase) and days 5 to 9 (treatment phase). Beginning on day 5, the 5 control cats received 1 drop (50 µL) of an artificial tear preparation2 in each eye and the treatment group cats received 1 drop (30 µL) of ophthalmic 2% dorzolamide hydrochloride solution2 in each eye at 7 AM, 3 PM, and 11 PM for 5 consecutive days (days 5 through 9 [treatment phase]). A final single treatment was administered at 7 AM on day 10. To mask the principal investigator (AJR) to which cats were in the treatment or control group, another investigator (WRC) administered the ocular medications (dispensed from identical sterile dropper bottles) to each cat.

Aqueous humor flow rate was measured, by use of anterior segment fluorophotometry, prior to drug administration (day 4) to obtain baseline data and after the single treatment administered on day 10 of the study (ie, after the treatment phase had been completed). One drop (50 µL) of 10% fluorescein2 was applied to each cornea of each cat at 5-minute intervals (total amount of fluorescein administered was 3 drops). Five minutes after administration of the third fluorescein drop, both eyes of each cat were rinsed thoroughly to ensure that fluorescein did not remain in the precular tear film. The forelimb paws and other areas of the body were also rinsed thoroughly to prevent fluorescein dye from being reintroduced into the tear film by the cat rubbing its eyes. Fluorescein concentrations were measured in the cornea and midcentral portion of the anterior chamber by use of a computerized scanning ocular fluorophotometerb with an anterior chamber adapter. Each cat was sedated with a combination of medetomidine (0.03 mg/kg) and ketamine (5 mg/kg) IM to facilitate proper positioning in front of the fluorophotometer. On the basis of results of a previous study,22 fluorophotometry was performed at 3, 6, 7, and 8 hours after the administration of the third drop of fluorescein on days 4 and 10.

AHFR calculation—At each predetermined time point on days 4 and 10 (ie, at 5, 6, 7, and 8 hours after the administration of the third drop of fluorescein), the corneal and aqueous humor fluorescein concentrations in each eye of each cat were determined. The fluorescein concentrations (in ng/mL) were natural log transformed and plotted, and regression analysis was performed to derive the slope. The following equations were used to determine the AHFR:

\[
K_{f} = -\Delta X (1 + [k_{d}^{\text{res, res}} VC_{c}^{1.2} VC_{d}]^{b})
\]

where \(V_{c}\) is anterior chamber volume, \(A\) is the slope of the decreasing cornea and aqueous humor fluorescein concentrations, \(k_{d}^{\text{res, res}}\) is a correction factor necessary to compensate for underestimation of corneal fluorescence inherent to fluorophotometry measurements, \(V_{c}\) is corneal volume, \(C_{c}\) is corneal fluorescein concentration, \(C_{d}\) is anterior chamber fluorescein concentration, \(b\) is the natural logarithm, \(Q\) is 0.90622, \(B\) is 1.848, and \(d\) is the thickness of the cornea (in mm). A value of 0.546 mm has been reported for the corneal thickness in cats30 and was used as the value of \(d\) in the present study; as a result, \(k_{d}^{\text{res, res}} = 1.51\). Reported values for the anterior chamber volume (820 µL) and corneal volume...
(165 µL) in cats were used. The denominator value of 1.2 represents a second correction factor designed to account for an inherent difference in fluorescence between the cornea and the aqueous humor.

Data analysis—For the measured variables, mean values of the right and the left eyes of each cat were calculated and used in the statistical analysis of the data. Regression analysis of the natural log-transformed corneal and aqueous humor fluorescein concentrations was used to create linear decay curves. Correlation coefficients were calculated to assess the fit of the data obtained at the 4 assessment times to the approximated straight line. The slopes of these curves were then compared to ensure they were decreasing in a reasonably parallel fashion. Eyes were excluded from flow calculation if correlation or slope ratio values represented extreme outliers. Aqueous humor flow rates were calculated for all eyes both before and after treatment. A repeated-measures ANOVA was used to compare AHFRs before and after treatment in the control and dorzolamide-treated groups. Intraocular pressure measurements before and during treatment in the control and dorzolamide-treated groups were analyzed by use of a hierarchical linear mixed-model ANOVA with repeated measures and a Tukey-Kramer adjustment. Intraocular pressures were also analyzed by use of a repeated-measures ANOVA with a Tukey-Kramer adjustment for multiple comparisons to evaluate time-matched IOPs before and during treatment in both the control and dorzolamide-treated groups at each measurement time point. Values of P ≤ 0.05 were considered significant.

Results

The mean AHFR for the clinically normal study cats prior to treatment was 4.27 ± 1.8 µL/min in the control group and 3.90 ± 2.2 µL/min in the dorzolamide-treated group. There was no significant (P = 0.131) difference between the 2 groups. Aqueous humor flow rates after the treatment phase for the control and dorzolamide-treated groups were 4.33 ± 1.1 µL/min and 3.47 ± 1.5 µL/min, respectively. The difference in AHFRs before and after treatment was significant (P < 0.001) for the dorzolamide-treated group and was not significant (P = 0.992) for the control group (Table 1). The mean overall decrease in AHFR in clinically normal cats after ocular administration of 2% dorzolamide solution was 2.43 µL/min, a decrease of 41%.

The mean IOP for the clinically normal study cats prior to treatment was 15.5 ± 1.1 mm Hg in the control group and 14.9 ± 1.0 mm Hg in the dorzolamide-treated group. There was no significant (P = 0.929) difference between the 2 groups. Intraocular pressure values during the treatment phase for the control and dorzolamide-treated groups were 13 ± 1.0 mm Hg and 11.1 ± 1.0 mm Hg, respectively. The difference in IOP before and during treatment was significant (P < 0.001) for the dorzolamide-treated group and was not significant (P = 0.720) for the control group (Table 1). The mean overall decrease in IOP in clinically normal cats during ocular administration of 2% dorzolamide solution was 3.8 mm Hg, a decrease of 26%. Intraocular pressures were significantly (P < 0.001) decreased at all time points in the cats in the dorzolamide-treated group, compared with their baseline values. No significant (P ≥ 0.968) change from baseline value was detected at any time point in the control group (Figure 1).

Discussion

Fluorophotometry estimates the AHFR on the basis of serial measurements of corneal and aqueous humor fluorescein concentrations following corneal fluorescein loading. The fluorophotometer is es-

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Table 1—Mean ± SD AHFR and IOP in 20 clinically normal cats before and after or during ocular administration of ophthalmic 2% dorzolamide hydrochloride solution (n = 15) or an artificial tears preparation (control solution; 5) for 5 days.

<table>
<thead>
<tr>
<th>Group</th>
<th>AHFR (µL/min)</th>
<th>IOP (mm Hg)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Before treatment</td>
<td>After treatment</td>
</tr>
<tr>
<td>Dorzolamide</td>
<td>5.90 ± 2.2</td>
<td>3.47 ± 1.5*</td>
</tr>
<tr>
<td>treatment</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>4.27 ± 1.8</td>
<td>4.53 ± 1.1</td>
</tr>
</tbody>
</table>

The duration of the study was 10 days, which included a pretreatment phase (days 1 to 3), performance of pretreatment fluorophotometry (assessment of pretreatment AHFR, day 4), a treatment phase (days 5 to 9), and performance of posttreatment fluorophotometry (assessment of posttreatment AHFR, day 10). Beginning on day 5, 1 drop (50 µL) of control solution or 2% dorzolamide solution was instilled in each eye of each cat at 7 AM, 3 PM, and 11 PM daily during the treatment phase; a final single treatment was administered at 7 AM on day 10 (prior to posttreatment AHFR assessment). On days 4 and 10, fluorophotometry for determination of AHFR was performed at 5, 6, 7, and 8 hours after ocular administration of fluorescein (3 drops over a 10-minute period); IOP was assessed at 7 AM, 10 AM, 1 PM, 5 PM, and 9 PM.

* For a given variable within a group, the value is significantly (P < 0.001) decreased, compared with the pretreatment value.
sentially a modified slit lamp with a fiber optic probe that emits a focused beam of blue light (wavelength, 480 nm). A barrier filter allows only the green light (wavelength, 520 nm) that indicates peak fluorescein to be evaluated, thereby reducing the effect of scatter. The digital radiometer then compares the emitted fluorescein fluorescence curves to determine the fluorescein concentration. The decrease in fluorescein concentration over time is attributed to the drainage of the fluorescein-aqueous humor mix and further dilution as it is replaced by newly produced aqueous humor. One of the assumptions of fluorophotometry is that a steady state is achieved as the fluorescein passes from the cornea into the aqueous humor and exits via the aqueous outflow pathways. The slopes of the decay curves generated from serial measurements of corneal and anterior chamber fluorescein concentrations are the basis for aqueous humor flow measurement and subsequent calculation of AHFR.

In the present study of clinically normal cats, ocular administration of ophthalmic 2% dorzolamide solution 3 times daily for 5 days reduced AHFR by 2.43 µL/min, which corresponded to a 41% reduction in aqueous humor flow. The reduction in aqueous humor flow in the cats in the present study was similar to findings in clinically normal dogs (43%) and monkeys (29%) and higher than the values reported for rabbits (17%) and humans (13% to 19%). In the rabbit study, 1 drop of 2% dorzolamide solution was administered only once 2 hours prior to fluorophotometry, which may account for the less dramatic reduction in aqueous humor flow, compared with the results of the other studies. Wide variability in AHFRs in normotensive humans has been reported, with ranges of 0.2 to 32 µL/min. For dogs, variability in baseline AHFR has also been reported, with ranges of 2.2 to 9.8 µL/min and 1.47 to 10.69 µL/min. Similarly, in the cats of the present study, there was a range of baseline AHFRs from 1.17 to 10.69 µL/min. Despite the variability in AHFRs, the difference in baseline AHFR between the dorzolamide-treated group (5.90 ± 1.8 µL/min) and control (4.27 ± 1.61 µL/min) groups was not significant.

Intraocular pressure was measured in the cats of the present study by use of a rebound tonometer, which estimates the IOP on the basis of the deceleration of the probe as it rebounds from the corneal surface. The tonometer has an internal calibration curve for small animals (dogs and cats). In a recent study involving enucleated cats’ eyes, IOP determined by use of the rebound tonometer was compared with IOPs determined via direct manometry and applanation tonometry. The rebound tonometer findings correlated well with those of direct manometry in the range of 25 to 50 mm Hg. Based on rebound tonometer measurements, the mean IOP in clinically normal cats has been reported to be 20.74 mm Hg (range, 11 to 33 mm Hg). Ocular administration of an anesthetic agent was not used to obtain IOP readings in the present study. Ocular administration of an anesthetic agent prior to use of the rebound tonometer does not significantly affect IOP readings in clinically normal cats and dogs. In the present study, the mean pretreatment IOP values for the dorzolamide-treated group (14.9 mm Hg) and the control group (13.5 mm Hg) were within the range of values considered to be normal for cats as determined by use of the rebound tonometer.

Ocular administration of 2% dorzolamide solution 3 times daily for 5 days in ocularly normotensive cats in the present study caused a mean decrease in IOP of 3.8 mm Hg, compared with baseline values, which corresponded to a 26% decrease in IOP. In the dorzolamide-treated group, the IOP was significantly (P < 0.001) different from the baseline value at each of the 5 time points. In the control group, IOP did not differ significantly (P ≥ 0.968) from the baseline value at any of the time points evaluated. Previous studies evaluating the effects of 2% dorzolamide solution administered in the eyes of normotensive cats also revealed a significant treatment-associated decrease in IOP. Twice-daily administration of 2% dorzolamide solution in the eyes of normotensive cats for 5 days resulted in a decrease in IOP of 2.9 mm Hg (24.2%). In another study in clinically normal cats, IOP significantly decreased from baseline values as a result of ocular administration of 2% dorzolamide solution either 2 (decrease of 1.6 mm Hg [8.8%]) or 3 (decrease of 2.2 mm Hg [12.1%]) times daily. Concomitant ocular administration of 2% dorzolamide solution and 0.3% timolol maleate solution twice daily to cats in that same study did not decrease the IOP to a greater extent than did ocular administration of dorzolamide solution alone 3 times daily. In comparison, the application of 2% dorzolamide solution to eyes of normotensive monkeys, humans, and dogs reduces IOP from baseline values by 11%, 8.5% to 13%, 20,30 and 24.3%, respectively.

The magnitude of the effect of dorzolamide on IOP in normotensive cats may not be an accurate representation of the effect of this medication on cats with glaucoma. Following ocular or systemic administration of CAs, dogs with glaucoma typically have a greater decrease in IOP, compared with that observed in normotensive dogs. A recent study revealed a dramatic decrease in IOP in 7 cats with primary congenital glaucoma that received topical treatment of the eyes with 2% dorzolamide solution 3 times daily. In that study, the circadian fluctuations in IOP were dampened during the treatment period, compared with the baseline values.

On the basis of decreases in AHFR and IOP, results of the present study have suggested that the mechanism of action of 2% dorzolamide solution following ocular administration in normotensive cats involves the suppression of aqueous humor production. Ocular administration of 2% dorzolamide solution may be useful for treating cats with glaucoma or ocular hypertension.

a. SL-14 Biomicroscope, Kowa Co Ltd, Tokyo, Japan.
b. BioGlo HUB Pharmaceuticals LLC, Rancho Cucamonga, Calif.
c. Domitor, Orion Corp, Espoo, Finland.
d. Trusopt, Bausch & Lomb Inc, Tampa, Fla.
e. LiquiTears, Major Pharmaceuticals, Livonia, Mich.
g. AK-Fluor10%, Akorn Inc, Lake Forest, Ill.
h. HEINE Omega 180 Ophthalmoscope, HEINE Optotechnik, Herrsching, Germany.
i. VetaKet, IVX Animal Health Inc, St Joseph, Mo.

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References


