Effects of topical 2% dorzolamide hydrochloride alone and in combination with 0.5% timolol maleate on intraocular pressure in normal feline eyes

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Abstract

Objective  To evaluate the effect of topical 2% dorzolamide alone, and in combination with 0.5% timolol, on intraocular pressure (IOP) in normal cats.

Animals  Twenty-four healthy Domestic Short-haired cats.

Procedure  Baseline values of IOP were established at 7 AM, 10 AM, 1 PM, 5 PM and 9 PM during pretreatment phase (days 1–2). During treatment phase (days 3–10) cats received 2% dorzolamide HCl q 12 h in group A (n = 6), q 8 h in group B (n = 6), and combined with 0.5% timolol maleate q 12 h in group C (n = 6). Cats in control group D (n = 6) received artificial tears q 8 h. During treatment phase IOP measurements were continued at the same time-points as in the pretreatment phase.

Results  Mean pretreatment IOP in all cats was 18.46 ± 2.99 mmHg. Mean IOP decreased significantly (P < 0.0086) in all treatment groups compared to pretreatment values (group A: 16.40 ± 0.49 mmHg, group B: 16.04 ± 0.49 mmHg, group C: 17.76 ± 0.49 mmHg). IOP did not decrease in control group D (18.55 ± 0.49 mmHg). The difference in IOP between treatment groups (A, B, C) was not statistically significant, but comparison of IOP between each treatment group and the control group was statistically significant (A–D; P = 0.0057; B–D, P = 0.0012; C–D, P = 0.0212).

Conclusion  Topical 2% dorzolamide significantly lowers IOP in normal cats but the effect is mild. Concomitant application of 2% dorzolamide and 0.5% timolol does significantly decrease IOP, but the effect is not significantly greater than q 8 h administration of dorzolamide alone.

Key Words: carbonic anhydrase inhibitor, cats, dorzolamide, glaucoma, intraocular pressure, timolol maleate
agent in a twice or three-times-daily application. Additionally, the efficacy of concomitant administration of 2% dorzolamide and 0.5% timolol on IOP in cats was evaluated. Timolol is a β-adrenergic blocking agent that has shown efficacy in reducing IOP in normal cats. Both timolol and dorzolamide reduce intraocular pressure by decreasing aqueous humor flow. The commercially available combination drug of timolol and dorzolamide (Cosopt®, Merck & Co., Whitehouse Station, NJ, USA) may be advantageous in animals, as the recommended application dose of q 12 h may improve patient and client compliance.

The additive effect of dorzolamide and timolol on aqueous flow and intraocular pressure has been observed in humans and monkeys but to our knowledge not in cats.

MATERIALS AND METHODS

Animals
Twenty-four healthy Domestic Short-haired cats (19 spayed female, five castrated male) were used in the experiment. All animals were enrolled in a nonocular research project, which was carried out after completion of this study. Age of the cats ranged between 6 and 12 months. They were housed individually in two separate rooms, in a temperature-controlled environment at 21–23 °C. Cats were exposed to an automated 12-h light/dark cycle (light phase from 7 AM to 7 PM, dark phase from 7 PM to 7 AM), and were acclimatized to the environment for 1 week prior to initiation of the research trial. This study was reviewed and approved by the Animal Use and Care Committee of the College of Veterinary Medicine, The University of Georgia. An initial ophthalmic examination, including slit-lamp biomicroscopy, gonioscopy (17-mm Koepp goniolens, Ocular Instruments Inc., Bellevue, WA, USA), tonometry (Tono-pen™XL, Mentor Ophthalmics, Norwell, MA, USA) and indirect ophthalmoscopy, was normal for all cats.

Pretreatment phase
The length of the study was 12 days, divided into a pretreatment phase (days 1–2), treatment phase (days 3–10), and post-treatment phase (days 11–12). Prior to the study, the applanation tonometer was internally calibrated by the manufacturer and was calibrated daily throughout the course of the study. During the pretreatment phase (day 1–2), baseline IOP was recorded in all animals five times daily (7 AM, 10 AM, 1 PM, 5 PM and 9 PM). For the 9 PM measurement the dark phase was interrupted for the length of the IOP measurements, which lasted approximately 45 min per room. The cats tolerated IOP measurements with gentle manual restraint. Topical anesthetics were not applied prior to measuring IOP. On average three measurements were performed, and the first reading with < 5% variance was recorded.

Treatment phase
At the initiation of the research trial the cats were randomly divided into four groups of six animals each: three treatment groups A, B, C and one control group D. One drop (50 µL) of 2% dorzolamide hydrochloride (Trusopt®, Merck & Co., West Point, PA, USA) was applied to cats in group A q 12 h, and to cats in group B q 8 h. Group C received a combination of 2% dorzolamide q 8 h and 0.5% timolol (Timolol maleate, Bausch & Lomb Pharmaceuticals Inc., Tampa, FL, USA) q 8 h. If both dorzolamide and timolol were given at the same time (7 AM) the application of timolol was followed by the administration of dorzolamide 5 min later. All cats were monitored for signs of discomfort (tearing, squinting) or conjunctival hyperemia after receiving medications. IOP measurements were performed daily at the same time-points as during the pretreatment phase and by the same investigators (UD, MC). Cats were measured in a random order. The same person restrained the animals throughout the study. The 7 AM IOP measurement on days 3–10 was immediately followed by the first treatment.

Post-treatment phase
Measurement of intraocular pressure in all cats was continued at the above-mentioned time-points for 2 days after cessation of treatment (days 11–12). This time period was used to monitor the return of IOP to baseline values.

Statistical analysis
The ‘two-eye-design’ applied in this study involved treatment of both eyes of the same individual. For statistical analysis a linear mixed model was employed, which accounts for the longitudinal data obtained in this study and accommodates repeated observations among individuals from the same eye. Animal was a random factor in the mixed model. Fixed factors in the model were baseline value on each day (for treatment and post-treatment phases only), eye, day and time, and their interactions. IOP measurement at 7 AM on each day in each group was considered the baseline value. IOP measurements in each treatment group and at different time-points were compared and set in relation to the baseline IOP (7 AM) on each day (these values were therefore called adjusted mean values). F-test was employed to test the significance of fixed effects. T-test was applied to perform multiple comparisons within the treatment period and to test overall time trend and trends within days in the post-treatment phase. All analysis was performed at 5% significance level.

RESULTS

Pretreatment phase
Mean IOP ± SEM of all cats was 18.46 ± 2.99 mmHg (in group A, 18.00 ± 0.61 mmHg; in group B, 18.25 ± 0.61 mmHg; in group C, 19.62 ± 0.61 mmHg; and in group D,
17.97 ± 0.61 mmHg). The variation in IOP between days 1 and 2 and between different groups was not statistically significant. However, there was a significant difference (P < 0.0001) in the adjusted mean IOP (± SEM) between the left and right eye, with a mean value of 1.08 mmHg (± 0.25). A difference in IOP < 5 mmHg between the right and left eye is usually not considered to be clinically important. Therefore, a mean value of the right and left eye was established for each cat, and used in all subsequent statistical analysis. A significant variation in IOP (P < 0.0001) was identified during each day at different time-points, with the highest IOP in the morning and early afternoon between 7 AM and 1 PM and the lowest IOP late in the afternoon at 5 PM. This diurnal fluctuation in IOP was observed in all cats (P = 0.0004) and is illustrated in Fig. 1.

Treatment phase
Topical medications were well tolerated and no ocular side effects were observed. During treatment phase (days 3–10), mean IOP significantly decreased in all treatment groups (P < 0.0086) compared to pretreatment values, except in control group D. Mean treatment IOP ± SEM was 16.40 ± 0.49 mmHg in group A; 16.04 ± 0.49 mmHg in group B; 17.76 ± 0.49 mmHg in group C; and 18.55 ± 0.49 mmHg in group D. The mean IOP values for all groups during the treatment period are shown in Fig. 2.

Difference in IOP between treatment groups (A, B, C) was not significant, but comparison of IOP between each treatment group and the control group was significant (A–D; P = 0.0057; B–D, P = 0.0018; C–D, P = 0.018). In groups A and B monotherapy with topical 2% dorzolamide, either administered twice or three times daily, appeared to be equally efficient in lowering IOP (P = 0.62). The combination of 2% dorzolamide and 0.5% timolol (group C) did significantly reduce IOP compared to baseline values (1.86 mmHg; P = 0.31), but the effect was not greater than the three-times-daily application of dorzolamide in group B (P = 0.31).

Post-treatment phase
On day 11, mean IOP values increased significantly over time (P < 0.0001). The overall change in IOP from pretreatment to post-treatment phase is illustrated in Fig. 2.
DISCUSSION

The baseline intraocular pressure (mean ± SEM), established during the pretreatment period for all cats, was 18.46 ± 2.99 mmHg. This is comparable to previously reported normal values in cats of 19.7 ± 5.6 mmHg.26 Diurnal fluctuation in intraocular pressure followed a distinct pattern, which was consistently observed in all 24 cats. This was not an unexpected finding, as variation in IOP throughout the day has been previously described in cats27 and also exists in humans,28 rabbits,29 monkeys30 and dogs.31 In cats the lowest IOP has been reported in the morning, followed by a rise during the day and with the highest IOP occurring in the evening.27 In contrast, IOP fluctuations in the present study were different from the above-mentioned pattern and this was consistently observed in all cats: a low pressure in the morning was followed by a steady rise of IOP, until a peak value was reached at 1 pm. A gradual decrease in IOP was then observed in the afternoon with the lowest value at 5 pm, followed by a slow rise of IOP again later in the evening (Fig. 1). IOP was not measured during the night-time, which would have provided additional information. In humans fluctuations in IOP have been attributed to many endogenous or exogenous factors, including plasma hormone levels, sleep, age and health, environmental light, postural changes, or blood pressure.32,33 In cats highest IOP readings in the evening have previously been explained by nocturnal behavior of this species.27 In the present study the housing environment did not reflect a normal behavior pattern of the research cats and it is more likely that exogenous factors, such as artificial light cycles, as well as handling and feeding times, may have influenced fluctuations in IOP during the day.

Topical application of 2% dorzolamide significantly decreased intraocular pressure in groups A and B, with the maximum effect occurring 3–6 h after the first treatment. This finding is comparable with a peak reduction of 2–7 h observed in humans,21 rabbits,8 and dogs.12 As listed in Table 2, reduction in IOP by 1.6 mmHg (8.8%) was observed with the twice-daily, and 2.2 mmHg (12.1%) with the three-times-daily application compared to the mean baseline values. This effect was slightly less than the results reported in a recent study,14 where q 12 h application of dorzolamide in normal cats reduced IOP by 2.3 mmHg compared to baseline values.14 Direct comparison of both studies is problematic, as investigators of the aforementioned study used fewer (client-owned) and older cats (median age 9.5 years), measured IOP at different time-points, and evaluated data using a different statistical method. However, despite the differences in study design, the absolute drop in IOP over the treatment period was within a similar range and it can be concluded from both studies that the IOP-lowering effect of dorzolamide in cats is much lower than it is in other species. In comparison, the application of 2% dorzolamide as a single therapeutic agent reduced IOP by 9–24% in normotensive humans,34 by 19% in monkeys,13 and by 24.3% in normal dogs.7 The very modest response of the feline eye to the pharmacologic effect of topical carbonic anhydrase inhibitors is also supported by a previous study with brinzolamide,35 which showed that a 1% solution did not have a significant IOP-lowering effect in normal cat eyes if given q 12 h. Interestingly, horses appear to be similarly resistant to the pharmacologic effect of topical CAIs. In one study the mean IOP-lowering effect of 2% dorzolamide in normal equine eyes was < 2 mmHg compared to baseline values, which is almost identical to the results obtained in the present study.9 Topical carbonic anhydrase inhibitors effectively inhibit CA II, the main enzyme of aqueous humor production.3,2 It is possible that different CA isoenzymes in the feline ciliary body are responsible for aqueous production and that the lack of response to the IOP-lowering effect of topical CAIs may be related to incomplete enzyme inhibition or insufficient absorption of the drug at the effector site. It is further possible that the amount of ocular pigmentation may play a role in the effect of TCAIs in different species, as eyes of cats are usually less pigmented than eyes of dogs.37 TCAIs are known to bind to ocular pigment.18 A study comparing the IOP-lowering effect in a group of albino rabbits and pigmented rabbits found that the hypotensive effect of 2% dorzolamide was greater in magnitude and of a longer duration in the pigmented than in the albino rabbit.38

It has previously been hypothesized that a three-times-daily application may increase the pharmacologic effect on IOP in cats14 as this effect has been observed in studies with humans10,11 and dogs.12 This hypothesis is not supported by the results of the present study, as the effect between twice-daily and three-times-daily application of dorzolamide was not statistically significant. It does, however, support the theory that lack of response to topical CAIs in normal cats may be caused by incomplete enzyme inhibition and that topical CAI do not sufficiently decrease IOP in normal cats regardless of concentration or dose frequency.

It is well documented that dogs with glaucoma show an increased sensitivity to IOP-lowering drugs compared to normal individuals.10,11 The reason for this increased sensitivity of glaucomatous eyes to IOP-lowering drugs is unknown, but it is possible that dorzolamide has a more profound effect in cats with glaucoma than found in our

Table 2. Changes in intraocular pressure (mean ± SEM) in all groups during the pretreatment, treatment and post-treatment phase and changes expressed as a percentage

<table>
<thead>
<tr>
<th>Group</th>
<th>Pre-treatment (mean IOP in mmHg)</th>
<th>Treatment (mean IOP in mmHg)</th>
<th>Post-treatment (mean IOP in mmHg)</th>
<th>Change (% )</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>17.99 (0.61)</td>
<td>16.40 (0.49)</td>
<td>17.50 (0.63)</td>
<td>–8.8</td>
</tr>
<tr>
<td>B</td>
<td>18.24 (0.61)</td>
<td>16.04 (0.49)</td>
<td>17.54 (0.63)</td>
<td>–12.1</td>
</tr>
<tr>
<td>C</td>
<td>19.64 (0.61)</td>
<td>17.76 (0.49)</td>
<td>18.58 (0.62)</td>
<td>–9.4</td>
</tr>
<tr>
<td>D</td>
<td>17.97 (0.61)</td>
<td>18.55 (0.49)</td>
<td>19.43 (0.63)</td>
<td>3.2</td>
</tr>
</tbody>
</table>

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study with normal cats. This is supported by the results of several clinical reports in cats with glaucoma. One study found an IOP-lowering effect of nearly 30% when 2% dorzolamide was administered q 8 h.\textsuperscript{15} In another study 2% dorzolamide (q 8 h) decreased IOP from 27 to 15 mmHg in a cat with aqueous humor misdirection syndrome (AHMS), and deepening of the anterior chamber was observed.\textsuperscript{16} Another clinical report suggested a good or partial/temporary response of 2% dorzolamide in some cats, with IOP being maintained between 25 and 30 mmHg.\textsuperscript{17} Again, the reason for this increased sensitivity to dorzolamide in the glaucomatous eye in cats or in other species is not known. One may speculate that regulation of aqueous humor production is a well-balanced system in the normal eye, rendering it somewhat resistant to drug effects. In the glaucomatous eye this autoregulatory mechanism may be compromised and hence the eye becomes more sensitive to the therapeutic effect of the drug.

In the study reported here, the concomitant administration of dorzolamide and timolol had no greater IOP-lowering effect than the effect achieved with dorzolamide given as a single agent every 8 h. This was surprising, as the additive effect of timolol and dorzolamide on IOP is well documented in humans.\textsuperscript{22-24} Both timolol and dorzolamide lower IOP by decreasing aqueous production.\textsuperscript{22} This is achieved through different mechanisms: dorzolamide inhibits carbonic anhydrase, the key enzyme of aqueous production;\textsuperscript{1,2} timolol binds to β-adrenergic receptors of the ciliary body and decreases aqueous production through inhibition of cyclic AMP synthesis.\textsuperscript{39} In humans, timolol is a much stronger suppressor of aqueous flow and IOP than dorzolamide, but both drugs in combination have an additive effect that is stronger than the effect of either drug given alone.\textsuperscript{21,22} In one study dorzolamide further reduced IOP by 13 to 21% in patients with glaucoma already receiving 0.5% timolol twice daily.\textsuperscript{21} A similar observation was recently made in the glaucomatous Beagle.\textsuperscript{40} The fixed dorzolamide-timolol combination was more effective in reducing IOP in the affected dogs than was either timolol or dorzolamide alone.\textsuperscript{40}

The effect of topical timolol on IOP in normotensive cats has previously been evaluated and the drug was able to reduce IOP by 22.3% if given once daily.\textsuperscript{57} Thus, it is possible that the IOP-lowering effect in the combination group C observed in the present study was mainly attributed to the effect of timolol and not to dorzolamide. However, we did not determine the effect of timolol independently from the effect of the combination of dorzolamide-timolol, which is necessary to confirm the efficacy of timolol in our cat population.

Based on the analysis of data reported here, 2% dorzolamide (alone or in combination with 0.5% timolol) has a significant IOP-lowering effect in normal feline eyes; however, the hypotensive effect of this drug was less than the effect observed in other species and in animals with glaucoma. Further studies are needed to investigate the effect of dorzolamide in cats with glaucoma.

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