Comparison of the effects of topical administration of a fixed combination of dorzolamide–timolol to monotherapy with timolol or dorzolamide on IOP, pupil size, and heart rate in glaucomatous dogs

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Abstract
Objective To determine whether the combination multiple-dose dorzolamide–timolol administered topically has any greater effects on the reduction of intraocular pressure, pupil size, and heart rate in dogs with glaucoma than do either timolol or dorzolamide alone.

Procedure Applanation tonometry, pupil size, and heart rate measurements were made at 7 a.m., 1 p.m., and 7 p.m. daily of 12 laboratory Beagles with inherited primary open-angle glaucoma during each active phase of this study. Timolol 0.5% was administered first twice daily for 4 consecutive days. Dorzolamide 2.0% was administered next three times daily for 4 consecutive days. The fixed combination of the two (timolol 0.5% and dorzolamide 2.0%) was administered twice daily for 4 consecutive days during the final week of the study. Between administration of each drug, a withdrawal period of at least 10 days was instituted. Statistical comparisons between the effects of the three drugs were performed.

Results Intraocular pressure (IOP) was decreased with the administration of all three drugs: timolol alone, dorzolamide alone, and the combination of the two decreased IOP after 1 day of treatment $2.83 \pm 0.70$ mmHg, $6.47 \pm 0.32$ mmHg, and $6.56 \pm 0.37$ mmHg, respectively. After 4 days of treatment, the IOP decreased even further: timolol alone, $3.75 \pm 0.88$ mmHg, dorzolamide alone, and the combination of the two decreased IOP $7.50 \pm 0.29$ mmHg and $8.42 \pm 0.58$ mmHg, respectively. Heart rate was significantly decreased with timolol ($-11.9 \pm 2.0$ bpm) and the combination preparation ($-8.6 \pm 2.4$ bpm), but not with dorzolamide ($-3.7 \pm 1.8$ bpm) alone. Pupil size was significantly decreased with timolol ($-1.42 \pm 0.40$ mm) and the combination preparation ($-1.3 \pm 0.33$ mm), but not with dorzolamide ($0.97 \pm 0.36$ mm) alone.

Conclusions The combination dorzolamide–timolol appears to be more effective at reducing intraocular pressure in glaucomatous dogs than is either timolol or dorzolamide alone.

Key Words: Cosopt®, dog, dorzolamide, glaucoma, timolol

INTRODUCTION
Glaucoma is an insidious disease in humans and dogs associated with an increase in intraocular pressure (IOP) that is incompatible with the health of the eye.1,2 Vision loss and pain are the almost invariable sequelae in dogs.2 Finding efficacious medications to treat glaucoma is important because it is a leading cause of blindness in dogs and uncontrolled IOP can threaten life quality as a result of the discomfort that results.2

The primary aim in glaucoma management is the reduction of IOP, either by decreasing the production of aqueous humor or by increasing the outflow or drainage of that fluid from the eye.2,3 Carbonic anhydrase inhibitors (CAI) and beta-adrenergic antagonists have been mainstays in the treatment of glaucoma in humans and in dogs for many years.3,4 Both classes of drugs decrease IOP by decreasing the production of aqueous humor, although by different mechanisms.3–6 Topical formulations of each have been developed in an effort to capitalize on their IOP-lowering effects while minimizing systemic side effects.4,7–11 Dorzolamide hydrochloride, a topical CAI, and timolol maleate, a nonselective beta blocker, have both been found to be safe and effective for the treatment of the glaucoma.5–8,10,11 It is generally accepted that in dogs, dorzolamide has a stronger effect on reduction of IOP than timolol; however, its ideal dosing schedule is three times daily, whereas timolol is
usually administered only twice.\textsuperscript{6–8} Unfortunately, even with consistent therapy with either of these medications, the disease in both humans and dogs is progressive, necessitating the addition of another medication or some sort of surgical procedure.\textsuperscript{2,4,12} Therefore, the search continues for medications and dosing schemes that will aid in the control of this frustrating disease process.

A fixed combination of 2\% dorzolamide 0.5\% timolol (\textit{Cosopt\textsuperscript{®}}, Merck, West Point, PA, USA) is available that has been shown in humans to decrease IOP more than either timolol or dorzolamide alone.\textsuperscript{13–17} This combination appears to have few side effects and may even decrease IOP more than the expected individual contributions of either drug alone, suggesting a potential synergistic effect.\textsuperscript{13–17} No studies to date have evaluated the potential therapeutic effects of this combination product in the control of glaucoma in dogs. This study compares the effects on IOP, pupil aperture size (PS), and heart rate (HR) of each of these medications: timolol alone, dorzolamide alone, or the fixed combination of both. This combination product may be useful for long-term control of ocular hypertension in dogs and also for increasing client compliance, as it is administered only twice daily.\textsuperscript{12–17}

\section*{MATERIALS AND METHODS}

Twelve healthy adult laboratory Beagles with various stages of primary open-angle glaucoma were used (five males; seven females). The animals used in this study were tested with three different drugs; one drug at a time for a period of 4 days according the manufacturer’s recommendations. Between instillations of each consecutive drug, a withdrawal period of at least 10 days was instituted to allow for the previous drug to leave the animals’ systems and for the recorded parameters to return to baseline levels. Before administration of any medications, the IOP OU, PS OU, and HR of each dog were measured three times a day for 3 consecutive days in order to establish a baseline for comparison. Recorded measurements included IOP by applanation tonometry (\textit{Tono-Pen-XL, Mentor O and O, Norwell, MA, USA}), PS by Jameson calipers and HR with stethoscope placed upon the chest over the point of maximal intensity of the heart. The IOP of each eye (treated and untreated control) was measured after the administration of a topical anesthetic agent (0.5\% tetracaine HCL, Alcon, Ft. Worth, TX, USA). The study parameters were measured three times daily at 7 a.m., 1 p.m., and 7 p.m. The drugs were administered topically to one eye of each study dog on schedule according to the recommended dosing regimen for each agent. The contralateral (untreated) eye of each animal served as control. Treated and control eyes for each dog were chosen at random. Timolol maleate 0.5\% (Akorn, Inc., Buffalo Grove, IL, USA) was administered twice daily (BID), dorzolamide hydrochloride 2.0\% (\textit{Trusopt\textsuperscript{®}}, Merck, West Point, PA, USA) three times daily (TID) and the fixed combination of the two (\textit{Cosopt\textsuperscript{®}}, Merck) twice daily (BID). After each separate medication trial, all antiglaucoma medications were discontinued to allow for a washout period to ensure that each drug was acting independently. The study parameters continued to be measured three times daily even after the cessation of therapy. After sufficient time had been allowed, the next drug was given with TID daily measurements. The same individual (CEP) performed all the measurements.

Each of the measured parameters was compared between each product administered and the untreated controls to determine if there was a difference in the effects of the combination product compared to the effects of each agent individually.

The drug comparisons were performed using SAS programs utilizing Tukey’s HSD and \textit{ANOVA} tests for repeated measurements.\textsuperscript{21} Within each test week, the average measurements for IOP, PS, and HR for each day were compared with subsequent measurements to detect significant changes ($P < 0.05$) using the Tukey tests and \textit{ANOVA} for repeated measurements. Each measured parameter for drug-treated eyes was compared both to baseline and to the values for untreated eyes. Measured parameters for treated eyes were compared between drugs as well.

\section*{RESULTS}

\subsection*{Timolol 0.5\%}
The mean $\pm$ SEM changes in IOP for each of the tested drugs are summarized in Fig. 1. After 1 day of treatment, timolol decreased IOP in the treated eye by 2.83 $\pm$ 0.70 mmHg and in the untreated control eye by 2.53 $\pm$ 0.94 mmHg. The decrease in IOP in the treated eye was 3.75 $\pm$ 0.88 mmHg and in the untreated eye 2.31 $\pm$ 0.89 mmHg after 4 days of treatment. The IOP decrease from baseline was significant for the treated eye ($P = 0.0472$), but not for the untreated eye ($P = 0.1209$). The difference in IOP decline between the treated and untreated eyes was significant ($P < 0.0001$).

The mean $\pm$ SEM changes in PS for each of the tested drugs are summarized in Fig. 2. Pupil aperture was decreased from baseline by 1.42 $\pm$ 0.40 mm in the treated eye. This decrease was significant ($P = 0.0056$). The pupil of the untreated eye did not significantly change in size during the course of this study ($P = 0.9517$).

The mean $\pm$ SEM changes in HR for each of the tested drugs are summarized in Fig. 3. Heart rate was significantly decreased with the administration of timolol by 11.9 $\pm$ 2.0 bpm ($P = 0.0005$).

\subsection*{Dorzolamide 2.0\%}
After 1 day of treatment, dorzolamide decreased IOP in the treated eye by 6.47 $\pm$ 0.32 mmHg and in the untreated control eye by 2.19 $\pm$ 1.21 mmHg. The decrease in IOP in the treated eye was 7.50 $\pm$ 0.29 mmHg and in the untreated eye 3.50 $\pm$ 0.83 mmHg after 4 days of treatment. The IOP decrease from baseline was significant for the treated eye ($P < 0.0001$), but not for the untreated eye ($P = 0.1701$). The difference in IOP decline between the treated and untreated eyes was significant ($P < 0.0001$).
Heart rate was not significantly affected by the administration of dorzolamide ($-3.7 \pm 1.8$ bpm, $P = 0.4533$), nor was the pupil aperture significantly changed in either eye (treated eye $P = 0.0819$; untreated eye $P = 0.9986$).

**Timolol 0.5% – dorzolamide 2.0% combination**

After 1 day of treatment, Cosopt decreased IOP in the treated eye by $6.56 \pm 0.37$ mmHg and in the untreated control eye by $3.67 \pm 0.78$ mmHg. The decrease in IOP in the treated eye was $8.42 \pm 0.59$ mmHg and in the untreated eye was $7.03 \pm 0.61$ mmHg after 4 days of treatment. The IOP decrease from baseline was significant for the treated eye ($P < 0.0001$), and for the untreated eye as well ($P < 0.0001$).

The difference in IOP decline between the treated and untreated eyes was significant ($P < 0.0001$).

Heart rate was significantly decreased with the administration of Cosopt by $8.6 \pm 2.4$ bpm ($P = 0.0003$) and pupil aperture was significantly decreased by $1.3 \pm 0.33$ mm in the treated eye ($P = 0.0196$). The pupil of the untreated eye was not significantly affected by the administration of Cosopt in the fellow eye ($P = 0.6138$).

**Drug comparisons**

Values for IOP, PS, and HR were significantly different among each of the tested drugs ($P < 0.0001$ for each). The decline in IOP was significantly different for each drug.
tested. All three drugs significantly decreased IOP in treated eyes. The fixed-combination product decreased IOP to a significantly greater degree than did either dorzolamide alone \((P=0.05)\) or timolol alone \((P=0.05)\). Both the fixed-combination product and timolol alone significantly decreased PS \((P<0.0001)\) in the treated, but not in the untreated eyes. Both drugs decreased PS to a similar degree (the PS change in the treated eyes between the two drugs was not significant). The fixed-combination product and timolol alone both significantly decreased HR in dogs \((P<0.0001)\), but the difference in decline of HR between the two drugs was not significant.

**DISCUSSION**

Many veterinary ophthalmologists have already begun using the combination product of timolol and dorzolamide on their glaucomatous canine patients with favorable results. Timolol, a nonselective beta-adrenergic antagonist that lowers IOP by decreasing production of aqueous humor, has been the historical topical first line against an increasing IOP in both humans and small animal patients. Bilateral miosis and relative bradycardia result from the systemic uptake of timolol and occur because of the inhibitory effects of the drug on the beta-adrenergic fibers in the canine iris sphincter muscle and on those within the cardiovascular system. Both a decrease in pupil size in the treated eyes and a decrease in heart rate were noted with administration of both timolol alone and the combination product in this study. Newer drugs have shown promise for the even greater reduction of IOP by effecting different mechanisms of either aqueous humor production or outflow. Dorzolamide, a topical CAI, was developed in order to take advantage of the reduction of aqueous production that was noted to occur with the administration of systemic CAIs while avoiding the systemic side effects including metabolic acidosis and gastrointestinal upset that often either require the cessation of therapy or negatively affect compliance rates. No changes were noted with pupil size or heart rate with this topical CAI. The relative bradycardia and miosis noted in the dogs treated with the combination product in this study was most likely the result of the timolol component, as these changes have not previously been noted with the use of topical CAIs. The fixed combination of the two agents has been shown to have similar safety profiles for each of the individual drugs. No adverse effects were noted in any of the dogs included in this study. Human studies have proved that the fixed combination of timolol and dorzolamide is more effective at controlling elevated IOP than either of the component drugs alone.

Although the efficacy of dosing the combination product three times daily was not investigated, it is reasonable to infer that increasing the frequency of administration will be at least as effective as the twice-daily schedule and perhaps more so as previous studies have shown that dorzolamide on its own works best when given three times daily because of the pharmacokinetics of the agent. Previous studies have shown that the reduction of IOP after the administration of CAIs is dose responsive and that the maximum reduction occurs when there is 98–99% inhibition of the enzyme carbonic anhydrase within the nonpigmented ciliary body epithelium, the site of the production of aqueous humor. This study also did not compare the efficacy and equivalence of treating with the fixed combination to the concomitant use both timolol and dorzolamide. Human studies have shown that the use of the combination product is at least as effective as both of the individual drugs together and one study even reported an even greater reduction in IOP with the fixed combination. Of course, it is very likely that...
greater compliance was a contributing factor to the greater efficacy in that report, as it is much easier to properly administer one BID drug than it is to administer one BID drug and one TID drug. Additionally, when two separate products are administered if an appropriate amount of time has not passed between the two drops (at least 5 min), the first agent is likely to be washed out of the conjunctival cul-de-sac before it has a chance to effect its action. It is also possible that the different effects of each drug on the ocular tissues may have an effect on the penetration of the other increasing their potential availability for action.

Interestingly, the combination product produced a significant decrease in IOP of the untreated fellow eye at the same time it decreased IOP in the eye into which the drug was directly administered. This is somewhat puzzling as neither of the parent component drugs produces a significant decline in contralateral IOP, although both do show a trend toward a lowered IOP in the fellow eye, especially timolol. This could be the result of increased systemic absorption or some thus far unclassified synergistic effect of the two agents upon one another.

Clients, as well as patients, appreciate the single product BID dosing regimen. The cost of administering the combination product, of course, is higher than either single agent, but is now comparable to administering both of the separate products concurrently. Use of the fixed-combination product is more effective at lowering IOP than either individual drug and this, along with its convenient dosing scheme, may warrant the additional cost.

In summary, the fixed combination of timolol–dorzolamide is efficacious at reducing IOP in glaucomatous dogs, especially when compared to either timolol or dorzolamide alone and should be considered as an option for the medical treatment of glaucoma in the canine.

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