Effects of topical administration of latanoprost, timolol, or a combination of latanoprost and timolol on intraocular pressure, pupil size, and heart rate in clinically normal dogs

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Objective—To determine effects after topical administration of latanoprost, timolol, or a commercially available latanoprost-timolol combination twice daily on intraocular pressure (IOP), pupil size (PS), and heart rate (HR) in clinically normal dogs.

Animals—17 clinically normal dogs.

Procedures—A randomized controlled clinical trial was performed with a treatment (n = 9) and saline (0.9% NaCl) solution group (8). Each dog in the treatment group received 3 treatments (latanoprost, timolol, and the latanoprost-timolol combination), with a 14-day washout period between treatments. Baseline values were established on day 1 of each treatment period. On days 2 through 5, drugs were administered topically every 12 hours to 1 eye of each dog in the treatment group. In both groups, IOP, PS, and HR were measured at 0, 2, 4, 6, 8, and 9 hours on days 2 and 5.

Results—Eyes treated with latanoprost or the latanoprost-timolol combination had a significant decrease in IOP and a significantly smaller PS, compared with results for dogs receiving only timolol or dogs in the saline solution group. Timolol and the latanoprost-timolol combination both significantly lowered HR, compared with HR following administration of latanoprost and the saline solution.

Conclusions and Clinical Relevance—Topical administration of latanoprost alone was as effective at lowering IOP as was administration of the latanoprost-timolol combination when both were given every 12 hours to clinically normal dogs. Timolol, either alone or in combination with latanoprost, appeared to have little or no effect on IOP in clinically normal dogs but was associated with a reduction in HR. (Am J Vet Res 2010;71:1055–1061)

Glaucoma comprises a group of diseases that are unified by the common theme of progressive death of the retinal ganglion cells and their axons, which results in vision loss. An increase in IOP is the principal risk factor for glaucoma, and the primary goal of treatment is to reduce the IOP to values that will halt the death of retinal ganglion cells. Although clinicians have numerous medical and surgical options at their disposal, vision loss and decreased quality of life are frequent sequelae to glaucoma in domestic animals. Ideally, topical application of a single ocular hypotensive agent is the preferred treatment for patients with glaucoma; however, treatment with a single agent alone often does not achieve the desired degree of IOP reduction, and treatment with a combination of 2 or more antiglaucoma drugs is required. However, topical treatment with multiple drugs or > 2 dosing intervals/day is associated with significantly reduced compliance in human patients with glaucoma. This reduced compliance is not a trivial issue because 1 large study in humans with glaucoma revealed that approximately 45% of patients using an electronic monitoring device who knew they were being monitored and were provided free medication used the medications < 75% of the time. Products that combine 2 antiglaucoma drugs in the same bottle have been introduced. Examples of such combination treatments include dorzolamide-timolol and brimonidine-timolol.

Timolol maleate is a β-adrenergic receptor antagonist that reduces IOP by decreasing production of
aqueous humor. It is a safe and effective drug for the treatment of glaucoma in humans and for a number of years was typically regarded as the drug of choice in the treatment of primary open-angle glaucoma in humans. However, efficacy for the commercially available concentration of timolol (0.3%) in domestic animals is not clear. A single dose of 0.5% timolol resulted in a mean decrease in IOP of 4.14, 2.5, and 4.2 mm Hg in cats, dogs, and horses, respectively. Three other studies with single and multiple doses of 0.25% and 0.5% timolol revealed no decrease in IOP of clinically normal dogs. A single application of higher concentrations of timolol (2%, 4%, and 6%) failed to decrease the IOP in clinically normal Beagles. Because of systemic uptake and the crossover effect, it has been suggested that installation of timolol in 1 eye of a dog can lower IOP and induce miosis in the contralateral eye of that dog.

Latanoprost 0.005% is a topical preparation of a prostaglandin F₂α analogue first introduced in the United States in 1996. Latanoprost was one of the first commercially available prostaglandin analogues used in the treatment of open-angle glaucoma and ocular hypertension in humans. The presumed mechanism of action is to increase outflow of aqueous humor via the uveoscleral pathway, although a more recent study in dogs suggests that latanoprost is also capable of reducing production of aqueous humor. Topical administration of latanoprost has no systemic effects because it is rapidly metabolized and acts only locally. Producing hypertension in humans.

In the treatment of open-angle glaucoma and ocular hypertension in humans more effectively than can either agent used alone. Latanoprost is effective at lowering IOP in clinically normal dogs and cats and in Beagles with primary open-angle glaucoma.

As popular antiglaucoma medications become available in generic formulations, combination products are introduced to the market. These products typically increase patient compliance and, at times, have synergistic effects. A latanoprost-timolol combination product was approved for use in humans by the FDA in 2001. Two 6-month-long double-blind studies conducted in the United States and Europe revealed that the latanoprost-timolol combination administered once daily in humans was minimally but significantly more effective at lowering IOP than was use of latanoprost or timolol alone. In the US study, the mean reduction in IOP from baseline values for the latanoprost-timolol combination was 2.2 mm Hg, whereas use of latanoprost or timolol alone caused a mean reduction in IOP of 2.1 and 0.3 mm Hg, respectively. Given that latanoprost and timolol both cause a reduction in production of aqueous humor, greater synergistic effects might be expected in dogs.

Use of a latanoprost-timolol combination product once daily in humans is as effective as use of latanoprost once daily and use of timolol twice daily. A study in France in which investigators evaluated satisfaction and compliance in human glaucoma patients who used the latanoprost-timolol combination drops indicated an increase in satisfaction of 15% and an increase in compliance of 25% when patients were changed to the combination product. The purpose of the study reported here was to determine effects of latanoprost, timolol, or a combination of latanoprost and timolol administered topically on the reduction of IOP, PS, and HR in clinically normal dogs.

Materials and Methods

Animals—Healthy, socialized, staff-owned pets (n = 17 dogs [34 eyes]) were used in the study. None of the dogs had signs of ocular disease as determined on the basis of results of slit-lamp biomicroscopy, indirect ophthalmoscopy, Schirmer tear testing, rebound tonometry, and gonioscopy. Eleven dogs were spayed females, and 6 were neutered males. Dogs ranged from 6 months to 8 years of age (mean ± SD, 3.7 ± 2.4 years). Dogs comprised 2 Beagles, 2 German Shepherd Dogs, 2 Great Pyrenees, 2 Terrier-crossbred dogs, and 1 each of American Staffordshire Terrier, Border Collie, Catahoula Leopard Dog, Chow-crossbred dog, English Pointer, Lhasa Apso, Miniature Pinscher, Rottweiler, and Whippet. Consent was obtained from each owner for use of their dog in the study. The study was conducted in accordance with guidelines set forth by the Association for Research in Vision and Ophthalmology.

Procedures—Dogs were assigned by use of blocked randomization to a saline (0.9% NaCl) solution group (n = 8 dogs) or a treatment group (9). Dogs remained in their respective group throughout the study. The study consisted of 3 treatment periods. Each treatment period lasted 5 days, and there was a 14-day washout period between successive treatments. On day 1 of each treatment period, dogs were admitted at 7 AM (ie, 1 hour prior to the start of the treatment) to provide time for them to acclimate to the surroundings.

Treatments—On days 2 through 5 of each treatment period, drugs (latanoprost [0.005%]-timolol [0.5%] combination, latanoprost [0.005%], or timolol maleate [0.3%]) were administered topically twice daily (8 AM and 8 PM) to 1 randomly selected (repeated fair coin-tossing) eye of each dog in the treatment group. The contralateral eye served as a control eye and received 1 drop of saline solution at the same time points. Dogs in the saline group received 1 drop of saline solution in each eye twice daily (8 AM and 8 PM). After the drug administration at 8 PM on days 2 and 5, eyes were reexamined with a slit lamp to evaluate drug-induced toxicosis (blepharospasm, conjunctival hyperemia, or aqueous flare).

Measurement of variables—Measurements were obtained on day 1 (baseline), 2 (the first day of treatment), and 5 (the last day of treatment) of each treatment period. Baseline IOP, PS, and HR were obtained for each dog on day 1, 2, and 5 of each treatment period at 8 AM (time 0) and at 2, 4, 6, 8, and 9 hours. All measurements were acquired by the same observer (LNS), who was unaware of the treatment administered to each dog.

Dogs were gently manually restrained. Topical anesthesia was not used. The IOP measurements were acquired by use of a rebound tonometer that was used with the dog calibration table in accordance with the manufacturer’s recommendations. The instrument was programmed to perform 6 sequential rebounds and dis-
play the mean value. A single mean value was recorded for each eye at each time point during this study. The IOP measurement was repeated when an error message was displayed as a result of an excessive deviation between the 6 measurements, a problem with probe motion, or misalignment with the central portion of the cornea. Horizontal PS measurements were performed by placing a Jameson caliper adjacent to the cornea. These measurements were performed in the same room for all 3 treatment periods, and lighting was maintained at 2.6-foot candles. The HR was acquired by auscultation for 60 seconds by use of a stethoscope placed over the point of maximal intensity.

Statistical analysis—A multivariate repeated-measures ANOVA was used for the analysis of the IOP and PS data. Data collected from both eyes of a dog and over time on the same dog are likely to be correlated; thus, 2 within-subject covariance structures are required for proper inference. To accommodate this, the direct (Kronecker) product structure designed for multivariate repeated measures was used. These structures are constructed by use of the direct product resulting from an unstructured matrix (modeling covariance across the multivariate observations) and an additional covariance matrix (modeling covariance across time). The autoregressive option was used for time dependency within days. Residuals from the model were examined to evaluate the assumption of normality of the error terms. The model used included 3 factors (drug, day [1, 2, or 5], and time [0, 2, 4, 6, 8, or 9 hours]). When there was evidence of a significant interaction involving all 3 factors, pairwise comparisons were performed between pairs of drugs, with day and time held constant. These comparisons were calculated by use of least squares means. To adjust P values for multiple tests, the false discovery rate adjustment was used. Differences were considered significant for adjusted values of \( P < 0.05 \). Values for measured variables of the treated eyes were compared with those of the saline solution group and those of the contralateral eyes treated with saline solution. Measured variables for treated eyes were also compared among drugs. Repeated-measures ANOVA methods were also used to compare HR between the saline solution and treatment groups and among drugs. Analyses were implemented in statistical software by use of the mixed model procedure.

Results

HR—No significant differences in HR were detected on day 1 of any treatment period of the study (Figure 1). The latanoprost-timolol combination and timolol alone both caused significant decreases in HR, compared with results for latanoprost alone and the saline solution group on days 2 and 5. The decrease in HR induced by the latanoprost-timolol combination was not significantly different from the decrease in HR induced by timolol alone. The HR of dogs treated solely with latanoprost did not differ significantly from the HR of the saline solution group.

PS—No significant differences in PS were detected between eyes (left vs right) within the dogs of the saline solution group or among contralateral eyes receiving saline solution among dogs in the treatment group at any time during the study (Figure 2). On days 2 and 5, timolol administration resulted in a PS that was smaller (but not significantly different) in the treated eye than in the contralateral saline solution eye or in the eyes of the saline solution group. Compared with PS for the saline solution group and the contralateral eyes treated with saline solution, significant differences were detected for the PS of dogs treated with latanoprost or the latanoprost-timolol combination at 2 through 9 hours on days 2 and 5. The PS in dogs treated with latanoprost or the latanoprost-timolol combination was significantly smaller than that for dogs treated with timolol and for the saline solution group. The largest decrease in PS was at 2 hours after administration of latanoprost and the latanoprost-timolol combination. Compared with results for the saline solution group at 2 hours on day 2, PS was 7.6 and 7.9 mm less in eyes treated with the latanoprost-timolol combination and latanoprost, respectively. Compared with results for the saline solution group at 2 hours on day 5, PS was

![Figure 1](image-url)
8.2 and 8.1 mm less in eyes treated with the latanoprost-timolol combination and latanoprost, respectively. No significant difference was detected for the degree and duration of miosis induced by the latanoprost-timolol combination, compared with results for latanoprost alone. The PS had returned to baseline values in all treated eyes (the latanoprost-timolol combination and latanoprost alone) by 12 hours (as determined by PS measured at 8 AM on day 5 after drug administration at 8 PM on day 4).

IOP—No significant differences in IOP were detected within the eyes of the saline solution group during any treatment period of the study (Figure 3). Significant differences also were not detected between eyes treated with timolol and the saline solution group. No significant difference was found in IOP of the contralateral eyes treated with saline solution during any treatment period of the study. At 2 through 9 hours on days 2 and 5, eyes treated with latanoprost and the latanoprost-timolol combination had a significant decrease in IOP, compared with results for the saline solution group and eyes treated with timolol alone. The latanoprost-timolol combination typically caused the IOP to decrease by 1 to 2 mm Hg more than the decrease for latanoprost; however, the values did not differ significantly. Of interest, although PS returned to baseline values 12 hours after administration of latanoprost or the latanoprost-timolol combination, IOP did not. Mean ± SD IOPs measured at time 0 on day 2 for the latanoprost-timolol combination and for latanoprost alone were 17.1 ± 4.3 mm Hg and 16.2 ± 3.7 mm Hg, respectively. Mean IOPs measured at time 0 on day 5 for the latanoprost-timolol combination and for latanoprost alone were 12.7 ± 3.9 mm Hg and 12.7 ± 3.1 mm Hg, respectively. The maximum ocular hypotensive effect during the treatment period was detected on day 5 for both the latanoprost-timolol combination and latanoprost. The greatest mean ± SD reduction in IOP was detected at 2 hours for the latanoprost-timolol combination (5.6 ± 0.3 mm Hg) and at 6 hours for latanoprost (5.5 ± 1.2 mm Hg). Dogs were not monitored after day 5; thus, the specific time needed for IOP to return to pretreatment values was unknown. However, IOP had returned to pretreatment values in all dogs by the end of each washout period (14 days).

Adverse effects—Mild conjunctival hyperemia was detected in 6 of 9 eyes treated with the latanoprost-timolol combination and 5 of 9 eyes treated with latanoprost-timolol combination and treated in the contralateral eye with saline solution (saline solution group). The PS had returned to baseline values in all eyes treated with latanoprost or the latanoprost-timolol combination by 12 hours after administration. §Within a time point, values for latanoprost and the latanoprost-timolol combination were significantly (P < 0.05) different from the values for timolol or the saline solution group. See Figure 1 for remainder of key.
prost alone. Hyperemia typically developed on day 2 and remained through the end of day 5. One dog had an increase in lacrimation when receiving the latanoprost-timolol combination or latanoprost alone. Aqueous flare and blepharospasm were not observed in any eyes of dogs in the treatment or saline solution groups.

Discussion

Timolol and latanoprost are used routinely on glaucomatous patients in veterinary medicine. Timolol maleate is a nonselective β-adrenergic receptor antagonist that decreases production of aqueous humor by binding to β-adrenergic receptors in the ciliary body and inhibiting cAMP synthesis. Timolol has no effect on outflow of the aqueous humor. In dogs, β-adrenergic fibers of the iris are inhibitory to the sphincter muscle and stimulation leads to relaxation and mydriasis. In 1 study, all eyes treated with 1 drop of timolol (0.5%) had a reduction in pupillary diameter (mean, 31.4%) at 30 minutes after administration, with the effect lasting for 12 hours. In the present study, we detected that there was a nonsignificant pattern toward miosis in the treated eyes. In the aforementioned study, miosis was also detected in the contralateral eye, with a duration between 4 and 12 hours after treatment, but we were unable to identify a comparable change in the study reported here. Topical treatment with timolol alone in normal eyes of dogs reduced IOP by a mean of 2.5 mm Hg (16.1%), with a maximum of 3.7 mm Hg (23.8%) in the treated eye and 1.4 mm Hg in the contralateral untreated eye. In the present study, we were unable to detect a significant decrease in IOP and PS by use of the same concentration (0.5%) of timolol. Timolol also did not cause a measurable effect on the contralateral eye. In other studies, topical application of 0.5% timolol in 1 eye of clinically normal dogs and cats also did not cause a significant decrease in IOP in the contralateral untreated eye. Our results are consistent with those in 2 studies conducted to evaluate timolol use in clinically normal dogs; neither of those studies revealed a reduction in IOP in dogs treated with timolol concentrations between 0.25% and 6%. The explanation for these differences includes a large reduction in β-adrenergic tone in the socialized pets used in the present study (compared with results of another study in which investigators used laboratory Greyhounds), heterogeneity in the responsiveness of individual humans and dogs to antiglaucoma medications, different formulations of 0.5% timolol, and differences in tonometers. Because β-adrenergic fibers are also found in the canine cardiovascular system, systemic uptake of timolol can cause bradycardia. Consistent with results of other studies, we were able to detect timolol-induced bradycardia with topical administration of timolol alone or with the latanoprost-timolol combination. More recently, veterinarians have turned to prostaglandin analogues for treatment of glaucoma in select patients because they have been found to induce an even greater reduction in IOP than did previously available medications. Latanoprost is a prostanoid-selective prostaglandin F-receptor agonist, and it reduces IOP in humans by increasing outflow of aqueous humor through the uveoscleral pathway. The main resistance in the uveoscleral pathway is the ciliary body musculature. In primates, latanoprost enhances metalloproteinase activity in the ciliary body, reduces the extracellular matrix material between muscle bundles, and enlarges the intermuscular spaces, which thus increases uveoscleral outflow. There is also remodeling of the trabecular meshwork, which suggests that latanoprost increases trabecular outflow. Preliminary research in dogs suggests that latanoprost may reduce production of aqueous humor and may result in a small increase in the outflow pathway by mildly increasing the surface area of the ciliary cleft. Latanoprost is absorbed through the cornea, where the isopropyl ester prodrug is hydrolyzed to the acid form and becomes biologically active.

Latanoprost has been evaluated in normotensive eyes of dogs. In that study, a mean decrease in IOP of 25% (3 mm Hg) was detected, with the maximum decrease at 6 hours after topical administration. In another study, investigators reported a decrease in IOP of 50% to 60% after latanoprost administration for 4 days in Beagles with primary open-angle glaucoma. Twice-daily dosing induced a significantly lower IOP and the least IOP fluctuations. The HR was not reported for either of those studies. We detected a decrease in IOP of 5.3 mm Hg with latanoprost administered alone or in combination with timolol. There was no significant difference between the 2 treatments. Administration of latanoprost alone did not significantly reduce HR in the study reported here. We did not compare the efficacy and equivalence of treatment with the latanoprost-timolol combination with those for concomitant use of latanoprost and timolol. A study in humans revealed that the use of the combination product is at least as effective as both of its components administered separately. In the present study, 2 treatment periods involved the administration of timolol (0.5%). Administration of timolol alone did not cause a reduction in IOP in clinically normal dogs, and it appears from our data that timolol does not act synergistically with latanoprost to further decrease IOP in clinically normal dogs, compared with results for latanoprost alone. Results would likely differ in glaucomatous dogs. Timolol (0.5% to 8%) significantly decreases IOP in Beagles with primary open-angle glaucoma, whereas similar concentrations of timolol have no effect on the IOP in clinically normal Beagles.

Investigators in 1 study detected extreme miosis, with the pupil diameter decreasing from 8.6 mm at baseline to 1.1 mm at 1 hour after treatment; however, this was not accompanied by characteristics of ocular pain. We detected a similar decrease in PS with latanoprost and the latanoprost-timolol combination at 2 hours after treatment. The PS was not measured at 1 hour in the present study. No signs of substantial ocular pain were observed in the dogs of our treatment group. The most frequently detected adverse effects reported in humans prescribed prostaglandin analogues include conjunctival hyperemia, iris pigmentation, hypertrichosis, increased eyelash pigmentation, cystoid macular edema, and superficial punctate epithelial erosions. Except for conjunctival hyperemia, simi-
lar adverse effects have not been reported in domestic animals.\textsuperscript{28} The study reported here confirmed that mild conjunctival hyperemia is an adverse effect after latanoprost is administered alone or in combination. The conjunctival hyperemia may be attributable to endothelial-derived nitric oxide–mediated vasodilatation and not associated with inflammation. This would explain the reason that aqueous flare and blepharospasm were not observed in any eyes of the treatment or saline groups.\textsuperscript{30} On the other hand, an adverse effect detected in this study was increased lacrimation in 1 dog, which most likely represented an extension of mild conjunctival irritation. Because latanoprost is a prostaglandin analogue, it can cause ocular irritation, breakdown in the blood-aqueous barrier, aqueous flare, and miosis.\textsuperscript{19,36,40–42} Recently, investigators detected a breakdown of the blood-aqueous barrier by use of anterior chamber fluorophotometry after administration of 1 drop of latanoprost in clinically normal dogs.\textsuperscript{43} The percentage increase in fluorescein concentration was modest (50%), compared with the increase of 200% resulting from paracentesis or the increase of 360% following phacoemulsification surgery.\textsuperscript{44} Although prostaglandins can mediate inflammation in the anterior segment, the exact mechanism for latanoprost-induced disruption of the blood-aqueous barrier is unclear because the prostaglandin F receptor has a limited role in intraocular inflammation.\textsuperscript{45–46} Signs of aqueous flare were not observed by use of slit-lamp biomicroscopy for any drug combination in the present study; however, fluorophotometry would be a more sensitive method to use to monitor for breakdown of the blood-aqueous barrier.

Additional studies are needed to determine the effects of administration of the latanoprost-timolol combination in glaucomatous dogs. On the basis of other studies that involved use of timolol, we hypothesize that administration of the combination product would likely lower IOP to a greater degree than would administration of latanoprost alone. However, in clinically normal dogs, the latanoprost-timolol combination and latanoprost alone had similar efficacy in reducing IOP. For patients currently prescribed both timolol and latanoprost, the combination product may be considered for use because it will likely increase client compliance and appears to be tolerated well.

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