The effect of a single dose of topical 0.005% latanoprost and 2% dorzolamide/0.5% timolol combination on the blood-aqueous barrier in dogs: a pilot study

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Abstract
Objective To determine the effects of 0.005% latanoprost and 2% dorzolamide/0.5% timolol on the blood-aqueous barrier (BAB) in normal dogs.

Animals studied Eight mixed-breed and pure-breed dogs.

Procedures Baseline anterior chamber fluorophotometry was performed on eight normal dogs. Sodium fluorescein was injected and the dogs were scanned 60–90 min post-injection. Seventy-two hours following the baseline scan, one eye received one drop of latanoprost. Fluorophotometry was repeated 4 h after drug administration. Following a washout period, the identical procedure was performed 4 h after the administration of dorzolamide/timolol. The degree of BAB breakdown was determined by comparing the concentrations of fluorescein within the anterior chamber before and after drug administration. BAB breakdown was expressed as a percentage increase in the post-treatment fluorescein concentration over the baseline concentration: %INC = [(Fl)post − (Fl)baseline]/(Fl)baseline) × 100. The percentage increase in fluorescein concentration in the treated eye was compared to that in the nontreated eye using a paired t-test with significance set at P ≤ 0.05.

Results Following administration of latanoprost, the fluorescein in the treated eyes increased 49% (± 58%) from baseline compared to 10% (± 31%) in the untreated eyes (P = 0.016). Following administration of dorzolamide/timolol, the fluorescein concentration increased 38% (± 54%) compared to baseline vs. 24% (± 38%) in the untreated eyes (P = 0.22).

Conclusions The results of this study show that topical latanoprost may cause BAB disruption in normal dogs while topical dorzolamide/timolol may have no effect on the BAB in normal dogs.

Key Words: anterior chamber fluorophotometry, blood-aqueous barrier, dog, dorzolamide/timolol, glaucoma, latanoprost

INTRODUCTION

Topical latanoprost and dorzolamide/timolol both lower the IOP in normal and glaucomatous dogs and these two compounds are commonly used in clinical veterinary medicine.1–5 While the side effects of these drugs have been rigorously evaluated in humans, the same is not true for dogs and the effect of these drugs on the blood-aqueous humor barrier (BAB) in dogs has yet to be determined. The effect of these drugs on the BAB is especially important to understand since they are often considered for use in dogs with concomitant IOP elevation and intraocular inflammation.

The BAB is formed by tight junctions in both the non-pigmented ciliary body epithelium and the endothelial cells in the iris vasculature.6 Aqueous humor flare, indicating increased permeability of the BAB, is a universal sign of anterior uveitis and results from structural changes in the tight junctions in both the iris and ciliary body.7–9 Because the amount of fluorescein that enters the anterior chamber following intraocular administration is proportional to the degree of BAB disruption, anterior chamber fluorophotometry provides a reliable and noninvasive method of evaluating the integrity of the BAB.10 The amount of fluorescein within the anterior chamber is measured by exciting the fluorescein molecules
with the appropriate wavelength of light and quantifying the subsequent emission.\textsuperscript{10,11} Other methods of assessing the BAB include aqueous humor protein measurements (following anterior chamber paracentesis) and subjective evaluation of aqueous flare. Anterior chamber fluorophotometry eliminates reliance on insensitive and unstandardized subjective evaluation of clinical signs and the need for anterior chamber paracentesis which can promote BAB breakdown.\textsuperscript{10,12} Laser flaremetry, which measures aqueous flare and cells by analysis of laser beam light-scattering within the anterior chamber, is another reliable method of assessing BAB breakdown.\textsuperscript{13} The purpose of the current study was to determine if a single drop of topical latanoprost or dorzolamide/timolol affects the stability of the BAB in dogs as measured by anterior chamber fluorophotometry.

MATERIALS AND METHODS

Eight client-owned dogs (seven spayed females, one neutered male, ranging from 4 to 9 years of age) were used in the study. Five of the dogs belonged to a recognized breed (Dachshund (3), Boston terrier (1), American Staffordshire terrier (1)) and the remaining three were mixed-breed dogs. Informed written consent was obtained from the owner of each dog prior to the study.

Results of pre-study ophthalmologic exams including Schimer I tear test, applanation tonometry, slit-lamp biomicroscopy, and indirect ophthalmoscopy were normal with the exception of mild corneal lipid dystrophy in one dog. Anterior chamber fluorophotometry was performed prior to the application of eye drops to establish a baseline. For each dog, 20 mg/kg of 10% sodium fluorescein was injected intravenously.\textsuperscript{10} Butorphanol 0.4 mg/kg was administered subcutaneously 30 min prior to fluorescein injection to limit nausea occasionally noted with intravenous fluorescein administration and to provide mild sedation for the fluorophotometry reading. Sixty to 90 min following injection of fluorescein, the fluorescein concentration in the mid-axial anterior chamber was measured using a computerized scanning ocular fluorophotometer with an anterior chamber adapter (Fluorotron Master, Coherent Radiation Inc, Palo Alto, CA). Obtaining a fluorophotometry reading 60–90 min postinjection ensures a stable concentration of fluorescein within the anterior chamber as determined by previous studies.\textsuperscript{10,11}

A 72-h period was then allotted to allow for washout of fluorescein. Fluorescein is metabolized very rapidly in dogs, and the anterior chamber concentration is typically barely detectable by 5–6 h after intravenous injection.\textsuperscript{10} To confirm the absence of fluorescein within the anterior chamber, fluorophotometry was performed on both eyes of two dogs 72 h after the baseline scan. Fluorescein was not detectable in either of the dogs’ eyes at that time, confirming that 72 h was an adequate washout period.

One eye was then randomly chosen as the study eye and one drop of 0.005% latanoprost (Xalatan\textsuperscript{®}) was administered in an open label manner. Intravenous 10% sodium fluorescein (20 mg/kg), preceded by subcutaneous butorphanol (0.4 mg/kg), was administered 3 h after the administration of latanoprost. Fluorophotometry readings were obtained 60 min following fluorescein injection (4 h after latanoprost administration). Two of the dogs did not require premedication with butorphanol. Sedation has been shown to have no effect on anterior chamber fluorescein concentrations.\textsuperscript{10}

Following a 72 h washout period, fluorophotometry readings were again obtained in two eyes of two dogs to confirm the absence of fluorescein. The eye that had not received latanoprost was given one drop of 2.0% dorzolamide/0.5% timolol (CoSopt\textsuperscript{®}) in an open label manner. Intravenous 10% sodium fluorescein (20 mg/kg), preceded by subcutaneous butorphanol (0.4 mg/kg), was administered 3 h after the administration of dorzolamide/timolol. Fluorophotometry readings were obtained 60 min following intravenous fluorescein injection (4 h after dorzolamide/timolol administration). Again, two of the dogs were not premedicated with butorphanol.

All fluorophotometry readings were obtained between 1:00 and 3:00 p.m. to limit the effect of diurnal fluctuations in aqueous humor production. Adverse reactions to the injection of intravenous fluorescein were recorded.

For each fluorophotometry reading, the degree of BAB disruption was determined by comparing the concentration of fluorescein within the anterior chamber of the eye in which the drug was administered to that eye’s baseline fluorescein concentration. Blood-aqueous barrier breakdown was expressed as a percentage increase in the post-treatment fluorescein concentration over the baseline concentration:

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%\text{INC}[F1] = \frac{([F1]_{\text{post}} - [F1]_{\text{baseline}})}{[F1]_{\text{baseline}}} \times 100.
\]

The percentage increase in fluorescein concentration (%INC [F1]) in the treated eye was compared to that in the nontreated eye using a paired \textit{t}-test. A \textit{P}-value of 0.05 was considered significant.

RESULTS

After a single dose of latanoprost, the fluorescein in the treated eyes increased by a mean (\(\pm\) SD) of 49\% (\(\pm\) 58\%) from baseline compared to 10\% (\(\pm\) 31\%) in the eyes that did not receive the drug (\(P = 0.016\)). Following a single dose of dorzolamide/timolol, the fluorescein concentration increased 38\% (\(\pm\) 54\%) compared to baseline vs. 24\% (\(\pm\) 38\%) in the untreated eyes (\(P = 0.22; \text{power} = 0.77\)). Adverse effects of fluorescein administration were limited to vomiting and ptalism immediately following injection in one dog and ptalism in another dog.

DISCUSSION

The results of this study show that topical 0.005\% latanoprost may cause BAB disruption while topical 2\% dorzolamide/0.5\% timolol does not result in BAB disruption as measured by anterior chamber fluorophotometry following a single application of the test drug in the eyes of normal dogs.
Latanoprost, an FP receptor agonist prodrug, lowers IOP in dogs through incompletely defined mechanisms. In primates, latanoprost enhances metalloproteinase activity in the ciliary body, reducing extracellular matrix material between ciliary muscle bundles and enlarging intermuscular spaces, thus increasing uveoscleral outflow.\textsuperscript{14–17} Some remodeling of the trabecular meshwork also occurs, suggesting a modest increase in trabecular outflow as well.\textsuperscript{15,17} In dogs, preliminary research suggests that latanoprost may reduce aqueous humor production and, in addition, the latanoprost-induced miosis may correct a ‘reverse pupillary block.’\textsuperscript{18,19}

Because prostaglandins are mediators of anterior segment inflammation, the finding that latanoprost may be capable of engendering BAB disruption is perhaps not surprising.\textsuperscript{20–22} However, the exact mechanism for how this occurs has not been determined and the FP receptor appears to have a limited role in intraocular inflammation.\textsuperscript{23} Latanoprost has been associated with a disruption in the BAB in humans in some reports, but the majority of studies have shown no measurable disruption of the BAB.\textsuperscript{17,24–29} Dogs may be more susceptible to BAB disruption following treatment with latanoprost because of their more labile BAB.\textsuperscript{30} There are also variations among species in the role that specific prostaglandins play in anterior segment inflammation.

The combination of dorzolamide, a carbonic anhydrase inhibitor, and timolol, a nonselective β-adrenergic antagonist, lowers IOP through a decrease in aqueous humor production. Timolol reduces aqueous humor production via nonselective binding of β-adrenergic receptors in the nonpigmented epithelium of the ciliary body and potentially through interactions with 5-HT\textsubscript{1A} receptors, while dorzolamide exerts its effect by inhibiting carbonic anhydrase in the nonpigmented ciliary body epithelium.\textsuperscript{31–34} The increased effect of combining the two drugs indicates that they are synergistic.\textsuperscript{35} We did not detect a statistically significant difference in anterior chamber fluorescein concentration relative to the baseline following a single dose of dorzolamide/timolol, indicating that this combination of drugs may have no effect of the BAB. This finding was not unexpected based on our clinical experience with this combination of drugs. However, it is interesting to note that both 0.5% timolol and benzalkonium chloride, the preservative used in both dorzolamide/timolol and latanoprost, resulted in breakdown of the BAB in humans.\textsuperscript{36,37}

Both latanoprost and dorzolamide/timolol are commonly used in veterinary medicine to treat dogs with elevated IOP. Because the results of this study indicate that latanoprost may result in BAB disruption, caution should be exercised when using latanoprost in dogs with glaucoma secondary to anterior uveitis or in dogs with ocular hypertension following intraocular surgery. However, two factors should be considered when weighing the clinical significance of our findings. First, the percentage increase in fluorescein concentration measured following latanoprost administration was relatively modest, with a mean of approximately 50%. By comparison, experimental disruption of the BAB using a controlled paracentesis model in dogs increased mean fluorescein concentrations by nearly 200%.\textsuperscript{10} In addition, we have measured an average %INC[Fl] of 360% following phacoemulsification in cataractous dogs (unpublished data). Given the smaller increase in fluorescein concentration caused by topical latanoprost administration, the degree of BAB breakdown may not be of great clinical significance. The second consideration is the possibility that the increase in anterior chamber fluorescein concentration we measured following latanoprost application may not be due to BAB disruption, as recent studies convincingly demonstrate that miotics and aqueous humor suppressants increase anterior chamber protein concentrations in the absence of BAB disruption. Freddo et al. have shown that in normal eyes protein exits the fenestrated capillaries in the ciliary body stroma and diffuses through the stroma of the iris root to enter the anterior chamber, albeit at a modest rate.\textsuperscript{38,39} Miosis following topical application of pilocarpine causes thinning of the iris stromal root, forcing this protein into the peripheral anterior chamber. Simultaneous contraction of the ciliary musculature results in reduced uveoscleral outflow and redirection of protein anteriorly into the peripheral anterior chamber.\textsuperscript{40} Lastly, compounds that reduce aqueous humor production concentrate all aqueous humor solutes, including protein, explaining the occasional flare seen clinically in humans following the application of timolol.\textsuperscript{38,40} These concepts raise the possibility that some or all of the increase in fluorescein concentration found after the administration of latanoprost could be due to latanoprost-induced miosis and decreased aqueous humor formation rather than true breakdown of the BAB.

This study was carried out in an open label manner because the profound miosis associated with latanoprost administration in dogs made it impossible to mask the observer. While open label studies can introduce bias, the data in this study were quantitative and it is unlikely that the open label design meaningfully impacted the findings.

In conclusion, latanoprost administration causes increased anterior chamber fluorescein concentrations, which may reflect a breakdown in the BAB. The clinical significance of this finding is unclear. In contrast, dorzolamide/timolol produced no change in anterior chamber fluorescein concentrations and thus may be a superior initial choice in dogs with an elevated IOP in association with anterior uveitis. However, because latanoprost caused a relatively minor increase in the anterior chamber fluorescein concentration, its use may be justified in dogs with an elevated IOP with anterior uveitis if dorzolamide/timolol alone does not sufficiently lower IOP.

REFERENCES


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