Effects of topical application of a 2% solution of dorzolamide on intraocular pressure and aqueous humor flow rate in clinically normal dogs

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Objective—To evaluate effects of topical application of a 2% solution of dorzolamide on intraocular pressure (IOP) and aqueous humor flow rate in clinically normal dogs.

Animals—15 Beagles.

Procedure—The IOP was measured in both eyes of all dogs for 3 days to determine baseline values. In a single-dose study, 50 µl of dorzolamide or control solution was applied in both eyes at 7:00 AM, and IOP was measured 7 times/d. In a multiple-dose study, dorzolamide or control solution was applied to both eyes 3 times/d for 6 days, and IOP was measured 4 times/d during treatment and for 5 days after cessation of treatment. Aqueous humor flow rate was measured for all dogs fluorophotometrically prior to treatment and during the multiple-dose study.

Results—In the single-dose study, dorzolamide significantly decreased IOP from 30 minutes to 6 hours after treatment. Mean decrease in IOP during this time span was 3.1 mm Hg (18.2%). Maximal decrease was detected 6 hours after treatment (3.8 mm Hg, 22.5%). In the multiple-dose study, dorzolamide decreased IOP at all time points, and maximal decrease was detected 3 hours after treatment (4.1 mm Hg, 24.3%). Mean aqueous humor flow rate decreased from 5.9 to 3.4 µl/min (43%) after treatment in the dorzolamide group.

Conclusions and Clinical Relevance—Topical application of a 2% solution of dorzolamide significantly decreases IOP and aqueous humor flow rate in clinically normal dogs. Therefore, topical administration of dorzolamide should be considered for the medical management of dogs with glaucoma. (*Am J Vet Res* 2001;62:859–863)

Systemically administered **carbonic anhydrase inhibitors (CAI)** have been used for > 40 years in the treatment of humans with glaucoma.[1-3] It was first documented in 1954 that systemic administration of CAI decreases **intraocular pressure (IOP)** in humans.[1] The need arose for a topical formulation of CAI because of the adverse effects associated with systemic use, including anorexia, lethargy, diarrhea, decreased libido, blood dyscrasias, and teratogenesis.[4-8] Systemically administered CAI have been used for dogs with all types of glaucoma and include such drugs as acetazolamide, methazolamide, dichlorphenamide, and ethoxzolamide.[9] Adverse effects have been reported in dogs and include vomiting, diarrhea, lethargy, and an increased respiratory rate.[10]

Dorzolamide hydrochloride[a] is a recently introduced CAI for topical administration. The most common adverse effect in humans is mild transient ocular irritation with burning or stinging.[11-13] Other reported effects include transient blurred vision, photophobia, and a bitter taste after instillation.[12,13] The most common adverse clinical sign reported is punctate epithelial erosions.[12] A 2% solution of dorzolamide, when used as a single therapeutic agent, maximally decreases IOP in humans by 17 to 28%[11,12,14-16] and in monkeys by approximately 19%.[17] An investigational topically administered CAI (MK-927) decreases IOP in dogs by approximately 23%[18]; however, to our knowledge dorzolamide, which is the only commercially available topical formulation of CAI, has not been evaluated in dogs.

Mechanism of action of a 2% solution of dorzolamide is to decrease aqueous humor production in monkeys and humans, but this has not been documented in dogs.[15,17] We hypothesized that topical administration of 2% dorzolamide 3 times/d to clinically normal dogs would significantly decrease IOP and aqueous humor flow rate. The study reported here was designed to evaluate the effects of topical administration of 2% dorzolamide on IOP in clinically normal dogs as well as to determine the mechanism of action of dorzolamide, using anterior segment fluorophotometry. Anterior segment fluorophotometry has been used extensively in humans to measure aqueous humor flow rate.[19-23] Fluorophotometry directly measures aqueous humor production and provides an estimate of facility of aqueous humor outflow; it is the method of choice for delineating mechanisms of action of IOP-altering drugs.[19-23] This technique recently has been adapted for use in dogs.[24]

Materials and Methods

Dogs—Fifteen adult Beagles (9 males and 6 females) weighing between 10 and 16 kg were used in the study. Dogs were purchased from a commercial supplier.[b] Physical examinations were performed at the beginning of the study. All dogs had normal results for ophthalmic examinations includ-

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ing slit lamp biomicroscopy, applanation tonometry,[c] indirect ophthalmoscopy, and gonioscopy. The study was conducted in accordance with guidelines established by a university animal care and use committee.

Experimental design—This experiment was conducted as a randomized study. Ten dogs were assigned to receive a topical 2% solution of dorzolamide, and 5 received an artificial tear preparation and served as control dogs. The study was conducted in 3 phases, including pretreatment, single-dose, and multiple-dose phases. Also, anterior segment fluorophotometry was performed, using a computerized scanning ocular fluorophotometer,[d] during the pretreatment and multiple-dose phases.

During a 1-week acclimation period, IOP in all dogs was measured, using applanation tonometry without the aid of topical anesthetics. The same investigator (MAC) performed all tonometry readings. The first day of measurement was day 1. In the pretreatment phase, IOP was measured at 7:15, 7:30, 8:00, and 10:00 AM and 1:00, 6:00, and 9:00 PM for 3 consecutive days. These values were used to allow for comparisons of values before treatment with values after treatment while accounting for the typical diurnal pattern of IOP in dogs.[25] On day 4, the single-dose study was conducted, and 50 µl of dorzolamide or the control preparation was applied to both eyes of assigned dogs at 7:00 AM, and IOP was measured 7 times/d at the previously described time points.

A multiple-dose study then was conducted. On days 5 to 10, 50 µl of dorzolamide or the control preparation was applied to both eyes of assigned dogs at 7:00 AM and 3:00 and 11:00 PM, and a final treatment was applied at 7:00 AM on day 11. This dosing regimen was chosen on the basis of other studies[11-14] in humans that documented efficacy of 3-times-daily usage. During the multiple-dose study and continuing on days 11 to 15, IOP was measured at 10:00 AM and 1:00, 6:00, and 9:00 PM. Those time points were selected on the basis that they enabled relatively consistent monitoring of the IOP before and after treatments.

Aqueous humor flow rate was measured, using anterior segment fluorophotometry, to obtain baseline data prior to drug administration and data on day 6 of the multiple-dose study. The fluorophotometric protocol was similar to standard protocols used in humans,[20] but it was optimized for application to dogs.[24] One drop (50 µl) of 10% fluorescein was applied to both corneas of all dogs every 5 minutes for 10 minutes (a total of 3 applications). Fluorescein was applied between 8:00 and 8:10 AM. Five minutes after the last fluorescein application, the eyes of each dog were rinsed thoroughly. Then, using a computerized scanning ocular fluorophotometer with an anterior chamber adapter, emitted fluorescence of the cornea and aqueous humor were measured approximately 5, 6.5, and 8 hours after the last administration of fluorescein. Dogs were sedated for each fluorophotometric scan, using a combination of tiletamine hydrochloride-zolazepam (6.5 to 11 mg/kg of body weight) and butorphanol tartrate (0.4 mg/kg) administered IM. The times for fluorophotometric scans were chosen to correlate with the time when corneal fluorescein staining was homogenous, and fluorescein concentrations in cornea and aqueous humor were decreasing and parallel.[24] With each subsequent scan, diminishing fluorescence was recorded. For each dog, results for the 3 scans were plotted, and the slopes were calculated (**Fig 1**). Analysis of these graphs also was used to derive the mean ratio for fluorescein concentrations in cornea and aqueous humor. These slopes and ratios were used in calculating the aqueous humor flow rates, using the following equation[20]:

$$\text{Flow} = K_o V_a$$

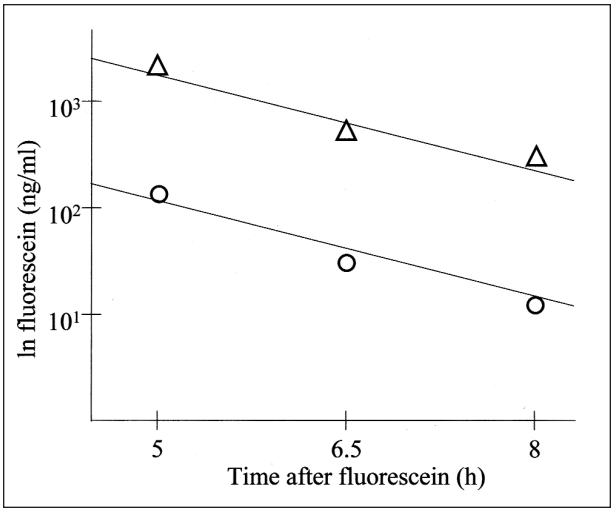

Figure 1—Graph of the logarithm of the emitted fluorescence in the cornea (triangle) and aqueous humor (circle) measured 5, 6.5, and 8 hours after fluorescein application in 15 clinically normal Beagles. Values were used to calculate the slopes of the diminishing fluorescence and ratios of concentrations

where $K_o = -A \times (1 + [1.53\ V_c C_c / 1.2 V_a C_a])$, A is the slope of lines of diminishing corneal and aqueous humor fluorescence, V_c = corneal volume, V_a = anterior chamber volume, C_c = corneal fluorescein concentration, and C_a = anterior chamber fluorescein concentration. A value of 100 µl was used as the mean corneal volume for all calculations. This value was determined by excising the cornea at the limbus from 3 weight-matched dogs, submerging them in a flask filled with saline (0.9% NaCl) solution, and measuring the volume that was displaced. A value of 400 µl was used as an approximation of the mean anterior chamber volume in all calculations.[10] Fluorescein concentrations for the cornea and aqueous humor were obtained from results of anterior segment fluorophotometry.

Statistical analysis—A *t*-test was used to compare the IOP of the control and dorzolamide groups during the pretreatment phase. During the single-dose study, a paired *t*-test was used to evaluate each IOP before treatment with its time-matched IOP after treatment for the control and dorzolamide groups. A paired *t*-test was used to compare each value before treatment with the mean of its time-matched value after treatment during the multiple-dose study (days 5 to 10). An ANOVA was used to compare values before treatment with time-matched values after cessation of dosing (days 11 to 15). A paired *t*-test was used to compare aqueous humor flow rates before treatment with flow rates during the treatment phase of the multiple-dose study for both groups. Values of $P < 0.05$ were considered significant.

Results

Values for IOP before treatment did not differ significantly between the control and dorzolamide groups (**Fig 2**). For the single-dose study, IOP did not change significantly in the control group. However, the dorzolamide group had a significant decrease in IOP from 30 minutes to 6 hours after treatment. Mean IOP decrease during this time span was 3.1 mm Hg (18.2%). By 11 hours after administration, IOP had returned to baseline values (**Fig 3**). During the multiple-dose study, only the dorzolamide group had a significant decrease in IOP, and this was evident for all of the times points (**Fig 4**). The maximal decrease in

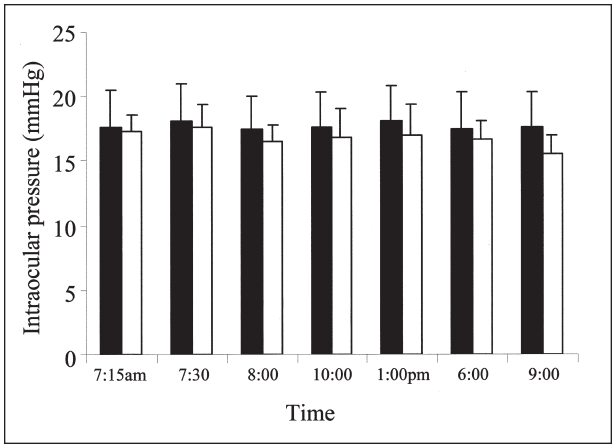

Figure 2—Mean ± SD values of intraocular pressure (IOP) obtained before treatment of clinically normal Beagles. Values did not differ significantly ($P > 0.05$) between the control group (black bar) and dorzolamide group (white bar) at any of the time points, nor was there significant ($P > 0.05$) diurnal variation.

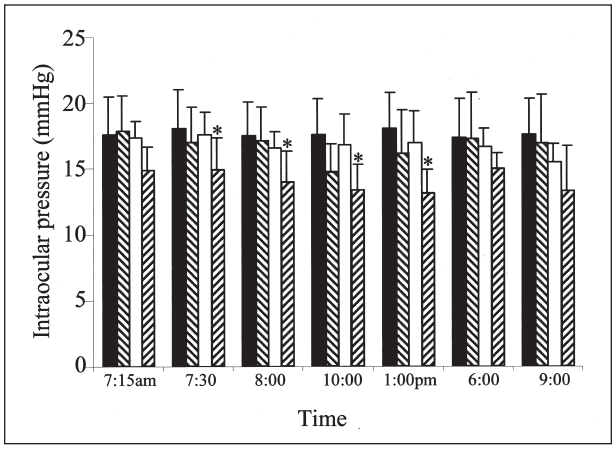

Figure 3—Mean ± SD IOP for groups before (control, black bar; dorzolamide, white bar) and during (control, diagonal bar descending from left to right; dorzolamide, diagonal bar ascending from left to right) the single-dose phase of the study. The IOP before treatment for each group represents baseline values. *Value for the dorzolamide group is significantly ($P < 0.05$) decreased, compared with baseline value for that group.

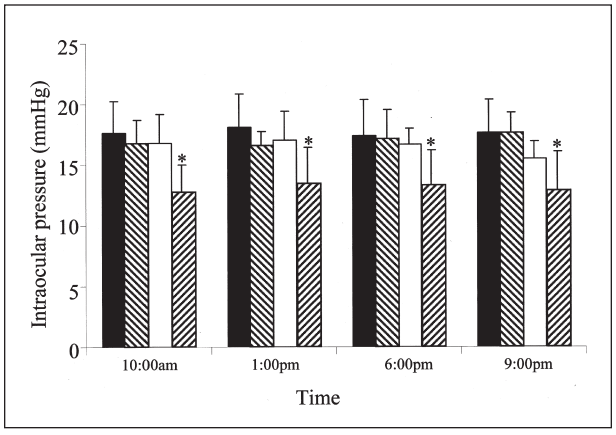

Figure 4—Mean ± SD IOP for the control and dorzolamide groups during the multiple-dose phase of the study. The IOP before treatment for each group represents baseline values. Notice the maximal decrease of 24.3% (4.1 mm Hg) at 10 AM during administration of dorzolamide. *See* Figure 3 for key.

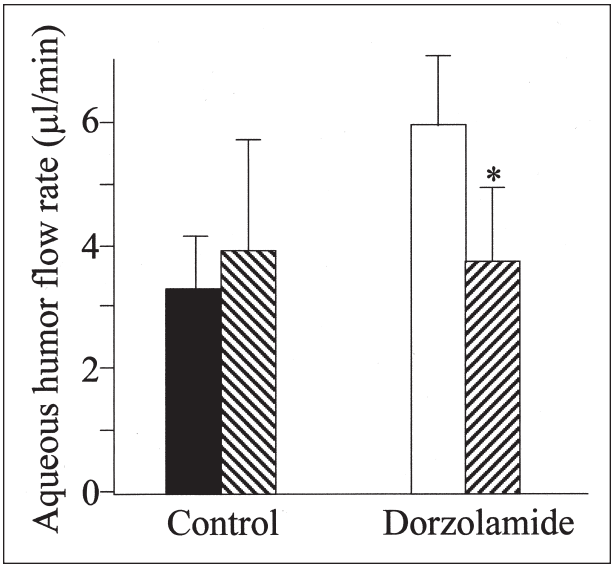

Figure 5—Mean ± SD aqueous humor flow rates for the control and dorzolamide groups. Flow rates before treatment represent baseline values. *See* Figure 3 for key.

IOP of approximately 4.1 mm Hg (24.3%) was detected 3 hours after treatment.

Aqueous humor flow rate in the dorzolamide group had a significant decrease from 5.9 to 3.4 µl/min (43%; **Fig 5**). Changes in aqueous humor flow rate were not detected in the control group.

Discussion

Finding efficacious medications for use in treating dogs with glaucoma is important, because glaucoma is one of the major causes of irreversible blindness in dogs, affecting 1 of every 200 dogs.[e] Therapeutic recommendations for the treatment of dogs with glaucoma often are extrapolated from the human literature. The study reported here allowed us to gain information about the effects of 2% dorzolamide in clinically normal dogs. Dorzolamide hydrochloride is a CAI formulated for topical use that inhibits carbonic anhydrase isoenzymes I, II, and IV.[f] Isoenzymes I and II are soluble cytoplasmic enzymes located in various ocular tissues.[26] Dorzolamide is most selective for isoenzyme II,[f] which is the predominant isoenzyme in the ciliary processes where aqueous humor is produced.[26] Further investigation of isoenzyme IV, the only ocular isoenzyme that is membrane-bound, is warranted, because inhibition of this isoenzyme can cause a decrease in IOP.[g,h]

Analysis of results of the study reported here confirmed that a 3-times-daily dosing regimen is appropriate in dogs. In the single-dose phase, the effects of dorzolamide had diminished by 11 hours after treatment. In the multiple-dosing phase, IOP remained significantly lower than baseline values throughout the study. Therefore, applying dorzolamide 3 times/d will provide more desirable drug concentrations. Dorzolamide maximally decreased IOP by 4.1 mm Hg (24.3%) in clinically normal dogs. To a similar extent, IOP decreases in humans (17 to 28%[11,12,14-16]) and monkeys (19%[17]). The magnitude of effects that dorzolamide

had on the IOP of our normotensive dogs may not fully represent the effect for dogs with glaucoma. For systemically administered and experimental topical formulations of CAI, dogs with glaucoma typically have a larger decrease in IOP, compared with normotensive dogs.[9,18]

Dorzolamide significantly decreased aqueous humor flow rate by approximately 43%. This is similar to the decrease of 38% for aqueous humor flow rate reported in monkeys.[17] Analysis of our data indicated a wide degree of variability, with baseline values ranging from 2.2 to 9.8 µl/min. There is wide variability in aqueous humor flow rates in normotensive humans (0.2 to 32 µl/min),[27] and similar variability in dogs would be predicted on the basis of wide variability in facility of outflow.[28] The large difference in mean baseline flow rates between the dorzolamide and control groups can be attributed to the fact that randomization placed many of the dogs with lower flow rates in the control group. In retrospect, it would have been better to randomize dogs by use of a block design after assessing baseline flow rates. However, our conclusions are not affected, because we compared values after treatment with baseline values within dorzolamide or control groups.

Baseline flow rates that we obtained (combined value for the 2 groups of approx 5 µl/min) differed from published values. Anterior segment fluorophotometry and tonography are the 2 methods most commonly used for evaluating aqueous humor dynamics in dogs. Fluorophotometry directly measures aqueous humor flow rate by measuring the rate of egress of fluorescein from the anterior chamber. Conversely, tonography measures the facility of aqueous humor outflow. This value then can be used indirectly to calculate flow rate, provided investigators use assumptions of IOP, episcleral venous pressure, and rate of uveoscleral outflow.[29] On the basis of these assumptions, estimated aqueous humor flow rate in clinically normal dogs is 2.5 µl/min.[10] Additionally, tonography is affected by ocular rigidity, recording instrumentation and techniques, and rate of aqueous humor formation.[29] Fluorophotometry is not affected by any of these factors. The most important limitations with fluorophotometry involve assumptions necessary for the mathematic derivation of the K_o value and assumptions concerning the pathway of the flow of aqueous humor and the pathway of fluorescein after application to the cornea. These assumptions have been validated.[21,29] For these reasons, fluorophotometry is considered a more accurate technique than tonography for measuring aqueous humor flow rate.[21,29]

We can deduce that the mechanism of action of a 2% solution of dorzolamide is the suppression of aqueous humor production on the basis of decreases in IOP and aqueous humor flow rate. Dorzolamide appears to be well tolerated in dogs, and we are not aware of reports of adverse effects. Dorzolamide significantly decreased IOP in clinically normal dogs. It appears that dorzolamide is another drug that can aid in decreasing IOP in dogs with glaucoma.

[a]Trusopt 2% ophthalmic solution, Merck & Co, West Point, Pa.
[b]RW Johnson Pharmaceutical, Springhouse, Pa.
[c]Tono-Pen XL, Mentor O & O Inc, Norwell, Mass.
[d]FM-2 FluorotronMaster, OcuMetrics, Mountain View, Calif.
[e]Martin CL. Glaucoma (abstr), in *Proceedings*. 44th Annu Meet Am Coll Vet Ophthalmol 1977;301.
[f]Sugrue MF, Waheed A, Sly WS, et al. A study of the in vitro inhibition of human carbonic anhydrase isoenzymes I, II, and IV (abstr). *Invest Ophthalmol Vis Sci* 1993;34(suppl):930.
[g]Sears ML, Matsui H, Murakami M, et al. Localization of carbonic anhydrase (CA) mediated aqueous secretion to basolateral membranes of non-pigmented epithelium of ciliary process (abstr). *Invest Ophthalmol Vis Sci* 1994;35(suppl):1454.
[h]Sugrue MF, Funk HA, Lazarides E, et al. Evidence for a role of membrane bound carbonic anhydrase in intraocular pressure regulation in rabbits (abstr). *Invest Ophthalmol Vis Sci* 1994;35(suppl):1400.

References

1. Becker B. Decrease in intraocular pressure in man by a carbonic anhydrase inhibitor, Diamox. *Am J Ophthalmol* 1954;37:13–14.
2. Gonzales-Jiminez E, Leopold IH. Effects of dichlorphenamide on the intraocular pressure of humans. *Arch Ophthalmol* 1958;60:427–436.
3. Drance SM. The effects of ethoxzolamide (Cardrase) on intraocular pressure. *Arch Ophthalmol* 1959;62:679–684.
4. Lichter PR, Newman LP, Wheeler NC, et al. Patient tolerance to carbonic anhydrase inhibitors. *Am J Ophthalmol* 1978;85:495–502.
5. Epstein DL, Grant WM. Carbonic anhydrase inhibitor side effects. *Arch Ophthalmol* 1977;95:1378–1382.
6. Wallace TR, Fraunfelder FT, Petursson GJ, et al. Decreased libido—a side effect of carbonic anhydrase inhibitor. *Ann Ophthalmol* 1979;11:1563–1566.
7. Mogk LG, Cyrlin MN. Blood dyscrasias and carbonic anhydrase inhibitors. *Ophthalmology* 1988;95:768–771.
8. Maren TH. Teratology and carbonic anhydrase inhibition. *Arch Ophthalmol* 1971;85:16–17.
9. Gelatt KN, Gum GG, Williams LW, et al. Ocular hypotensive effects of carbonic anhydrase inhibitors in normotensive and glaucomatous Beagles. *Am J Vet Res* 1979;40:334–345.
10. Gum GG. Physiology of the eye. In: *Veterinary ophthalmology*. 2nd ed. Philadelphia: Lea & Febiger, 1991;124–161.
11. Lippa EA, Carlson L, Ehinger B, et al. Dose response and duration of action of dorzolamide, a topical carbonic anhydrase inhibitor. *Arch Ophthalmol* 1992;110:495–499.
12. Wilkerson M, Cyrlin M, Lippa EA, et al. Four-week safety and efficacy study of dorzolamide, a novel, active topical carbonic anhydrase inhibitor. *Arch Ophthalmol* 1993;111:1343–1350.
13. Simpson AJ, Gray TB, Ballantyne C. A controlled clinical trial of dorzolamide: a single-centre subset of a multicentre study. *Aust N Z J Ophthalmol* 1996;24:39–42.
14. Le HH, Chang MR, Cheng Q, et al. The effectiveness and safety of dorzolamide 2% in addition to multiple topical antiglaucoma medications. *J Ocul Pharmacol Ther* 1999;15:305–312.
15. Yamazaki Y, Miyamoto S, Sawa M. Effect of MK-507 on aqueous humor dynamics in normal human eyes. *Jpn J Ophthalmol* 1994;38:92–96.
16. Schmitz K, Banditt P, Motschmann M, et al. Population pharmacokinetics of 2% topical dorzolamide in the aqueous humor of humans. *Invest Ophthalmol Vis Sci* 1999;40:1621–1624.
17. Wang RF, Serle JB, Podos SM, et al. MK-507 (L-671,152), a topically active carbonic anhydrase inhibitor, reduces aqueous humor production in monkeys. *Arch Ophthalmol* 1991;109:1297–1299.
18. King TC, Gum GG, Gelatt KN. Evaluation of a topically administered carbonic anhydrase inhibitor (MK-927) in normotensive and glaucomatous Beagles. *Am J Vet Res* 1991;52:2067–2070.
19. Jones RF, Maurice DM. New methods of measuring the rate of aqueous flow in man with fluorescein. *Exp Eye Res* 1966;5:208–220.
20. Yablonski ME, Zimmerman TJ, Waltman SR, et al. A fluorophotometric study of the effect of topical timolol on aqueous humor dynamics. *Exp Eye Res* 1978;27:135–142.
21. Bloom JN, Levene RZ, Thomas G, et al. Fluorophotometry and the rate of aqueous flow in man: I. Instrumentation and normal values. *Arch Ophthalmol* 1976;94:435–443.
22. Coakes RL. Slit-lamp fluorophotometry of the anterior segment. *Trans Ophthalmol Soc U K* 1981;101:149–151.

23. Cunha-vaz J. Ocular fluorophotometry: standardization and instrumentation development. *Int Ophthalmol* 1993;17:147–153.

24. Ward DA, Cawrse MA, Hendrix DVH. Fluorophotometric determination of aqueous humor flow rate in clinically normal Beagles. *Am J Vet Res* 2001;62:853–858.

25. Miller PE, Nelson MJ, Rhaesa SL. Effects of topical administration of 0.5% apraclonidine on intraocular pressure, pupil size, and heart rate in clinically normal dogs. *Am J Vet Res* 1996;57:79–82.

26. Wistrand PJ, Schenholm M, Lönnerholm G. Carbonic anhydrase isoenzymes CA I and CA II in the human eye. *Invest Ophthalmol Vis Sci* 1986;27:419–428.

27. van Best JA, Boets EPM, Stolwijk TR. Simultaneous determination of corneal endothelial permeability value and anterior chamber flow. In: Cunha-vaz JG, Leite E, Raymos MC, eds. *Manual of ocular fluorometry*. Coimbra, Portugal: Coimbra, 1993;51–67.

28. Peiffer RL Jr, Gelatt KN, Gum GG. Determination of the facility of aqueous humor outflow in the dog, comparing in vivo and in vitro tonographic and constant pressure perfusion techniques. *Am J Vet Res* 1976;73:1473–1477.

29. Brubaker RF. Clinical evaluation of the circulation of aqueous humor. In: *Duane's clinical ophthalmology*. Philadelphia: JB Lippincott Co, 1992;3:1–11.